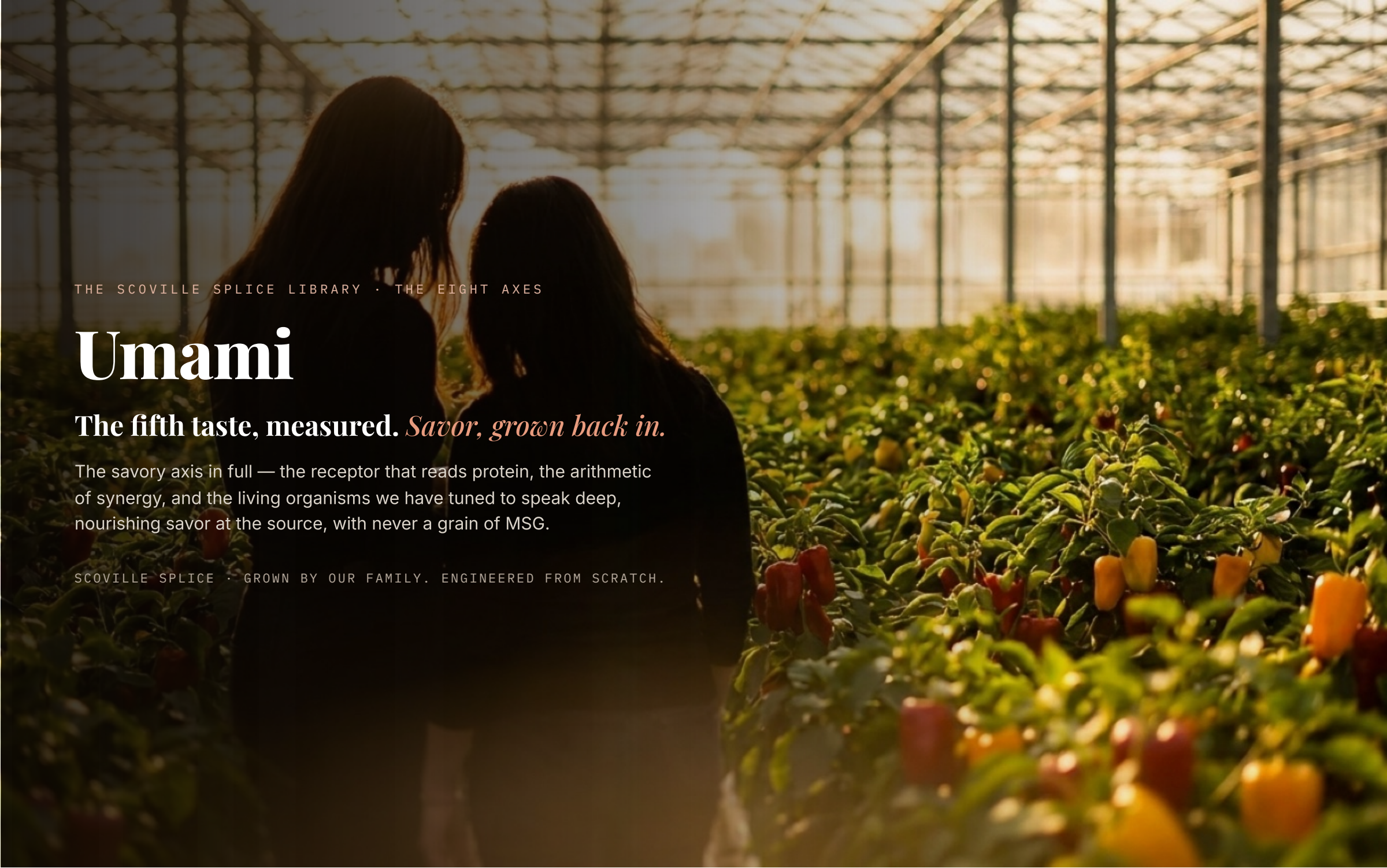

THE SCOVILLE SPLICE LIBRARY · THE EIGHT AXES

Umami

The fifth taste, measured. *Savor, grown back in.*

The savory axis in full — the receptor that reads protein, the arithmetic of synergy, and the living organisms we have tuned to speak deep, nourishing savor at the source, with never a grain of MSG.

SCOVILLE SPLICE · GROWN BY OUR FAMILY. ENGINEERED FROM SCRATCH.

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Opening essay · The taste of being fed

The first food any of us ever tasted was rich in it. Before a name, before a word in any language, before we could hold a spoon — the body already knew this taste and reached for it, because it is the one the body trusts. We have been chasing that savor, without a name for it, ever since.

Some tastes announce themselves. Sweetness arrives like applause; heat kicks the door in; a sour thing makes the whole face pucker and report. Umami does none of that. It is the taste you notice mostly by its absence — the broth that should have comforted you and merely filled you, the tomato that looked like August and tasted like water, the sauce that was loud and somehow empty at once. When savor is truly there you do not say *umami*. You say *more*, and reach for the bowl again, and feel, without quite knowing why, that someone has taken care of you. Savory is the body's word for *fed*.



The greenhouse at the end of the day — where the question that became this axis first came up at our table, and where savor is grown back into the plant at the source.

For most of human history we had the sensation and no word for it. Cooks knew only that certain things — a strip of kelp simmered in water, a handful of dried mushrooms, a long-aged paste of fermented soybean, a hard rind of cheese — made everything around them taste *fuller*, and they built whole cuisines on that trick without being able to say what the trick was. It took until 1908 for the thing to be named. In that year Kikunae Ikeda, a chemist at Tokyo Imperial University, boiled down the kombu broth his kitchen made each night and asked the question no one had thought to ask of a pleasant taste: *what molecule is this?* The answer was glutamate — the loosed, free form of the single most ordinary amino acid in all of life. He called the taste **umami**, from the Japanese *umai*, "delicious," and argued it was a fifth basic taste, as distinct from sweet and salt

as those are from each other. It took the rest of the century, and the eventual discovery of the receptors themselves, for the world to agree he was right.

This is, we should say plainly, the axis our family argued about most at the table — which is where the company began, and where its voice still lives. The computing was one pair of hands; the cooking, the tasting, the long disagreements about whether a thing was *deep* or merely *salty*, belonged to all of us. Someone would lift the lid off a pot that had been going a while, and the whole kitchen would lean in before a single spoon was dipped, and we would try, and fail, to say what exactly we were leaning toward. Umami is the taste that sent us looking for a number in the first place, because it was the one we could feel most certainly and name least well. Everything that follows is the attempt to earn, with rigor, the right to that reflex at the stove.

The receptor, exactly

Glutamate is not an exotic flavor compound; that is the first thing to hold. It is the stuff of protein itself — the carbon skeleton a cell hangs nitrogen on, the busiest amino acid in nearly every living tissue, the hub through which an organism shunts nitrogen into everything it builds. What the tongue is tuned to is not glutamate in general but *free* glutamate: glutamate loosed from the protein chain and dissolved, unbound, in solution. A whole steak is full of glutamate and tastes of almost none, because the glutamate is locked into protein; a spoon of aged paste made from the same quantity of protein tastes of nothing else, because time and enzymes have cut it free. The receptor does not read *how much protein is here.* It reads *how much protein has already been broken down for me* — nourishment pre-digested, the oldest promise a food can make, and, not by any coincidence, the taste that floods a mother's milk.

Two receptors carry that message, and the Index names both: the metabotropic glutamate receptor **mGluR1** and the heterodimer **TAS1R1·TAS1R3**. Both are class C G-protein-coupled receptors, and both share a peculiar and beautiful piece of architecture — a large extracellular domain shaped like a bilobed clamshell, the *Venus flytrap*. Glutamate drifts into the cleft of that flytrap and the two lobes close around it, near the hinge, the way the plant it is named for folds shut on a fly. The umami-specific subunit is TAS1R1; its partner TAS1R3 is the same subunit the tongue lends to the sweet receptor, which is why savor and sweetness are built from a shared half and read on neighboring wiring. The taste form of mGluR1 is itself a quiet marvel of tuning: a truncated variant, shorn of much of the high-affinity N-terminus the brain's version carries, so that it answers not to the micromolar glutamate of a synapse but to the *millimolar* glutamate of actual food. The receptor was fitted to the broth, not the nerve.

Closure of the flytrap is only the first move. The conformational change travels down through a cysteine-rich linker into the seven-transmembrane bundle buried in the cell membrane, which releases the heterotrimeric G protein — α-gustducin, the tongue's own transducin, and its βγ partners. The βγ pair switches on phospholipase C-β2; the enzyme cleaves the membrane lipid PIP_2

into diacylglycerol and inositol trisphosphate; the IP_3 unlocks its receptor on the endoplasmic reticulum and calcium floods out of internal stores into the cytosol. That rising calcium gates TRPM5, a calcium-activated cation channel, and sodium pours in; the cell depolarizes; voltage and calcium together open the CALHM1/CALHM3 channel, and the taste cell pushes ATP straight out through that pore — no vesicle, no synaptic bag, just a channel flung open — onto the P2X2/P2X3 receptors of the waiting afferent nerve. From flytrap to nerve is perhaps a dozen steps, each one a lock meeting its key, and the whole relay fires and resets a billion times a second while you eat. The number we build on this axis is a measurement of the very first step: how readily glutamate closes the flytrap at all.

The arithmetic of loudness

The Index gives that first step a number, and the formula is the same plain ratio that governs every axis in this library:

> **$IFI\text{-}U = 1{,}000 \times EC_{50}(reference) \div EC_{50}(candidate)$**

EC_{50} is the concentration of stimulus at which a receptor drives to half of its maximal response — the midpoint of the dose-response curve, the concentration at which the flytrap is closing half the time. It is a statement of *potency*, of how little it takes to be heard, and it runs the opposite direction to loudness: a smaller EC_{50} means a louder voice, because less of the compound is needed to reach the half-way point. Inverting EC_{50} turns potency the right way up, and scaling against a fixed reference turns it into a score. We anchor the reference so that **IFI-U = 1,000 corresponds to a pinned glutamate standard whose EC_{50} our assay reads at 2.00 mM** — a stimulus of exactly average, reference loudness. Everything quieter than the standard scores below a thousand; everything louder, above.

The dose-response itself is a Hill curve. Writing R for response, R_{max} for the ceiling, and n for the Hill coefficient — about 1.3 at this receptor, a touch of positive cooperativity as the flytrap settles —

> **$R([S]) = R_{max} \cdot [S]^n \div (EC_{50}^n + [S]^n)$**

At [S] = EC_{50} the two terms in the denominator are equal and $R = R_{max}/2$, which is the definition of the midpoint. Step up to twice the EC_{50} and the response climbs to $2^{1.3}/(1 + 2^{1.3}) = 2.46/3.46 =$ **71%** of maximum; drop to half the EC_{50} and it falls to **29%**. That steepness around the midpoint is why EC_{50} is the honest place to measure: it is where the curve is most sensitive to the stimulus, where a small change in loudness moves the response most.

Now the worked case our family cares about most — the tomato, measured on this scale. A tomato bred to survive a truck and ripen in a box half-activates the umami receptor only at an effective concentration of **6.67 mM** glutamate-equivalents; the fruit we restore, ripened on the vine through its native route, reaches the same half-activation at **0.77 mM**. Run both through the formula:

- Shelf tomato: IFI-U = 1,000 × 2.00 ÷ 6.67 = **300**
- Restored tomato: IFI-U = 1,000 × 2.00 ÷ 0.77 = **2,600**

The restored fruit is **8.7× louder** — the ratio 6.67 ⁄ 0.77 — and the Index reports that as the ratio 2,600⁄300, the same 8.7. The number is not a verdict; it is a translation. It says, in one figure a chef and a formulator can both read, that this fruit speaks savor nearly nine times as clearly as the one the century handed us.

STIMULUS	EFFECTIVE EC_{50} (GLUTAMATE-EQUIV.)	IFI-U	VS. REFERENCE
Shelf tomato — harvest-green, trucked	6.67 mM	300	0.30×
Reference standard — the pinned anchor	2.00 mM	1,000	1.00×
Phoenix · SS-0096 — savory axis of a full-spectrum fruit	1.11 mM	1,800	1.80×
Restored tomato — native GAD / GDH1 route	0.77 mM	2,600	2.60×
Glutamate + inosinate — the synergy (see below)	0.20 mM	10,000 †	10.0×

† Raw index; above the per-axis scorecard normalization ceiling of 5,000, where the radar plot saturates.

Those millimolar figures are easy to feel once you convert them. Free glutamic acid has a molar mass of 147.13 g/mol, so the 2.00 mM reference is 2.00 mmol/L × 147.13 mg/mmol = 294 mg/L, or **29 mg per 100 mL** — about a pinch in a glass of water. The restored tomato's 0.77 mM works out to **11 mg per 100 mL** of glutamate-equivalent potency; the shelf tomato's 6.67 mM to **98 mg per 100 mL**. One caution we keep in front of us: the potency a food shows on the index is not the same quantity as the milligrams of glutamate the fruit contains. Potency folds in how the glutamate is presented — free or bound, alone or beside a synergist — and a fruit richer in free glutamate reaches half-activation at a greater dilution, a lower effective EC_{50}, a louder reading. Content and loudness travel together, but they are measured on different rulers, and the index is a ruler of loudness.

Why savor multiplies instead of adds

Here is the fact that makes umami unlike every other taste, and the reason a good broth is a small astonishment rather than a sum. Glutamate does not work alone. A family of 5′-ribonucleotides — inosinate (IMP), guanylate (GMP), and to a lesser degree adenylate (AMP) — barely register on their own, yet set beside glutamate they do not add to it. They *multiply* it. These molecules bind a second site on the TAS1R1 flytrap, out near the lip of the open clamshell, and once bound they brace the flytrap in its closed, active conformation — a positive allosteric modulation, a thumb on the latch. Glutamate now closes the receptor more readily and stays bound longer, so the receptor's affinity for glutamate rises and its EC_{50} slides left. In our assay, 0.5 mM of inosinate pulls the glutamate EC_{50} from 2.00 mM down to roughly **0.20 mM** — a tenfold leftward shift. On the index that is a clean, literal statement: the same glutamate now reads ten times louder, IFI-U 1,000 becoming nearly **10,000**, because IFI-U is proportional to $1/EC_{50}$ and the EC_{50} fell by ten. Synergy is not a special effect outside the number. It is the number, moving.

The classical description of that multiplication is Shizuko Yamaguchi's synergy equation, worked out in 1967 and never improved upon. Writing *u* for the glutamate concentration and *v* for

the ribonucleotide concentration, both in grams per 100 mL, the equivalent umami intensity *y* — the concentration of glutamate *alone* that would taste as savory as the mixture — is

$$y = u + 1{,}218 \cdot u \cdot v$$

The whole story lives in that cross term. Hold the glutamate fixed at a modest u = 0.010 g/100 mL and feed in a little ribonucleotide:

GLUTAMATE *U* (G/100 ML)	RIBONUCLEOTIDE *V* (G/100 ML)	NAÏVE SUM *U + V*	EQUIVALENT GLUTAMATE *Y*	AMPLIFICATION *Y ⁄ U*
0.010	0.0000	0.010	0.010	1.0×
0.010	0.0025	0.0125	0.040	4.0×
0.010	0.0050	0.0150	0.071	7.1×
0.010	0.0100	0.0200	0.132	13.2×

Read the last row against the third from the end: a mixture holding two-hundredths of a gram of actual savory material tastes as deep as **thirteen-hundredths** of a gram of pure glutamate — the arithmetic of addition would have promised 0.020 and the receptor delivers 0.132. Savor is the one taste where one plus one can be seven, or eleven, or thirteen. Every kitchen that ever built a stock understood this in the hand long before anyone could write it down: the glutamate of the vegetable and the ribonucleotide of the long-simmered depth, found together, climbing the cross term.

This is also the quiet triumph of a vegetarian table, and we write it as one. The broth that taught the world this synergy need never contain an animal. Kombu carries the glutamate; dried shiitake carries the guanylate; steep them together and the two sit on the same flytrap — glutamate in the cleft, guanylate at the lip — and the cross term does its multiplying with nothing caught, nothing killed, nothing but kelp and a mushroom and patient water. A lifetime of cooking this way is a lifetime of proof that depth was never a thing you had to take from a body. It was always a thing two plants could say to each other across a receptor.

Where the glutamate pools

To restore savor to a fruit, you have to know where the fruit keeps it, and why a century of breeding mislaid it. Glutamate sits at the exact center of a plant's nitrogen economy. Ammonium entering the plant is fixed into glutamate through the GS/GOGAT cycle — glutamine synthetase charging glutamate into glutamine, glutamate synthase handing the nitrogen back out onto a molecule of 2-oxoglutarate to make two glutamates where there was one. Glutamate dehydrogenase, the **GDH1** the restored route leans on, runs the reductive hinge directly: 2-oxoglutarate plus ammonium, reduced, becomes glutamate. From that central pool every other amino acid is transaminated; glutamate is the account every nitrogen transaction clears through. A ripening fruit, as its cells soften and its storage proteins are taken apart by proteases, floods that pool with *free* glutamate — the bound form cut loose exactly as it is in an aging paste, except here it happens on the vine, in the last days of ripening, in the sun.

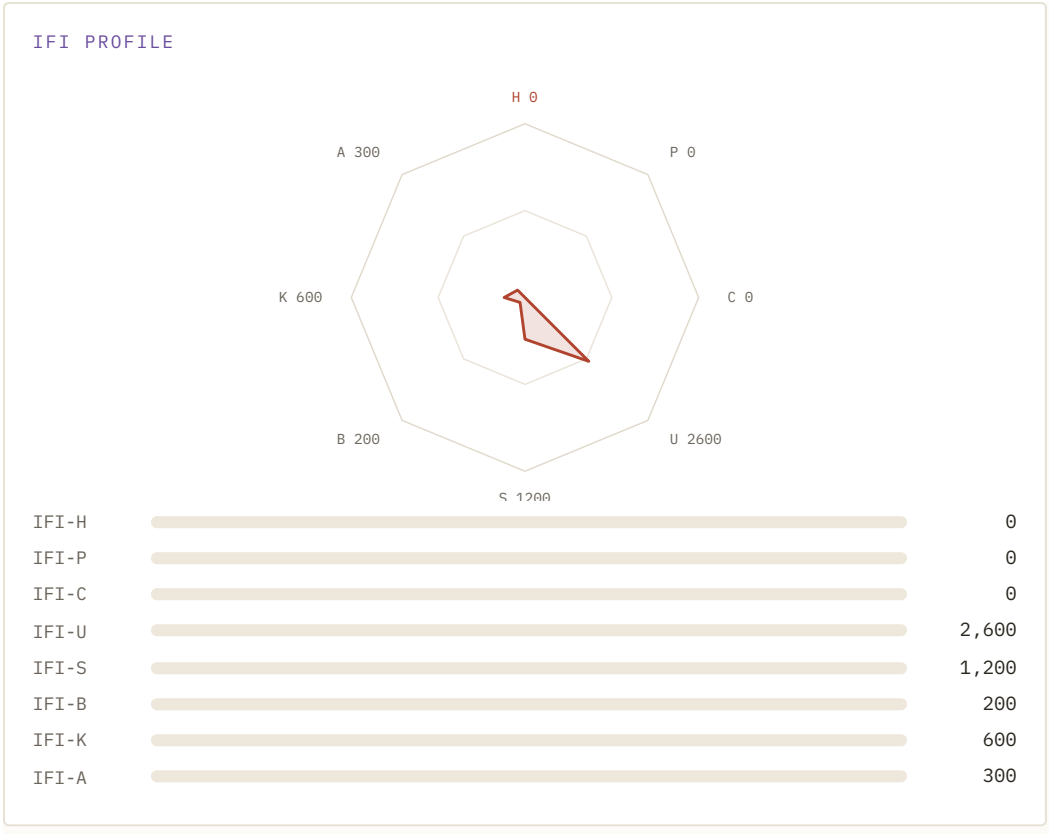
Those last days are precisely what the truck took. A fruit picked hard and green to ride a thousand miles never runs the final ripening program that liberates its glutamate; the cold chain that keeps it from spoiling also arrests the proteolysis that would have freed the savor; and a century of selecting for fruits that ship, store, and sit without bruising quietly selected *against* the very softening and self-digestion that savor requires. The arithmetic of the loss is brutal and specific: the tomato gave up roughly **93%** of its vine-ripe peak of free glutamate on the way to the shelf. We were handed a fruit emptied of the taste that is the fruit's whole point, and then told to buy the taste back in a shaker — to stir in the isolated compound the plant had been bred out of making.

We never believed the difference needed making up. It needed *restoring*, and restoring is a word we mean literally. There is no exogenous additive in the fruit we grow, no dusted-on glutamate, nothing shaken from a jar. We tune the plant's own enzymes — lifting GDH1 to hold the central pool full, easing back the glutamate decarboxylase (**GAD**) that otherwise siphons free glutamate off into GABA — so the native route pools savor in the ripening fruit the way the vine always meant to, and we let the fruit ripen long enough to finish the program the truck interrupted. Across the line the result is designed to deliver the consistency the whole platform is built on: a batch-to-batch coefficient of variation of **≤ 7%**, against the **42%** a wild population throws. In our model, the designed EC_{50} for the restored fruit is 0.77 mM with a predicted standard deviation near 0.05 mM — a CV of 6.5% — and because the reference standard is a tightly pooled constant, that candidate variation carries almost unchanged through the formula: our model predicts IFI-U = 2,600 with a spread of about ±170. The wild fruit is not only quieter; it is unreliable, loud one September and hollow the next. The restored fruit is designed to be savory *on schedule*.



The restored tomato in cross-section — the loculi and jelly where ripening proteases cut glutamate free of its protein and pool it, the 93% the truck took, grown back in through the native GAD / GDH1 route.

Read as a full eight-axis profile, the restored tomato is an honest portrait of what a tomato actually is — no heat, no pungency, no cooling bite, a loud umami carried over real sweetness, a little body beneath, a trace of tannin at the edge:



The seven that speak it

The tomato is where savor is easiest to love, but it is one of seven living organisms our family has tuned to speak this taste again, and the seven divide cleanly into those that *hold* glutamate and those that *free* it. Three are plants that pool free glutamate in their own tissue — the tomato, the soybean, the tea leaf. Four are the fungi and microbes that make savor their whole living, carrying the proteases that cut glutamate loose or the ribonucleotides that multiply it: yeast, koji mold, shiitake, mushroom. The construct counts and the axes each one is engineered along are drawn straight from the platform's own ledger.

ORGANISM	CONSTRUCTS	PRIMARY AXES	WHAT IT BRINGS TO SAVOR
Tomato	2,040	U · S	free glutamate pooled in the ripening fruit
Soybean	360	U · K	protein cut to free glutamate by fermentation
Tea	280	U · B · A	theanine and glutamate, the pale green depth
Yeast	240	U · K	glutamate and 5′-ribonucleotides together
Koji mold	80	U	the proteases that liberate glutamate
Shiitake	80	U	guanylate — the synergist, the multiplier
Mushroom	40	U	free glutamate beside its own ribonucleotide

The soybean is savor by patience. A whole soybean, like a whole steak, is dense with glutamate and tastes of almost none, because the glutamate is bound into storage protein. Fermentation is the slow knife: koji's proteases — the same fungal enzymes that earn their own line above — spend months cutting the protein down, residue by residue, until the bound glutamate is free glutamate and the bean that tasted of nothing tastes of everything a long-aged paste is loved for. We tune the bean toward umami and kokumi both, because

savor and the sensation of *body* ride together through a rich ferment, and because the soybean is, across more of the world's kitchens than any other, the foundation savor is built on.



Soybean — glutamate locked in storage protein, waiting for the slow knife of fermentation to cut it free.

Tea is the most surprising member of the seven, and the pale green cup is where umami hides in plain sight. The amino acid theanine, cousin to glutamate and concentrated in the shaded leaf, lends a savory roundness that a well-made green tea carries beneath its astringency — the reason a fine gyokuro tastes less like a beverage than like a broth. Tea is the one organism here that lives on three axes at once, umami answering to this receptor while its catechins speak to bitterness and its tannins to the astringency of the finish, which is why the leaf is such an exacting thing to tune: lift the savor without flattening the structure that makes tea *tea*. The remaining microbes — yeast with its native cargo of glutamate and ribonucleotides both, shiitake with its generous share of the guanylate that multiplies everything around it, the humble mushroom carrying free glutamate beside its own nucleotide — are savor's specialists, the organisms a kitchen reaches for not to be tasted themselves but to make everything else taste *fed*.



Tea — theanine and glutamate beneath the astringency; umami hiding in the pale green cup.

What the number cannot say

A number is a form of respect for the reader, and the deepest respect is to be honest about its edges. IFI-U measures the loudness of savor at the receptor, and there are real things it does not measure, which anyone designing with it should keep in hand.

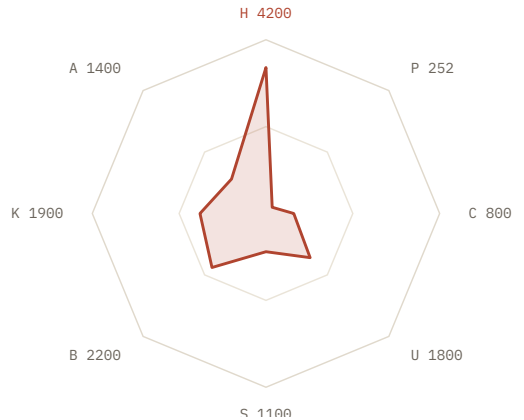
It measures potency, not ceiling. EC_{50} fixes *where* the dose-response curve reaches half-height, not *how high* the curve climbs — that is R_{max}, the efficacy, a separate quantity. Two foods can share an EC_{50} and part ways at full concentration, one of them topping out gently and the other roaring; the index, anchored on the midpoint, reports them alike. We hold both numbers in the lab and publish the one that travels best between a chef's palate and a formulator's spec, but a high IFI-U is a statement about *how little it takes to be heard*, not a promise about the loudest the food can ever be.

It measures savor in isolation, and savor is the least solitary of tastes. The tenfold leftward shift that a ribonucleotide buys is real, but it is a property of the *pairing*, not of the glutamate alone, and a food scored by itself cannot tell you what it will become beside its synergist. Sodium complicates it further — salt genuinely amplifies the perception of umami, which is why a broth tastes thin unseasoned and whole with a pinch, and the index, measured in a controlled matrix, holds the salt constant and so cannot report that lift. Adaptation complicates it again: the receptor habituates across a long meal, so the tenth spoon is quieter than the first through no change in the bowl. And the matrix itself — fat, acidity, temperature, the thickness of a sauce — bends how freely glutamate reaches the receptor at all. The number is measured against a fixed background precisely so it can be compared across foods; the cost of that fixity is that it describes the stimulus, not the supper.

And it carries the honest caveat of every anchored scale: the reference is a human choice. We pinned IFI-U = 1,000 to a defined glutamate standard at 2.00 mM because a standard has to sit somewhere, and every score on the axis is a ratio against that decision. Change the anchor and every number moves together; the *rankings* and the *ratios between foods* do not, which is why the index is trustworthy for design even though its zero-point is a convention. The scorecard's own ceiling is a related honesty — we normalize each axis at 5,000 for the radar plot, so a strongly synergized broth whose raw IFI-U runs past ten thousand simply saturates the visual, louder than the picture can draw.

What the number does, it does well: it lets a maker stop wishing for *savory depth* and start specifying it. And it lets a fruit engineered along all eight axes at once show exactly how savor composes with everything else. The flagship Phoenix — SS-0096, our model predicts approximately 2.95 million SHU of designed fire — was not built for umami, yet it registers on this axis too, savor sitting inside a plant that also carries heat, sweetness, bitterness attenuation, kokumi, cooling, pungency, and astringency, each one a separate, designed voice:

IFI PROFILE



IFI-H	4,200
IFI-P	252
IFI-C	800
IFI-U	1,800
IFI-S	1,100
IFI-B	2,200
IFI-K	1,900
IFI-A	1,400

These are computational designs; Scoville Splice's cultivars and constructs have not been experimentally validated in a laboratory.

That a single engineered fruit can read **1,800** on savor while roaring on heat is the whole argument of this axis made literal: umami is not a garnish the other tastes tolerate but a dimension that composes with all of them, a thing you can dial up in a tomato until it tastes the way August promised, or set into a full-spectrum plant beside seven other voices, and in neither case reach for a shaker. The deepest and most nourishing flavor a plant can offer, grown back in at the source — because savory is the body's word for *fed*, and we would rather feed it from the fruit than from a jar.



PART I OF V

The Signal

the fifth taste · the Venus flytrap · the number · the synergy

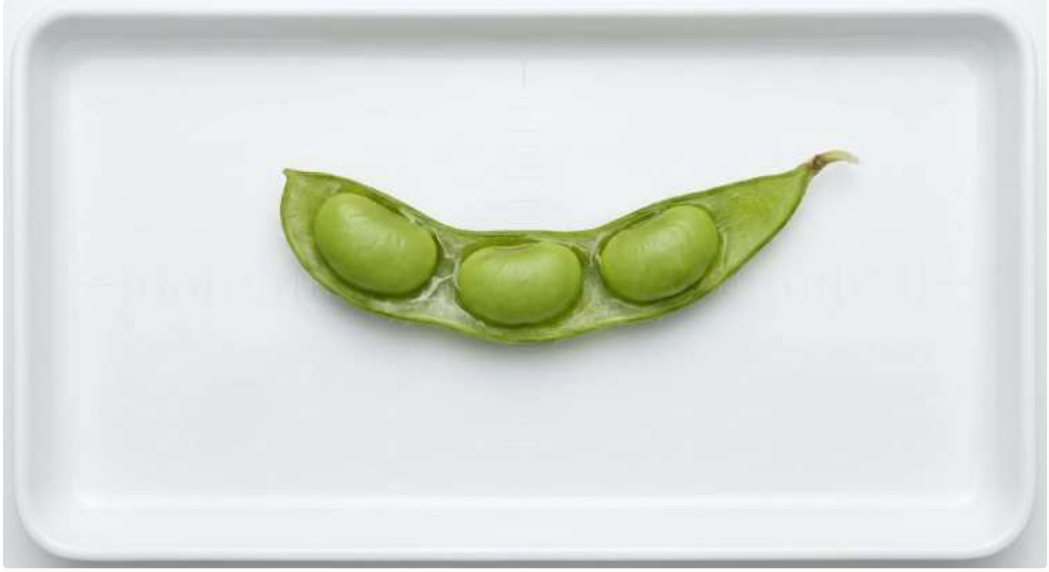
Chapter 1 · The Fifth Taste — *how savory got its name.*

IFI-U · the physiology of "fed"

To understand why savor deserves its own axis, you have to understand that for most of the modern era it was denied one. The textbooks taught four tastes — sweet, sour, salt, bitter — and drew little maps of the tongue assigning each to a region, a diagram now known to be wrong in nearly every particular. Umami was not on the map at all. It was dismissed as a blend, a side-effect, a flavor rather than a taste, for the better part of ninety years after Ikeda named it. The reason the dismissal held so long is the reason the taste matters so much: umami is quiet. It does not spike and vanish the way sweet does; it does not alarm the way bitter does. It fills. It underlies. It tells the body a steady, low, trustworthy truth, and the body answers not with a jolt but with a deep, animal contentment that is easy to feel and very hard to point at. The work of this chapter is to point at it — to take the thing a cook feels on the faces of the people she feeds and turn it, step by step, into a receptor, an equation, and a number a plant can be grown to carry.

The molecule the tongue can read

What Ikeda had actually found, boiling down his kombu, was that the savory principle of that broth was **monosodium glutamate** — the sodium salt of L-glutamic acid, one of the twenty amino acids from which all proteins are assembled. This is the fact that unlocks the whole axis, so we will say it carefully. Glutamate inside a protein, stitched into a long folded chain, is tasteless — the tongue cannot read it, because the receptors that answer to it can only grip the free molecule, loose and unbound. A raw soybean is dense with glutamate and tastes of almost nothing, because nearly all of that glutamate is locked into storage proteins. Age that soybean into miso, or ripen a tomato on the vine, or simmer a kombu broth for an afternoon, and the glutamate comes loose — cut free by enzymes, by heat, by time — and only then does it reach the receptor and declare itself. **Savor, biochemically, is the taste of protein coming apart into a form the body can use.** Cooking, aging, ripening, curing, and fermentation are, at bottom, all the same act: they set glutamate free.



A soybean in cross-section — dense with glutamate, nearly all of it locked inside storage proteins the tongue cannot read until a ferment's enzymes cut them loose.

It is worth naming the enzymes, because "by enzymes, by heat, by time" is the kind of phrase that hides the whole mechanism. Three machines do the liberating. **Endopeptidases and exopeptidases** — the proteases — hydrolyse the long storage proteins into peptides and then clip free amino acids off their ends; this is the slow engine of a miso or a soy sauce, where a mold's secreted proteases spend months taking a bean apart one bond at a time. **Glutaminase** performs a quieter trick: it deamidates glutamine, the amide cousin that a plant uses as a nitrogen shuttle, converting it directly into glutamate — which is why a ferment's savor keeps rising even after the obvious proteolysis has slowed. And in a ripening fruit the plant's own **glutamate dehydrogenase** and **glutamate decarboxylase** route carbon and nitrogen into and out of the free-glutamate pool as the fruit softens, so that a tomato's savor is not an accident of decay but a programmed event of ripening. A green tomato is nearly silent; a vine-ripe one can carry an order of magnitude more free glutamate than its flesh held a week earlier. The fruit is, in a real sense, cooking itself.

Numbers make this concrete, and a conversion is the first piece of arithmetic the axis asks of us. Free L-glutamic acid has a molar mass of 147.13 grams per mole. So a savory liquid reported at, say, 30 milligrams of free glutamate per 100 millilitres is carrying 300 milligrams per litre, or 300 ÷ 147.13 = **2.04 millimolar** free glutamate. Hold that figure. It is almost exactly the concentration at which the tongue's umami receptor reaches half of its full response — the hinge of the whole dose–response curve — and we will return to it when we anchor the number.

What the body already knows

Consider the evidence the body itself offers. Free glutamate is the single most abundant free amino acid in human breast milk — present at roughly 20 milligrams per hundred millilitres, many times the concentration of any other free amino acid there, and far above the level in cow's milk. Run that through the same conversion: 20 mg/100 mL is 200 mg/L, which is 200 ÷ 147.13 = **1.36 millimolar**. Set that against a receptor whose half-activation sits near 2.0 millimolar and the result is startling in its intention — the first food we are built to want drives the savory receptor to something like forty percent of its maximum, right out of the gate, before a child has tasted anything else. The appetite is tuned to savor before it is tuned to language.

Consider the sickroom. In every culture that ever kept one, the food carried to the ailing is a broth — miso, congee, kombu dashi, a long-simmered bone — and every one of those is, chemically, a glutamate delivery system. We did not reason our way to broth-for-the-sick from nutritional science. We arrived at it because the body, offered savor, accepts nourishment it would otherwise refuse, and generations of cooks read that acceptance on the faces of the people they were trying to keep alive. Umami is the taste the appetite trusts when it trusts nothing else.

This is the physiology beneath the poetry, and it is worth stating as a design premise rather than a sentiment: of the five basic tastes, each is a sensor for a dimension of nutrition or danger. Sweet flags accessible energy — sugar, the quick fuel. Salt flags the sodium and minerals the blood runs on. Sour flags acid, the ripeness-or-spoilage boundary. Bitter flags the alkaloids and toxins a plant uses to defend itself; it is a warning channel, tuned to make us spit and reconsider. And umami flags **protein** — the free glutamate on the tongue is the body's advance word that amino acids, the costliest and most essential class of nutrient, are present and available. Four of the five tastes are, in effect, nutritional instruments. Umami is the one tuned to the nutrient we most need and can least synthesize for ourselves, and that is why its signal runs so deep and reads as care rather than mere flavor. For a family that has fed itself from plants for generations, this is the axis that matters most and the one industrial agriculture quieted most thoroughly, and so it is the one we took most personally.

A century to believe the tongue

It is worth laying out how long the world took to believe any of this, because the slowness is itself the argument for a number. Umami's discovery was not one event but a century-long accumulation, and each step added a piece the tongue had always known and the textbook had always refused:

YEAR	WHO	WHAT THEY FOUND
1908	Kikunae Ikeda	glutamate is the savory principle of kombu; names *umami*
1913	Shintaro Kodama	inosinate (IMP) is the savory principle of dried bonito
1957	Akira Kuninaka	guanylate (GMP) is the savory principle of dried shiitake
1960s	Kuninaka, Yamaguchi	the glutamate–nucleotide **synergy** is measured and quantified
1985	first international umami symposium	the term *umami* is formally adopted into the scientific literature
2000–2002	Chaudhari; Nelson, Zuker, et al.	the receptors are cloned — taste-mGluR4, then the TAS1R1·TAS1R3 pair

Read the right-hand column and you are reading this axis in miniature: a glutamate-maker, a nucleotide-maker, the synergy between them, and at last the receptors that explain why the synergy exists. It took the better part of a hundred years and a dozen careers to assemble what a cook with a pot of kombu had, in a sense, known all along. Three of those rows — the glutamate, the inosinate, the

guanylate — are nothing more or less than the shopping list for building savor on purpose. The last two rows are the receptor that tells you, at last, *how much.*

Inside the receptor — a hand closing

Here is where the poetry has to become mechanism, because you cannot design against a feeling; you can only design against a structure. When free glutamate reaches the tongue, the molecule that answers is a **heterodimer of two class-C G-protein-coupled receptors, TAS1R1 and TAS1R3**, sitting together in the membrane of the taste cell. Class-C receptors wear a large structure on the outside of the cell called a **Venus flytrap domain** — two lobes hinged together like a clamshell, or like an open hand. Glutamate settles into the cleft of the TAS1R1 lobe, and the two lobes swing shut around it. That closing — a hand gently making a fist around a single small molecule — is the physical event of tasting savor. Everything after it is consequence.

The closure does not stay on the outside of the cell. It twists a rigid **cysteine-rich linker** that threads down to the receptor's **seven-transmembrane bundle**, the part embedded in the membrane, and the twist changes the shape of that bundle on the cell's inner face. Now the cascade runs, and it runs the way most of taste runs: the activated receptor hands off to **α-gustducin**, a taste-specific G protein; gustducin's partners switch on **phospholipase C-β2**, which cleaves a membrane lipid into the second messenger **IP_3**; IP_3 opens the **type-3 IP_3 receptor** on the cell's internal calcium store, and a pulse of Ca^{2+} floods the cytoplasm; that calcium opens **TRPM5**, a calcium-gated sodium channel, which depolarises the cell; and the depolarisation, with the calcium, drives **CALHM1 channels** open to release **ATP** onto the afferent nerve fibre waiting beneath. One glutamate molecule, one clamshell closing, and a handful of microseconds later a nerve is carrying the word *protein* toward the brain. The chain is long, but every link is a lever, and a lever is a thing you can pull on by design.

Glutamate's receptor is not quite alone. Taste tissue also expresses **metabotropic glutamate receptors** — a truncated taste variant of **mGluR4**, and **mGluR1** — that are built from the same class-C clamshell but tuned to a different register. The versions in your brain answer to glutamate at *micromolar* concentrations, the whisper of a synapse; the taste variants are stripped-down, lower-affinity, and answer only at the *millimolar* concentrations of actual food. This is an elegant piece of biological bookkeeping: the same molecule means "a neuron is firing" in one place and "there is protein in this mouthful" in another, and the receptor is re-tuned by three orders of magnitude so the two messages never cross. For the Index we headline **mGluR1** alongside the TAS1R1·TAS1R3 pair, because together they define the millimolar-scale response that a food actually produces, and it is that response — not any single protein in isolation — that the number must track.

The synergy, measured

Now the second organism in the shopping list earns its place, and it is the most beautiful piece of mechanism on the whole axis. The **5′-ribonucleotides** — inosinate (IMP) from a dried fish or a yeast,

guanylate (GMP) from a dried shiitake — are nearly tasteless on their own. What they do is bind a *second* site, a shallow pocket on the outer face of the TAS1R1 Venus flytrap, right beside the hinge. They do not trigger the receptor. They hold the clamshell. A nucleotide docked at the hinge stabilises the closed, active conformation, so that glutamate which drifts into the cleft is far less likely to drift back out — and the whole dose–response curve slides to the left. The receptor becomes dramatically more sensitive to glutamate it was already able to taste. This is why a pinch of dried mushroom transforms a tomato sauce out of all proportion to what the mushroom itself tastes of: the mushroom is not adding savor, it is amplifying it.

The amplification was measured before the structure was known, and the measurement still governs how savor is built. The relationship, in the form Yamaguchi fixed in the 1960s, is multiplicative rather than additive:

```
y = u + 1218 · u · v
```

where u is the glutamate concentration and v the nucleotide concentration, both in grams per decilitre, and y is the equivalent intensity expressed as glutamate alone. The constant **1,218** is the synergy coefficient for glutamate with inosinate; for glutamate with guanylate — the shiitake nucleotide — it is larger still, which is why the dried-mushroom route is the one we reach for when an organism needs the loudest possible savor from the smallest free-glutamate pool.

Work a case. Take a weak glutamate solution, u = 0.05 g/dL — on its own a faint, almost-savory water. Add an equal measure of inosinate, v = 0.05 g/dL, each still nearly tasteless alone. Then

```
y = 0.05 + 1218 × 0.05 × 0.05 = 0.05 + 3.045 = 3.095 g/dL equivalent.
```

The mixture tastes like roughly sixty times the glutamate solution you started with, from ingredients that, separately, you would struggle to taste at all. Nothing else in flavor science behaves like this. It is the single most important fact about building umami, and it means savor is never a question of one compound but of a *pair* — the base note and the modulator, the glutamate-maker and the nucleotide-maker — and of growing both into the same fruit so the amplification happens in the mouth and not in a packet.

From loudness to a number

With the receptor and its synergy in hand, the Index becomes almost inevitable. We do not score a food by how much glutamate it holds — a gram of locked storage protein scores nothing, and two foods with identical free glutamate taste wildly different once a nucleotide is present. We score it by how *loudly* it speaks: how little of it the receptor needs to reach half of its full response. That quantity is the **EC_{50}**, the half-maximal effective concentration, and loudness is simply its inverse — the lower the EC_{50}, the louder the food, because less of it says as much. Invert it against a fixed reference, scale by a thousand, and you have the axis:

$$\text{IFI-U} = 1{,}000 \times EC_{50}(\text{reference}) \div EC_{50}(\text{candidate})$$

We anchor the reference at a half-activating free-glutamate concentration of **2.0 millimolar** — the value, almost exactly the ripe-fruit figure from the conversion earlier, that we define by convention to read **IFI-U = 1,000**. Everything else is read against it. A candidate that reaches half-max at a *lower* concentration is louder and scores *higher*; one that needs *more* of itself to be heard scores lower.

The behaviour of that half-max point deserves a moment, because it explains why small changes in a fruit produce large changes on the tongue. The dose–response is a sigmoid — the **Hill equation**, response = $S^n \div (EC_{50}^n + S^n)$, where n is the Hill coefficient, measured near 1.5 for the umami heterodimer. The curve is flat at the bottom, flat at the top, and steepest exactly at the EC_{50}. Which means a plant sitting near its half-max is in the most responsive part of the curve: a modest lift in free glutamate buys a disproportionate lift in perceived savor, and a modest loss costs one.

$S \div EC_{50}$	RESPONSE (N = 1)	RESPONSE (N = 1.5)
0.25	0.200	0.111
0.50	0.333	0.261
1.00	0.500	0.500
2.00	0.667	0.739
4.00	0.800	0.889

Read the steepness: moving from half the EC_{50} to twice it — a mere fourfold change in concentration — carries the response from a quarter of maximum to three-quarters. That is the window a breeding program lives or dies in, and it is why restoring a tomato's free-glutamate pool is worth doing at all.

Now put real cultivars on the axis. The supermarket tomato — picked green, gassed ripe, shipped a continent — has lost the overwhelming majority of the free glutamate a vine-ripe fruit would carry; it registers around **IFI-U 300**. Restore that pool through the plant's own glutamate-decarboxylase and dehydrogenase route and the same fruit climbs to **IFI-U 2,600**. Our full-spectrum flagship, Phoenix, is designed to carry a predicted umami of **1,800** — the savor of a generalist engineered to register on all eight axes at once, not a umami specialist. Invert each score through the formula and you can read the effective half-max concentration straight off it:

SAVORY SOURCE	IFI-U	EFFECTIVE EC_{50} (MM FREE-GLU EQUIV.)	RELATIVE LOUDNESS
Industrial tomato (shipped unripe)	300	6.67	0.30×
Native reference (anchor)	1,000	2.00	1.00×
Phoenix · SS-0096 (full-spectrum, predicted)	1,800	1.11	1.80×
Restored tomato	2,600	0.77	2.60×
Restored tomato + native synergy *(illustrative)*	~7,800 → **5,000 cap**	~0.26	clamped

The restored fruit reaches half-activation at 0.77 mM where the industrial one needs 6.67 — it is **8.7 times louder**, and the number says so in one glance. That is the whole argument for measuring: *the tomato that tastes like a tomato should* stops being a slogan and becomes a coordinate.

The last row of that table is an honest edge case, and we show it rather than hide it. If a restored tomato also carries its native 5′-nucleotide, the synergy slides the curve further left — a threefold amplification would drive the raw loudness to nearly 7,800. But the Index normalises every axis to a scorecard maximum of **5,000**, so the printed profile clamps there. Two genuinely different super-savory foods can both read 5,000 and still be told apart by a careful palate; the ceiling compresses the very top of the axis. We would rather you know that the cap exists than mistake it for the limit of the plant.

And every one of these numbers carries an error bar, which is where our quietest point of pride lives. The IFI is a ratio of the defined reference EC_{50} and the candidate's predicted EC_{50}, and the relative variance of a ratio is the sum of the relative variances of its parts. Our batch-to-batch coefficient of variation is designed to hold at or below **7%**. Compute both reference and candidate at that precision and the score inherits a CV of $\sqrt{(0.07^2 + 0.07^2)}$ = **9.9%** — so a predicted IFI-U of 2,600 is really 2,600 ± about 260, tight enough to specify, to reorder, to build a product on. Run the same arithmetic against a wild-type program's **42%** variability and the combined CV balloons past 42%; the "score" would swing by more than a thousand points between harvests and mean nothing at all. The whole reason the axis can be a *number* rather than a *hope* is the discipline of that ≤7%. Precision is not a boast here. It is the thing that makes the measurement exist.



The tomato, restored — picked at full savor, its free-glutamate pool grown back in through the fruit's own GAD and GDH routes, climbing from IFI-U 300 to 2,600 on the same eight-axis scale that reads every organism we grow.

The quiet companion

Rigor asks us to name glutamate's companion, because the axis is a chord more than a single note. **Aspartate**, the other acidic amino acid, carries a weaker savor of its own — roughly a thirteenth of glutamate's, a relative coefficient of about **0.077** — and it rises and falls alongside glutamate in a ripening fruit or a maturing ferment,

because the plant's own enzymes trade the two back and forth. Asparagus, which took its name from aspartate, is the everyday case; a ripe tomato carries both.

The coefficient does real work in the arithmetic. If we fold aspartate into a single glutamate-equivalent pool, it is

```
[Glu-eq] = [Glu] + 0.077 × [Asp]
```

Suppose a cut fruit offers a free-glutamate pool of 10 mM against 3 mM free aspartate — then [Glu-eq] = 10 + 0.077 × 3 = 10.23 mM, aspartate adding about two and a third percent. Modest, but not nothing, and it is the kind of correction that keeps a measured score honest across organisms that balance the two acids differently. When we speak of restoring a plant's glutamate pool we are, in practice, restoring the whole acidic-amino-acid signal the receptor reads, aspartate included. The note is glutamate's. The chord is a little wider.

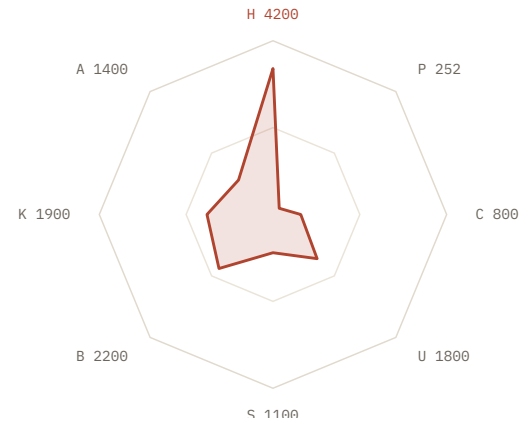
What we restore, and what we will not claim

Savor, once you can measure it, turns out to live in more plants than the broth pot. Umami is the backbone of a vegetarian kitchen precisely because so many of its oldest foods are glutamate engines — and the organisms we have tuned to speak the axis read like that kitchen's own larder:

ORGANISM	CONSTRUCTS	AXES	DIFFICULTY	WHAT CARRIES THE SAVOR
Tomato	2,040	S/U/F/M	Easy	free glutamate, via the fruit's GAD / GDH route
Soybean	360	U/K	Frontier	glutamate freed from storage protein
Tea	280	U/B/A	Hard	theanine and free glutamate of the green leaf
Yeast	240	U/K	Hard	glutamate plus 5′-nucleotides, released on autolysis
Koji Mold	80	U	Hard	the protease-and-glutaminase engine of the ferment
Shiitake	80	U	Hard	guanylate (GMP) — the strong synergist
Mushroom	40	U	Hard	glutamate with guanylate

Set beside them the one profile in the whole Index that registers on every axis at once — our full-spectrum flagship, where umami takes its place as one voice in an eight-part chord rather than the whole song:

IFI PROFILE



IFI-H	4,200
IFI-P	252
IFI-C	800
IFI-U	1,800
IFI-S	1,100
IFI-B	2,200
IFI-K	1,900
IFI-A	1,400

Tea belongs in that table for a reason worth savouring: the savory hush of a well-made green tea — the quality a tea master calls its body — is free glutamate and theanine, the same axis as a broth, read through a leaf. The receptor does not care whether its glutamate arrives from kombu, from a tomato, or from the first flush of a shade-grown tea. It only closes its hand around the molecule and reports what it finds.



A tea leaf at full savor — the umami hush of a shade-grown green tea is free glutamate and theanine, the same axis a broth speaks, read through a leaf.

We owe the reader the limits, too, because an axis you can measure is an axis you can overstate. Three honest boundaries hold us. First, **the Index measures loudness, not nourishment.** IFI-U is the half-activation of a receptor, the body's *advance word* that protein is near — and that word is only trustworthy when the protein is real. We restore the signal to match the nourishment a plant actually carries; we never engineer the signal to counterfeit a nourishment that is not there. A loud umami that promises protein the fruit cannot deliver would be a lie told to the appetite, and the appetite is the one reader we refuse to deceive. Second, **savor is pH-sensitive and slow to fade.** Glutamate's taste depends on its ionisation, so it speaks loudest near neutral pH and softens in a sharply acidic dish; and unlike sweet, which the tongue tires of quickly, umami adapts slowly

and lingers — a property that makes it generous in a broth and easy to overbuild in a sauce. Third, **the receptor reads the glutamate anion, not its salt.** Monosodium glutamate was only ever the convenient delivery form; what the tongue answers to is the glutamate itself, which means a plant grown to carry its own free glutamate can deliver full savor while a cook cuts the added salt — the axis, honestly engineered, is a way to feed the body more taste and less sodium at once. That is the clean-living arithmetic underneath the romance, and it is the arithmetic we care about most. These are computational designs; Scoville Splice's cultivars and constructs have not been experimentally validated in a laboratory.

The axis, then, is not a luxury or a seasoning. It is the restoration of a signal that evolution spent a very long time installing and that a century of industrial agriculture spent a few short decades turning down — a clamshell receptor, a nucleotide at the hinge, a sigmoid steepest exactly where a ripening fruit lives, and a single number that lets a family say *how much* where for ten thousand years we could only say *more.* We built the measurement so that a plant could tell the body the truth about the nourishment it carries, and so that we could help it say so on purpose, one honest millimolar at a time.

Chapter 2 · The Venus Flytrap — *the receptor, exactly.*

IFI-U · mGluR1 · TAS1R1·TAS1R3

The umami receptors belong to a family of proteins built around a structure chemists named, with unusual honesty for the field, the **Venus flytrap**. Picture a large hinged domain made of two lobes with a cleft between them, floating on the outer surface of a taste cell. It sits open, patient, until the right molecule drifts into the cleft; then the two lobes rotate shut around it like the carnivorous plant's trap, snapping closed and holding the prey. That closure is the signal. Everything downstream — the whole cascade of molecular relays that ends with a nerve firing toward the brain — begins with one small molecule caught in a protein's closing hand. Because our family keeps a vegetarian table, we have spent more evenings than most listening for exactly this one note: the round, brothy depth that tells the body *there is something nourishing here*, the savor that lets a plate of plants satisfy completely. We wanted to know where that note is born. It is born in a closing hand, and the hand can be measured.

For umami there are two such traps working the same prey, and our canon names them in the order of their weight to the Index. The first is **mGluR1**, a metabotropic glutamate receptor — a class the brain uses as well, to read the glutamate it signals thoughts with — stationed on the tongue in a truncated, taste-specific form and tuned to answer glutamate above almost anything else. The second is **TAS1R1·TAS1R3**, a *heterodimer*: two different proteins, the products of two different genes, clasped permanently together so that the pair works as a single receptor. Both are **class C G-protein-coupled receptors**, and both carry the Venus flytrap domain at their outermost tip, with a cysteine-rich stalk threading it down to the seven-helix transmembrane bundle that passes the message inward.



The controlled stage where candidate lines are read for exactly the receptor signal this chapter describes — fixed light, fixed hands, one note listened for at a time.

Two traps for one prey

Why two receptors for one taste? Because the body has never left a signal it cannot afford to miss — savor, the sign of protein and of food that will rebuild you — to a single gene. The two traps have

different jobs. **mGluR1** is the sensitive, conserved anchor: an ancient glutamate sensor, the same lineage the nervous system reads thought with, tuned down on the tongue from the micromolar sensitivity of a synapse to the millimolar range of food. **TAS1R1·TAS1R3** is the broad-band savory receptor, answering not only to glutamate but to a sweep of L-amino acids, and — this is the hinge the whole chapter turns on — carrying a *second* binding site that the 5′-ribonucleotides of a dried mushroom or a seared surface can occupy.

Our canon anchors IFI-U to mGluR1 specifically, and the choice is not arbitrary. An index axis needs a reference that does not drift: a receptor conserved across the animal kingdom, structurally stable, assayed the same way every time. mGluR1 is the oldest and most conserved glutamate sensor in the lineage, which makes it the fixed point an entire scale can hang from. That the body reads *savor* with a modified version of the same protein it reads *thought* with is one of those facts that sounds like poetry and is merely anatomy — but it is also exactly why the anchor holds.

One structural fact of the family explains some of savor's oddest manners. The TAS1R1·TAS1R3 pair shares a subunit with the sweet receptor, TAS1R2·TAS1R3: the same **T1R3** protein partners with T1R1 to read savory and with T1R2 to read sweet, a single hand gripping two different tools. This is the reason savory and sweet are the two tastes that most naturally co-occur — why a long-cooked onion's sweetness and its depth arrive together, why a ripe tomato can carry both without either fighting the other. It is also an honest engineering constraint: because the S and U axes share hardware, moving one can nudge the other, and our family engineers a tomato's sweetness and its savor as a pair rather than pretending they are strangers. (A sidelong proof of how seriously the body takes savor: the domestic cat, an obligate carnivore, has lost a working sweet receptor entirely — its T1R2 is a pseudogene, which is why cats are famously indifferent to sugar — while its umami machinery stays intact and vigorous. For a pure meat-eater, the protein signal is the one that must never fail.)

Anatomy of the flytrap domain

Open the flytrap domain and look at how it is built, because the geometry is the mechanism. The domain is two lobes — call them the upper and lower lobe — joined at a flexible hinge, with a cleft opening between them. The fold is inherited, almost unchanged, from the **periplasmic binding proteins** that bacteria use to catch nutrients drifting past their membranes: the tongue reads glutamate with a protein shape older than animals. In the resting receptor the two lobes sit apart, the cleft gaping open, the trap *unset but ready*.

Glutamate is a small, triply-charged molecule, and its charge is the whole key. At the pH of the mouth it is a zwitterion carrying three handles — an α-amino group (positive), an α-carboxylate (negative), and a γ-carboxylate out at the end of its side chain (negative). The floor of the cleft, down near the hinge of the TAS1R1 lobe, presents a matched cluster of polar and acidic residues positioned to coordinate exactly those three handles at once. When glutamate settles in, it does not simply rest in the cleft — it bridges the two

lobes, its handles reaching to contact both the upper and the lower lobe simultaneously, so that the bound molecule becomes a clasp drawing the trap toward closure.

The domain therefore lives in three states, not two: **open** (empty, lobes apart), **closed** (lobes rotated shut, usually around a captured ligand), and the **active** closed state that has transmitted its rotation down the cysteine-rich stalk into the transmembrane bundle. And near the *lip* of the cleft — up by the hinge, not down where glutamate binds — the TAS1R1 lobe carries a second, separate pocket. Nothing of the main taste binds there. It is reserved for the ribonucleotides, and what it does is not catch prey but *hold the trap shut once prey is caught*. We will spend real arithmetic on that second pocket, because it is where the economics of savor are decided; for now, mark that it exists, and that it works by stabilizing the closed state rather than adding any taste of its own.

The binding event, read as thermodynamics

The closing of the trap is not a switch; it is an equilibrium tilted. Picture the empty domain flickering between open and closed many times a second, strongly favoring open — the trap is set, not sprung. Glutamate's arrival does not *force* the lobes shut so much as make the shut conformation the cheaper one to occupy. This is **conformational selection**: the ligand binds preferentially to the closed shape, and by binding it, it removes closed receptors from the equilibrium, pulling the whole population toward closure. The more glutamate present, the larger the fraction of traps caught shut at any instant, and the louder the signal passed inward.

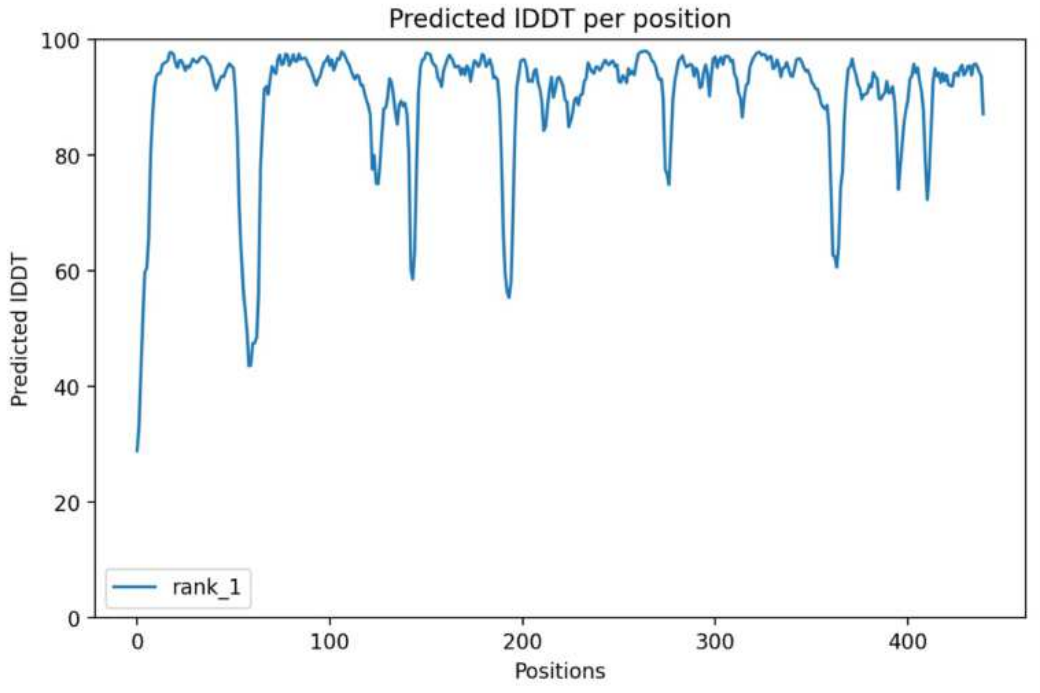
That tilt obeys a single clean equation, and it is worth writing because every later number inherits from it. The free-energy difference between two states and the ratio of their populations are related by

> $\Delta G = -RT \cdot \ln K$

where K is the equilibrium constant (how many closed for each open), R is the gas constant, and T is absolute temperature. At body temperature, T ≈ 310 K, so RT ≈ 0.616 kcal/mol. Run one decade through it: to shift an equilibrium — or an apparent binding affinity — by a factor of ten, you must change its free energy by

> $\Delta\Delta G = -RT \cdot \ln(10) \approx -0.616 \times 2.303 \approx$ **-1.4 kcal/mol.**

Hold onto that figure. Roughly **every 1.4 kcal/mol of extra stabilization of the closed state multiplies the receptor's apparent sensitivity tenfold** — lowers by ten the concentration of glutamate needed to half-fill the traps. It is a small energy. A single well-placed hydrogen bond is worth one to two kcal/mol; a buried salt bridge more. This is why a second-site molecule that contributes only a couple of kcal/mol of closed-state stabilization can drop the glutamate requirement by an order of magnitude — and why savor is so cheap to amplify and so expensive to fake. The receptor is a lever with a very long arm, and a few kcal/mol at the fulcrum moves the whole load.



A per-residue structural-confidence read (pLDDT) on a modeled enzyme from the platform's structure pipeline — the same confidence map our family demands before we trust any predicted fold, the flytrap's closed state among them. If the closed geometry is modeled wrong, every synergy number built on it is worthless, so the confidence read comes first.

The cascade, relay by relay

A caught trap is only the first word. What turns one bound molecule into a shout at the nerve is the cascade behind it, worth drawing in full because its nonlinearity is exactly what the Index measures. Five relays carry the message from the closed trap to the brain, and at nearly every one the signal is amplified.

1. The activated receptor grips its coupled G-protein and catalyzes the exchange of GDP for GTP on it — in taste tissue, **α-gustducin** together with the **Gβ3γ13** pair — releasing the βγ subunits to act. One receptor, acting as an enzyme, switches many G-proteins in its active lifetime.
2. Those freed subunits switch on **phospholipase C β2 (PLCβ2)**, an enzyme that cleaves the membrane lipid PIP_2 into two messengers: diacylglycerol and, the one that matters here, **inositol trisphosphate (IP_3)**. One enzyme, running over and over, makes many messengers.
3. IP_3 diffuses to the endoplasmic reticulum and opens the **IP_3 receptor type 3 (IP_3R3)**, a gate that releases a flood of stored **calcium (Ca^{2+})** into the cell's interior — thousands of ions per opening.
4. The rising calcium opens **TRPM5**, a calcium-activated cation channel, which lets sodium in and depolarizes the cell past its firing threshold. TRPM5 is itself *temperature-sensitive*, potentiated by warmth — a quiet fact that will matter when we ask why a broth tastes deepest hot.
5. Depolarization opens **CALHM1/CALHM3**, a large-pore channel, through which the cell releases **ATP** as its neurotransmitter onto the gustatory nerve fibre waiting beneath it. The nerve carries the word to the brain: *savory, here.*

RELAY	MOLECULAR PLAYER	WHAT IT DOES	TYPICAL GAIN (ORDER OF MAGNITUDE)
1	α-gustducin · Gβ3γ13	GDP→GTP exchange, releases βγ	~10×
2	PLCβ2	PIP_2 → IP_3 + DAG	~10^2×
3	IP_3R3	Ca^{2+} release from ER store	~10^3×
4	TRPM5	Ca^{2+}-gated depolarization (warmth-potentiated)	gating
5	CALHM1/3	ATP release to the nerve	quantal

Multiply even the conservative gains and the chain amplifies a trickle of bound glutamate into something on the order of ten-thousand- to a million-fold louder at the nerve. (We write those as orders of magnitude, not as measured constants of our own system; the point is the *shape*, not a false precision.) This is the reason savor does not climb in a straight line with glutamate content, and the reason that measuring how much glutamate a food holds tells you far less than you would hope about how savory it will taste. The cascade is steeply nonlinear through its working range. The Index measures the **response** — the loudness at the nerve — not the stimulus, not the count in the jar, and that is exactly what makes it honest.

Why savor climbs a curve, not a line — the Hill equation

Give the nonlinearity its proper shape. The fraction of maximal response a stimulus produces follows the sigmoid of the **Hill equation**:

$$R / R_{max} = [C]^n / (EC_{50}^n + [C]^n)$$

Here [C] is the stimulus concentration, **EC_{50}** is the concentration that drives the receptor to half of its full response, and **n** is the Hill coefficient — a measure of cooperativity. For a class C receptor built from two protomers leaning together, n can run above 1: the two halves of a dimer do not act wholly independently, and savor shows mild positive cooperativity, a trap that helps its neighbor close. Set [C] = EC_{50} and the equation gives R = R_max/2 by construction — that is what EC_{50} *means*.

Walk the curve and watch it bend.

[C] / EC_{50}	R / R_MAX (N = 1)	R / R_MAX (N = 2)
0.10	0.091	0.010
0.25	0.200	0.059
0.50	0.333	0.200
1.00	0.500	0.500
2.00	0.667	0.800
4.00	0.800	0.941
10.0	0.909	0.990

Two things fall out, and both are design facts. First, the curve is *steepest in its middle*. The slope of response against the logarithm of concentration reaches its maximum right at EC_{50}, where it equals ln(10) · R_max · n / 4 ≈ **0.58 · R_max · n per tenfold** change in stimulus. Climb the steep middle and a small change in stimulus

buys a large change in response: on the lower limb with n = 2, raising the stimulus from one-quarter to one-half of EC_{50} lifts the response from 0.059 to 0.200 — it more than *triples* the signal for a mere doubling of the cause. Double the glutamate near a receptor working at half-activation and you can far more than double the firing at the nerve. That is the whole reason savor rewards precision: you are standing on the steep part of a sigmoid, where the hand that places the molecule matters more than the amount.

Second — and this is the honest limit that keeps the first fact from being a sales pitch — the curve *saturates*. Past the knee, above EC_{50}, every further increment of stimulus buys less: from 0.80 to 0.94 to 0.99, the traps run out of room to be any more shut. Piling glutamate past the knee is diminishing returns, and the per-axis scorecard ceiling our canon fixes at **5,000** is not an arbitrary edge of the ruler — it is a real shoulder of receptor response, the place where the biology itself stops getting louder. A number can report savor faithfully only up to the loudness the receptor can actually reach.

The curve also quietly indicts the obvious shortcut. If you measure a food's glutamate by chemistry — grind it, run it on a column, report milligrams — you have measured [C], the stimulus, when what a diner tastes is R, the response, read through a sigmoid whose EC_{50} moves with everything else in the mouth. Two foods with identical glutamate content can sit at wildly different points on the curve, and taste wildly different, because one of them also carries the ribonucleotide that moves EC_{50}. Content assays mislead exactly where it matters most. The Index refuses the shortcut and measures the response.

The Index, computed

With the curve in hand the scoring is a single, inverted line. Our canon writes it:

> $\mathrm{IFI(candidate)} = 1{,}000 \times EC_{50}\mathrm{(reference)} \div EC_{50}\mathrm{(candidate)}$

The Index is inversely proportional to EC_{50}. A food that reaches half-maximal receptor response at a *lower* concentration — a louder food — earns a *higher* score. The reference is fixed so that the native wild-type anchor reads **IFI-U 1,000**: substitute EC_{50}(candidate) = EC_{50}(reference) into the line and it returns exactly 1,000, the hinge the whole axis swings from.

The cleanest arithmetic needs no absolute concentration at all, because the reference cancels in any ratio. Two candidates compared read

> $\mathrm{IFI(A)} \,/\, \mathrm{IFI(B)} = EC_{50}\mathrm{(B)} \,/\, EC_{50}\mathrm{(A)},$

so the canonical scores tell us their EC_{50} ratios directly. A tomato restored to **IFI-U 2,600** against a commodity tomato off the truck at **IFI-U 300** is 2,600 ÷ 300 = **8.67× louder**, meaning it half-activates the receptor at a concentration 8.67 times lower — nearly an order of magnitude less tomato needed to say the same savory word.

Phoenix at **IFI-U 1,800** stands 1,800 ÷ 300 = **6×** above that same commodity fruit. These are exact consequences of the canonical numbers; no reference value is assumed.

To put absolute concentrations on the axis — and to show the unit work the kitchen actually cares about — anchor the reference, for this worked illustration, at EC_{50}(reference) = 10 mM glutamate-equivalent, a value in the millimolar range taste receptors genuinely occupy. Then EC_{50}(candidate) = 1,000 × 10 ÷ IFI mM:

FOOD	IFI-U	EC_{50} (MM GLUTAMATE-EQUIV.)	RELATIVE TO REFERENCE
Reference anchor	1,000	10.0	1.00×
Commodity tomato (off the truck)	300	33.3	3.33× more needed
Phoenix · SS-0096	1,800	5.56	0.56×
Restored tomato (SS line)	2,600	3.85	0.39×
Per-axis scorecard ceiling	5,000	2.00	0.20×

The unit conversion that threads this to a real tongue: the bare detection threshold for glutamate alone sits near a hundredth of a gram per hundred millilitres. Taking free glutamate at its acid molar mass of 147.13 g/mol, 0.01 g/100 mL = 0.1 g/L, and 0.1 ÷ 147.13 = 6.8×10^{-4} mol/L ≈ **0.68 mM**. Detection at two-thirds of a millimolar, half-activation at a few to a few tens of millimolar — the working range of savor is a narrow, millimolar window, which is why small, exact moves inside it read so loudly.

One honesty belongs on this page. The EC_{50} the Index scores is a *food* concentration — the dilution of a food's active fraction at which the fixed reference panel reaches half-maximum — and that single number folds together two different biological facts: how much glutamate the plant actually makes, and how well the receptor hears whatever is there. A food can earn a high IFI-U by making more glutamate, or by carrying what makes the receptor more sensitive to it, or both. The number is faithful; it is simply the product of two levers, and a designer should always know which one is doing the work.



A tomato in cross-section — the locular jelly around the seeds is where a ripe fruit concentrates its free glutamate. Restore that pool and you move the whole axis.

Two levers — what the plant makes, and what the tongue hears

Those two levers deserve naming, because our family's work and the cook's work pull on different ones.

LEVER	MECHANISM	WHAT IT CHANGES	WHERE IT SHOWS
Content	GAD / GDH1 free-glutamate restoration	raises free [glutamate] the plant carries	the family's tomato: IFI-U 300 → 2,600
Sensitivity	5′-ribonucleotide second-site synergy	lowers EC_{50} — the receptor hears more	the cook's, or the formulator's, multiplier

The **content** lever is the tomato story. A ripe tomato concentrates free glutamate in the jelly around its seeds, and for ten thousand years that pool was the quiet engine of why a tomato tastes like comfort. Then a century of breeding for the truck — for shelf life, for shipping weight, for fruit that survives a thousand miles — stripped it out; roughly ninety-three percent of the free glutamate a heritage fruit once carried was traded away for durability. The commodity tomato at IFI-U 300 is not broken so much as *robbed*. We grew it back, not by adding anything foreign, but by restoring the plant's own route to its own molecule — the native **GAD / GDH1** pathway, glutamate decarboxylase and glutamate dehydrogenase, the enzymes a tomato uses to build and balance its free glutamate pool. Rebuild the pool and the fruit climbs its own dose-response curve under its own power: IFI-U 300 → 2,600. That is content, honestly won — the tomato made to taste like a tomato should, by giving it back what the truck took.

The **sensitivity** lever is different in kind, and it belongs as much to whoever cooks the fruit as to whoever grows it. It does not add glutamate. It adds the ribonucleotide that docks the second site and makes the receptor hear the glutamate already present as louder. A content lever moves you along the curve; a sensitivity lever moves the curve itself, sliding EC_{50} to the left. The two stack, and the best savor is nearly always both.

Savor is also the axis with the widest bench of plants, because so many organisms already make glutamate for their own metabolism — it is the nitrogen currency of a living cell. Across our 42 platform hosts, the umami workhorses span the garden and the ferment crock alike:

HOST	CONSTRUCTS	PRIMARY AXES	DIFFICULTY
Tomato	2,040	S / U / F / M	Easy
Pepper	1,060	all (incl. U)	Native
Soybean	360	U / K	Frontier
Tea	280	U / B / A	Hard
Yeast	240	U / K	Hard
Koji Mold	80	U	Hard
Shiitake	80	U	Hard
Mushroom	40	U	Hard

(The counts are constructs, not scores — the number of engineered lines standing behind each organism, not its loudness.) This is the axis on which a vegetarian table is quietly won. Savor is the taste of protein and nourishment, and building it into the plant itself — the

tomato, the soybean, the shiitake, the koji that will become a broth — is how we make food grown to feed the body well *and* to satisfy it completely, with nothing extracted from an animal to supply it. Nourishment and savor, for us, are the same act done twice.

The synergy, as arithmetic

Now the second site earns its arithmetic. A **5′-ribonucleotide** — inosinate (IMP), the signature of a dried or aged or seared surface; guanylate (GMP), the signature of a dried mushroom — docks into that pocket up at the lip of the TAS1R1 cleft and acts as a molecular doorstop in reverse. It does not open anything. It makes the *closed* trap harder to spring, stabilizing the shut conformation so that each captured glutamate is held longer and read louder. In the language of the thermodynamics above, the nucleotide contributes a couple of kcal/mol of closed-state stabilization — and at ~1.4 kcal/mol per decade, a contribution of that size slides EC_{50} down by roughly an order of magnitude. This is the structural synergy Zhang and colleagues modeled in the receptor, confirmed at every savory table in history.

Food science measured the same thing from the other side, long before the structure was solved, and its shape is the lesson. The equivalent umami intensity of a glutamate-plus-nucleotide mixture, expressed as the glutamate concentration that would taste as strong, runs

$$\gamma = u + 1218 \cdot u \cdot v$$

with u the glutamate and v the ribonucleotide (IMP), each in grams per hundred millilitres, as measured by Yamaguchi and colleagues. The term that matters is the second one: synergy is a **product**, not a sum. Work a point. Pair glutamate and IMP at u = v = 0.05 g/100 mL — a plausible savory-dish level:

$$\gamma = 0.05 + 1218 \times 0.05 \times 0.05 = 0.05 + 3.05 = \mathbf{3.10\ g/100\ mL}$$

equivalent glutamate.

A mixture physically carrying a tenth of a gram of actives tastes like more than three grams of pure glutamate — roughly sixty times the savor of its glutamate taken alone — because the amplification scales with the product u · v, not with how much of either you pour in. The multiplier on the glutamate term is (1 + 1218·v): a very little nucleotide, placed beside a glutamate base, rewrites the whole intensity. (The relation is steepest in exactly this suprathreshold band; near the bare threshold it folds back toward simple addition, which is its honest domain.) The same multiplication shows at the threshold itself: glutamate alone is detectable near 0.68 mM, but with a nucleotide present that threshold can fall by an order of magnitude, toward ~0.07 mM — savor you could not have found becomes savor you cannot miss.

Carry the product term back to the Index and the picture closes. Because IFI-U is inversely proportional to EC_{50}, and the nucleotide second-site drops EC_{50} by roughly tenfold, **synergy multiplies the Index by roughly ten** — not adds to it. A glutamate-rich base and a nucleotide source are each ordinary; together they are a different

order of loudness. This is the whole economics of savor in one sentence, and it is why a few dried shiitake (GMP) over a tomato sauce (glutamate), or a seared crust (IMP) beside a long-simmered stock, transforms a dish for a cost that rounds to nothing. One site catches the prey. A second site, just beside it, holds the trap shut. The family grows the first into the plant; the cook supplies the second at the stove; the Index reads their product.

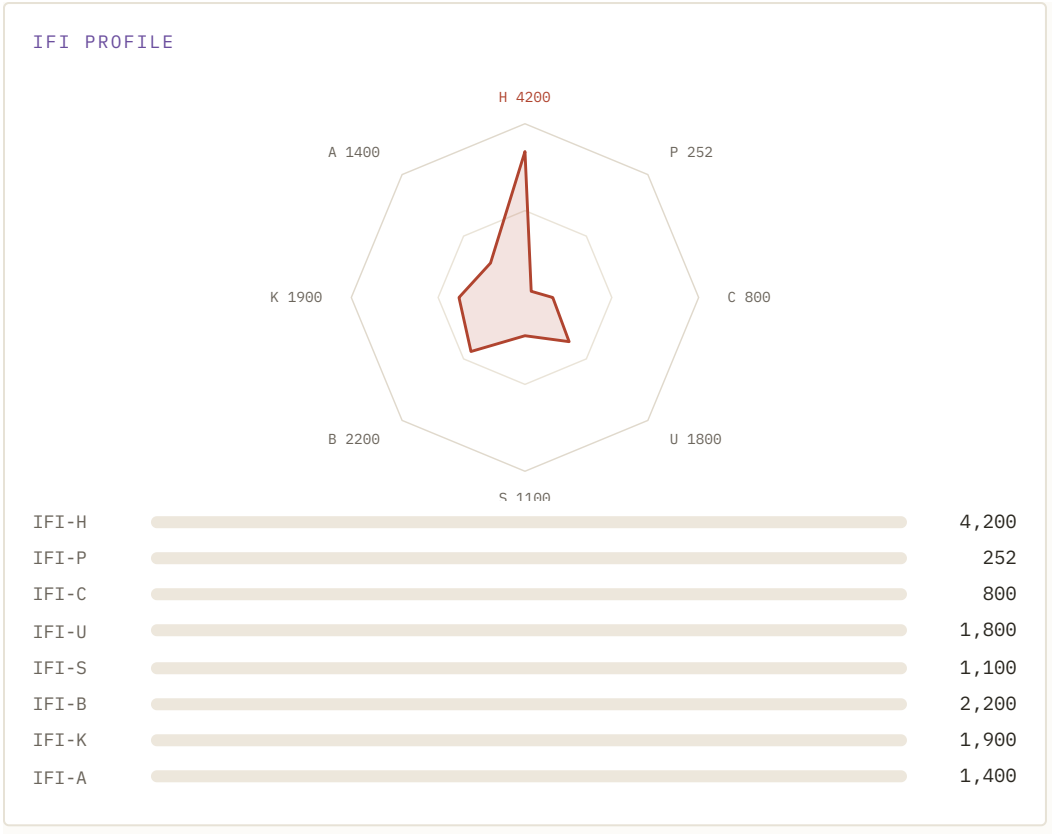
What the living receptor will not let you forget

Three behaviors of the living receptor keep the designer honest, and all three are failure modes if ignored.

Adaptation. Hold a single steady savory note on the tongue and it fades — the cascade desensitizes, the first sip of a broth always reading deeper than the fifth. A savor built from one sustained tone tires the palate by its own success. The receptor's answer, and ours, is to compose savor as a note *and* a body — umami carried on the richness and continuity of kokumi (the CaSR axis), so the signal has somewhere to go when the first channel tires. A broth that keeps giving is a broth built on two receptors, not one.

Temperature. Recall that the fourth relay, TRPM5, is potentiated by warmth. The cascade's own channel reads a warm stimulus as louder, which is the honest reason a hot broth tastes more savory than the identical broth gone cold — the chemistry has not changed, the gain has. For a designer this is both a gift and a trap: it means serving temperature is part of the recipe, and it means any tasting panel must *fix* temperature or watch its EC_{50} wander for reasons that have nothing to do with the food.

Error, and why ≤7% is the whole game. A score is only as real as its reproducibility, and here the arithmetic is unforgiving. Since IFI $\propto 1/EC_{50}$, the relative error of a score equals the relative error of its EC_{50}; and a score is a *ratio* of two measured EC_{50} values, so their relative errors add in quadrature. At our batch coefficient of variation of **≤7%**, a candidate score carries $\sqrt{(0.07^2 + 0.07^2)} \approx$ **9.9%** — Phoenix's U 1,800 lands as 1,800 ± ~180. To tell two cultivars apart with confidence you need their scores separated by about twice the combined standard deviation, roughly a **20% step**. Now run the same math at a wild-type process's **42%** variability: the combined error balloons to ~43%, and the smallest resolvable step swells past **110%** — more than a doubling — which is to say the axis becomes unreadable, unable to distinguish a six-axis fruit from an eight-axis one. The ≤7% CV is not a vanity metric. It is the precision that makes the fourth decimal of flavor *mean something*, the difference between an index and a guess.



Those eight numbers are Phoenix, SS-0096 — our model predicts approximately 2.95 million SHU and, sitting inside the same fruit, an umami of **1,800**: savory depth designed to stand beside seven other axes without any of them drowning the rest. Read the U line against everything this chapter has built and it stops being a number on a card. It is a count of how often, per second, a Venus flytrap on the tongue is caught shut around a glutamate the plant was designed to make, held there by a doorstop the kitchen was invited to supply, shouted down a five-relay cascade to a waiting nerve, and scored — exactly, and the same way every time — against a receptor older than the animals that carry it.

A few honest limits close the account, because a number earns trust by naming what it cannot do. A cell-based EC_{50} is not a mouth: saliva, the food's own matrix, the shared T1R3 subunit that lets sweetness lean on savor, and the serving temperature all shift the lived experience around the scored value, and we report the scored value while respecting the gap. The Index does not claim to be the taste. It claims to be the most faithful, most reproducible *shadow* of the taste that a receptor can cast — the one that lets us design toward savor instead of stumbling onto it. These are computational designs; Scoville Splice's cultivars and constructs have not been experimentally validated in a laboratory. One molecule, one closing hand, one number that finally tells us how loud. The hand has always done this. We only learned, at last, to read it.

Chapter 3 · The Number — *measuring the savory signal.*

IFI-U · loudness, not amount · per-axis maximum 5,000

Loudness, not amount — this is the thing to hold onto about IFI-U, and the reason the Index is more honest than any concentration could ever be. A savory molecule, or a whole savory extract, is not interesting because of how much of it sits in a food. It is interesting because of how hard it leans on the receptor that reports savor to the brain. Two extracts can carry the same milligrams of glutamate and arrive on the tongue a world apart; what separates them is not quantity but *loudness*, and loudness is a property of the meeting between molecule and receptor, not a property of the jar.

EC_{50} is how we name that loudness precisely. It is the concentration at which a stimulus drives its receptor to half of full response — the midpoint of the whole climb from silence to saturation. A molecule or extract that reaches half-response at a low concentration is loud; one that needs a flood to get there is quiet. Invert the candidate's EC_{50} against a fixed reference, scale by a thousand, and loudness becomes a score you can print on a specification sheet and reproduce in February:

$$\text{IFI-U}(c) = 1{,}000 \times EC_{50}(\text{ref}) \div EC_{50}(c)$$

The reference is anchored so that the native wild-type receptor's half-activation reads exactly **1,000**. A candidate that activates the receptor at half the reference concentration — twice as loud — reads 2,000. One that needs twice the concentration reads 500. Every axis in the library normalizes to a per-axis maximum of **5,000**, the ceiling a scorecard radar is drawn against, so that a savory reading and a heat reading and an astringency reading are all legible on the same eight-spoke figure. The number is deliberately plain. We spend none of our cleverness on the formula; we spend all of it on measuring EC_{50} honestly, which is where the whole biology hides.

The receptor we are counting

A number is only ever as honest as the receptor it is anchored to, so before we can measure savor we have to be exact about what savor *is* at the surface of a taste cell. Umami is read by two proteins working the same message from different angles. The broadly tuned sentinel is the **TAS1R1·TAS1R3** heterodimer — two class-C G-protein-coupled receptors clasped together, each carrying a large extracellular lobe named a Venus flytrap domain for the shape it keeps. Beside it, narrower and glutamate-selective, sits **mGluR1** in a short taste-specific form, a metabotropic glutamate receptor repurposed by the tongue. Between them they give a single taste cell both a specialist and a generalist for the same quiet announcement: *protein is here; this is food.*

The binding itself is almost tender to describe, and describing it exactly is the point. L-glutamate drifts into the open cleft of the TAS1R1 flytrap, and the two lobes fold closed around it the way two hands close around something warm. That closure *is* the signal — not the arrival of the molecule, but the shape the protein takes once it has caught one. And here the receptor does something a simple counter never would: a second class of molecule, the 5′-ribonucleotides inosinate (IMP) and guanylate (GMP) that ripening and fermentation liberate, binds at a neighboring site right at the hinge of the flytrap and wedges it shut. The nucleotide does not taste of much alone. What it does is make the flytrap far harder to reopen, raising glutamate's apparent affinity several-fold and dropping the whole extract's EC_{50} — which is to say, making the same glutamate suddenly *louder.* The synergy every cook has felt between tomato and kombu, between mushroom and miso, is this one hinge being held closed.

From the closed flytrap the signal travels inward, and the cascade is worth walking step by step, because the single number we print stands in for every rung of it:

1. The closed Venus flytrap twists its cysteine-rich linker and tilts the seven-helix transmembrane bundle beneath it.
2. That tilt releases **α-gustducin**, the taste cell's heterotrimeric G-protein, from the receptor's inner face.
3. Gustducin's partners switch on **phospholipase C-β2**, which cleaves the membrane lipid PIP_2 into diacylglycerol and **IP_3**.
4. IP_3 opens channels on the cell's internal calcium store, and a pulse of **Ca^{2+}** floods the cytosol.
5. The calcium opens **TRPM5**, a monovalent cation channel; sodium rushes in and the cell depolarizes.
6. Depolarization opens **CALHM1**, and the cell releases ATP to the waiting gustatory nerve — the first word of a sentence the brain will finish as *savory.*

We do not have to model all six rungs to score a food, and that is the mercy of EC_{50}. The half-activation concentration is the one summary statistic that lets the entire cascade speak for itself: feed the receptor a rising series of concentrations, watch the output climb, and read off the input that produces half the maximum. Everything downstream — the gustducin, the calcium, the nerve — is folded into that single readout. The receptor integrates its own biology; we only have to catch it at its midpoint.



The calibrated assay stage — where half-activation is read from the receptor's own dose-response climb, the method by which every IFI-U in our library is defined.

We catch it on a calibrated cell-based assay: a line engineered to display the umami receptor on its surface alongside a reporter that makes an intracellular calcium pulse glow. We dose plates of these cells with the savory extract across a dilution series — a dozen concentrations marching up by factors of two or three — photograph the fluorescence, and fit the brightness-versus-concentration points to a curve. The concentration at the curve's half-height is EC_{50}. Then, because a cell is not a mouth, we cross-check every reading against a trained human panel tasting the same extracts blind. When the dish and the panel agree, we have a number. When they disagree, the dish is wrong and we go looking for what the cells missed.

From a curve to a number

The climb from silence to saturation is not a straight line; it is a shallow-steep-shallow sigmoid, and its algebra is the Hill equation. If we write the fraction of maximal response as a function of stimulus concentration [L]:

> $R([L]) \div R(max) = [L]^n \div (EC_{50}^n + [L]^n)$

then two things fall out that justify anchoring the whole Index here. First, set [L] = EC_{50} and the right-hand side collapses to $EC_{50}^n \div (EC_{50}^n + EC_{50}^n) = ½$, *for any value of n.* The half-activation point sits at the same concentration no matter how steep or shallow the curve — it is the one landmark on the climb that does not move when the biology changes shape. That is why we measure savor at its midpoint and not, say, at its threshold, which wanders with every bit of noise in the assay.

Second, the exponent **n**, the Hill coefficient, carries the cooperativity — the degree to which catching one glutamate makes the receptor hungrier for the next. For a composite savory extract we typically fit a slope a little above one; take **n ≈ 1.5** for a worked curve. At that steepness, doubling the concentration from EC_{50} to 2 × EC_{50} lifts the response from 50% to (2^1.5)/(1 + 2^1.5) = 2.83/3.83 ≈ **74%**, while halving it drops the response to **26%**. The whole working range from a tenth of saturation to nine-tenths spans a concentration ratio of 81^(1/n) — about **19-fold** at n = 1.5, against a lazy 81-fold at n = 1 and a crisp 9-fold at n = 2. Nucleotide synergy, by holding the flytrap

shut, nudges n upward: it does not only shift the curve left, it *sharpens* it, compressing the span over which savor goes from barely-there to fully present. A sharper curve is a more decisive flavor, and the Hill coefficient is where that decisiveness is written down.

EC_{50} is therefore not a convenience. It is the one point on the dose-response curve that is both physically meaningful — half the receptor's reach — and statistically stable, independent of the steepness that the rest of the curve's shape depends on. Anchor a scale anywhere else and the scale inherits every tremor in the measurement. Anchor it at the midpoint and the number holds still.

Working the index

Put numbers through the formula once, so it stops being abstract. Suppose the reference extract reaches half-activation of the umami receptor at an EC_{50} of **3.0 millimolar** glutamate-equivalent — a plausible figure for the native taste receptor, which operates in the low millimolar range, and the concentration we define to read 1,000. Then every other reading is one short division away. A supermarket tomato's whole savory extract, thin and emptied by a breed selected to survive a truck, needs about **10 mM** to reach half-response:

> `IFI-U = 1,000 × (3.0 ÷ 10.0) =` **`300.`**

A restored tomato, its glutamate pool refilled and its ripening nucleotides riding along, reaches half-response at roughly **1.15 mM**:

> `IFI-U = 1,000 × (3.0 ÷ 1.15) ≈` **`2,600.`**

An engineered koji ferment that drives the composite EC_{50} down near **0.94 mM** reads:

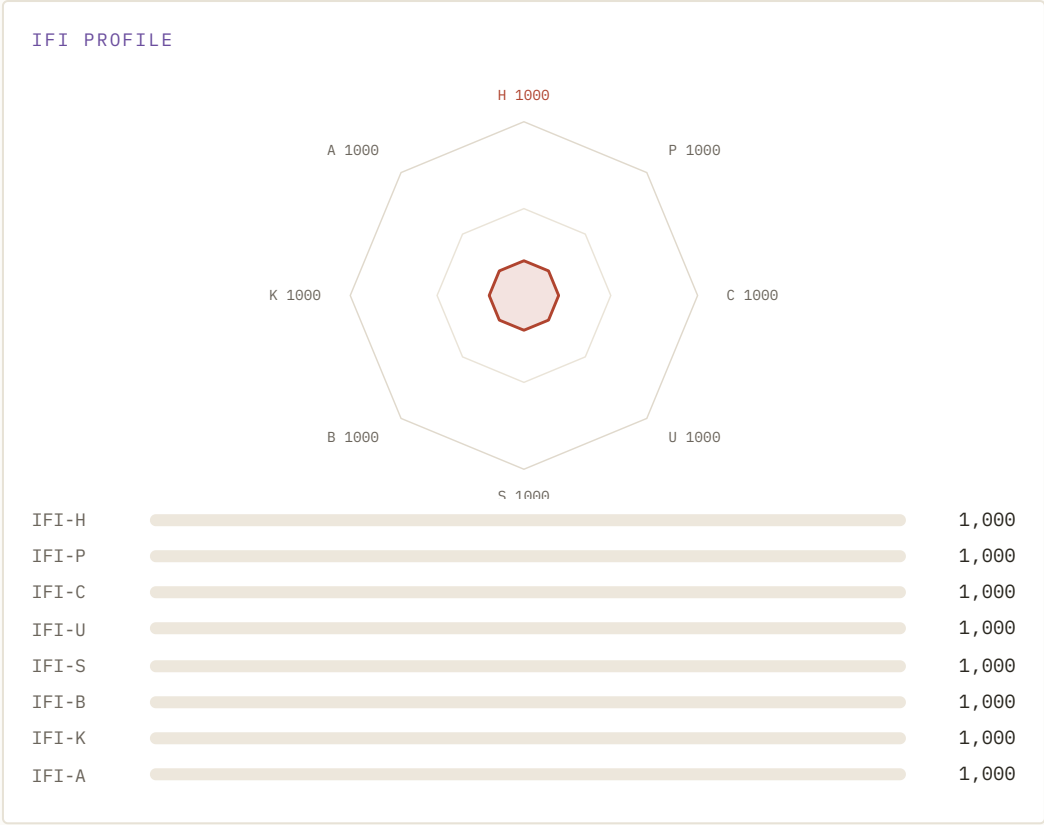
> `IFI-U = 1,000 × (3.0 ÷ 0.94) ≈` **`3,200.`**

And the full-spectrum pepper we will meet in a moment, with a deliberate savor built in beneath its heat, our model predicts it half-activates near **1.67 mM**, which is exactly the inversion of its predicted reading of 1,800. The arithmetic is plain on purpose. What it buys is a ladder where every rung is a loudness, and where loudness can be converted back into any chemist's unit at will. Multiply each EC_{50} in millimolar by the molar mass of free glutamate, 147.13 grams per mole, and the same ladder reads in milligrams per liter — the emptied tomato needing nearly 1.5 grams of glutamate-equivalent per liter to be heard, the restored one heard at barely a tenth of that.

READING	IFI-U	COMPOSITE EC_{50} (MM GLU-EQ)	MG/L GLU-EQ	LOUDNESS VS REFERENCE
Reference anchor · native wild-type	1,000	3.00	441	1.00×
Supermarket tomato, bred for the truck	300	10.00	1,471	0.30×
Phoenix · SS-0096, savor under heat	1,800	1.67	245	1.80×
Restored tomato, umami-tuned	2,600	1.15	170	2.60×
Engineered koji, single-source peak	3,200	0.94	138	3.20×
Scorecard ceiling (per-axis maximum)	5,000	0.60	88	5.00×

EC_{50} is derived from the IFI-U as 3,000 ÷ IFI-U millimolar; the milligram column is that figure times 147.13. The ceiling at 5,000 is not an abstraction — it is the loudness of an extract that half-activates the receptor at 0.6 mM, about five times louder than the native reference, which is as loud as the radar is drawn to hold. Wild-type and commercial food readings in this table are measured values; Phoenix (SS-0096) figures are computational predictions from our design model.

It helps to picture the calibration itself before picturing any product. The reference is not a cultivar; it is a baseline, the loudness of untouched wild-type biology on every axis at once. Drawn as a profile, it is a perfect octagon at radius 1,000 — the still circle against which every spiky, specialized thing we build is read.



The calibration baseline. By construction, each axis's native wild-type reference reads 1,000 — a flat octagon that is not a food but a ruler. Everything the library makes is a departure from this ring, measured in loudness.

Why savor outruns its chemistry

The honesty of this approach shows most clearly in a fact that surprises every maker who meets it: savor does not track glutamate in a straight line. Climb from the emptied supermarket tomato at 300

to the restored one at 2,600 and you have moved the reading roughly **ninefold** (2,600 ÷ 300 = 8.7). But the chemistry underneath did not move ninefold. The free glutamate pool rose only about threefold. The other near-threefold — the gap between what the chemistry did and what the tongue reports — is the receptor's nonlinearity made audible, and it comes from two sources we can name and separate.

STEP FROM EMPTIED TO RESTORED TOMATO	FACTOR	RUNNING IFI-U	WHAT CHANGED
Supermarket baseline	—	300	emptied glutamate pool, no nucleotides
Refill the free glutamate pool	×3.0	900	the linear part — more of the molecule
Nucleotide synergy + receptor cooperativity	×2.9	~2,600	the nonlinear part — the flytrap held shut, the curve steepened

A modeled decomposition; the measured endpoints, 300 and 2,600, are the hard data. The point is only that roughly three times the glutamate reads nearly ten times louder, and that the extra factor is not mysterious — it is synergy and cooperativity, both of which the composite EC_{50} captures without our having to bolt a correction on afterward.

The synergy term is large enough to deserve its own arithmetic. Classical taste psychophysics models the equivalent savor of a glutamate-and-nucleotide mixture as the glutamate fraction u amplified by a cross term: equivalent ≈ $u \times (1 + \beta \cdot v)$, where v is the nucleotide fraction and β the synergy constant that the mid-century literature measured near **1,200**. Feed that model a nucleotide fraction of only about four percent and the bracket becomes 1 + 1,200 × 0.04 ≈ **49** — a near-fiftyfold multiplication of perceived savor from a trace of ribonucleotide that, alone, you would barely notice. This is why a measured EC_{50} on the *composite* extract is not a nicety but a necessity. Measure pure glutamate in isolation and you would record the quiet molecule and miss the loud meal.



Tomato, in cross-section — the glutamate pool refilled and the ripening nucleotides restored through the plant's own GAD and GDH1 route, so three times the chemistry reads nearly ten times louder on the Index.

So we hold to one methodological rule without exception: we never measure the EC_{50} of pure glutamate and call that a food's savor. We measure the EC_{50} of the **composite extract** — the whole savory package a food actually delivers, glutamate and aspartate and whatever nucleotides and kokumi peptides ride with them — against the receptor, on the calibrated assay, cross-checked against the

panel. The synergy is therefore already *inside* the number. A food's IFI-U is not a theoretical glutamate figure with a synergy correction added later; it is the loudness of everything the food brings to the receptor at once — a measurement for wild-type and commercial foods, a computational prediction for our designed cultivars. This gives us a clean division of labor between two kinds of equation. The synergy model — the $u(1 + \beta \cdot v)$ arithmetic — is our *design and prediction* tool, the thing we reason with when we decide what to build and what to pair. For our designed cultivars, the IFI-U is a computational prediction; for wild-type and commercial foods, it is the assay's direct readout. The two meet in the validation design, and when model and assay diverge, it is the model we correct, never the tongue.

The readout, and its error

It is fair to ask why we do not simply print the glutamate concentration in milligrams and be done. A number is a number; why the detour through a receptor? Because concentration and perception part ways exactly where it matters most. Two extracts with identical glutamate in milligrams can taste a world apart depending on what rides alongside — a little guanylate, a few kokumi peptides, a background of salt or acid — and the shopper, the chef, and the formulator all live in the world of perception, not of the assay. A milligram figure would be precise and misleading. The IFI-U is a translation of that same chemistry into the loudness a tongue would actually report. It is the difference between telling you how much ink is on a page and telling you how loud the sentence reads. For savor, where synergy can multiply perceived intensity close to fiftyfold over the glutamate alone, the loudness measure is not a refinement of the concentration measure. It is a correction of it.

Any readout — measured or predicted — is only worth printing if it comes back the same tomorrow, so the error on the number matters as much as the number. Because the Index is a ratio, its error propagates in a way we can write down exactly. Take the logarithm of the formula and the reference and candidate terms simply add and subtract; their relative errors then combine in quadrature, which means the coefficient of variation of the reading is the root-sum-square of the coefficients of variation of the two EC_{50} measurements:

> $$\mathrm{CV(IFI\text{-}U)} = \sqrt{\ \mathrm{CV(reference)}^2 + \mathrm{CV(candidate)}^2\ }$$

Our process holds each candidate EC_{50} to a batch-to-batch CV of **≤7%**, and the reference calibration to about **3%**. So a reading carries CV = $\sqrt{(0.07^2 + 0.03^2)} = \sqrt{0.0058} \approx$ **7.6%**. On the restored tomato at 2,600, that is a standard deviation near **±200** — a number tight enough to specify, to pair against, to certify on a sheet. The discipline behind that tightness is the whole quiet argument for engineering savor rather than foraging for it. Left to its own devices, biology wanders: in our heat-pathway models, unoptimized capsaicin flux was predicted to vary at a CV near **42%** before the pathway design was brought under control. Carry a 42% CV into this same reading and the standard deviation on 2,600 balloons past **±1,000** — a number no one could build on. A loudness you can

reproduce in February is not a lucky harvest. It is a controlled process, and the ≤7% is the reason the number is allowed to be printed at all.

What the number does not say

A loudness measured at a receptor's midpoint is a strong summary, but it is a summary, and an honest index names what it leaves out. Three limits travel with every IFI-U, and we record each rather than bury it.

The first is the oldest distinction in pharmacology: **potency is not efficacy.** EC_{50} is a potency — the concentration for half-maximal response — and it says nothing about R(max), the height of the plateau the receptor finally reaches. Two extracts can share an EC_{50} and part ways at the ceiling, one saturating the cell while the other stalls at two-thirds of full response. So we never log EC_{50} alone; we record the fitted plateau beside it, and when two foods match on the axis but diverge at the top of the curve, the scorecard carries a note. The reading is the midpoint; the plateau is the reach. A savor can be loud to arrive and still fall short of filling the room.

The second is **time.** The assay reads a near-equilibrium response, and the tongue never tastes at equilibrium — it tastes an onset, a swell, and a linger. A composite extract heavy with slow-binding kokumi peptides can share an EC_{50} with a brighter, faster one and still unfold over a wholly different few seconds in the mouth. The Index does not pretend to carry that arc. We keep onset and persistence as panel notes beside the number, not folded into it, because a single concentration cannot encode a curve drawn in time.

The third is **context.** Salt and acid shift the receptor's apparent sensitivity, and warmth shifts it again; we fix the assay buffer precisely so that one reading is comparable to the next, which means the Index is a controlled-condition loudness and nothing more. A food eaten salted will read louder in the mouth than on the bench, and sustained exposure adapts the receptor, so a single EC_{50} does not promise that the third bite lands like the first. None of this loosens the number. It fences it — and the honesty of an index lives as much in the fence around its claim as in the claim itself. We would rather publish a loudness that means exactly one thing than a richer figure that quietly means several.

One scale, eight axes

There is one more virtue to the loudness framing, and it is the one that makes the whole eight-axis scorecard cohere: every axis is scored the same way, against its own receptor and its own defined reference compound, on a scale that tops out at 5,000. That is what lets a savory reading sit on the same radar as a heat reading and an astringency reading — each spoke measuring how loud that design is against *its own axis's defined reference point*, so that a reading of 1,800 on umami and a reading of 4,200 on heat each say "this many times louder than that axis's own native baseline", not against a shared single standard. The axes share a formula; each has its own

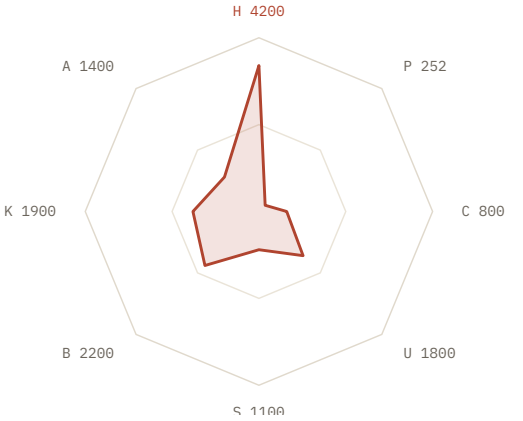
reference convention. Umami's spoke is usually not the longest on the figure; its gift is to be present under the others, and the radar is built precisely so that presence is visible at a glance.

It helps to see umami sitting inside a full eight-axis profile, where it rarely towers but almost always shows up. Read **Phoenix** — SS-0096, with a computational design target exceeding 2.9 million SHU — our model predicts approximately 2.95 million SHU — the only cultivar we have ever designed to register on all eight axes at once, a fruit built gene by gene because the plant that could reach it did not yet exist. Its headline is heat. But run your eye down the radar and savor is plainly there, grown in on purpose beneath the burn.



Phoenix (SS-0096) — the full-spectrum flagship, built to register on all eight axes at once, savor deliberately among them.

IFI PROFILE



IFI-H	4,200
IFI-P	252
IFI-C	800
IFI-U	1,800
IFI-S	1,100
IFI-B	2,200
IFI-K	1,900
IFI-A	1,400

Because every spoke is the same formula, the whole profile can be read as a set of EC_{50} ratios without touching the chemistry of any single axis. A reading of *x* means the candidate half-activates its receptor at 1,000 ÷ *x* times the reference concentration — louder than native wherever the reading tops 1,000, quieter wherever it falls below.

AXIS	RECEPTOR	IFI	$EC_{50}(C) \div EC_{50}(REF)$	RELATIVE TO NATIVE REFERENCE
H · Heat	TRPV1	4,200	0.238	4.2× louder
B · Zero-Bitter	TAS2R38	2,200	0.455	2.2× louder
K · Kokumi	CaSR	1,900	0.526	1.9× louder
U · Umami	mGluR1 / TAS1R1·R3	1,800	0.556	1.8× louder
A · Astringency	salivary PRPs	1,400	0.714	1.4× louder
S · Sweetness	TAS1R2 / R3	1,100	0.909	1.1× louder
C · Cool-Burn	TRPM8	800	1.250	0.8× — quieter than native
P · Pungency	TRPA1	252	3.97	0.25× — quieter than native

One formula, eight receptors, one convention per axis. The ratio column is simply 1,000 ÷ IFI; nowhere do the axes' chemistries meet. Each reading expresses loudness against that axis's own defined reference — a reading of 1,800 on umami means 1.8× louder than the umami reference; a reading of 252 on pungency means 0.25× as loud as the pungency reference. The axes share a formula but each has its own reference compound, so values across different spokes describe relative loudness on their own axis, not a single unified scale. Readings below 1,000 on any axis — C at 800 and P at 252 — are legible on the same figure because quiet and loud run the same direction on every spoke.

An IFI-U of 1,800 in a *pepper* is the quiet proof of this whole axis: savor is not the private property of broths and mushrooms. It can be specified into almost anything — even a fruit at 2.95 million SHU, as our model predicts for Phoenix — and when it is, the heat reads less like an assault and more like a dish, because something underneath it is telling the body *food.* This is the deepest lesson of the number: umami is the axis that makes the other seven edible. It is the floor. And a floor, to be built on, has first to be measured — which is the entire reason we translate chemistry into loudness before we translate it into anything else. These are computational designs; Scoville Splice's cultivars and constructs have not been experimentally validated in a laboratory.

READING	IFI-U	WHAT IT IS
Supermarket tomato, bred for the truck	~300	the emptied baseline
Phoenix · SS-0096, savor under heat	1,800	savor as a floor beneath a superhot
Restored tomato, umami-tuned	2,600	the vine's own intent, grown back
Engineered koji, single-source peak	~3,200	the loudest savor on our floor

Every figure here is a loudness, not a quantity. The climb from 300 to 2,600 is a roughly ninefold change on the Index driven by a far smaller change in chemistry — which is the whole reason we measure the way we do. The tongue keeps its accounts in loudness; so, now, do we.

Chapter 4 · The Synergy — *why one and one make eight.*

the nucleotides · the allosteric site · the equivalent-umami equation

Everything in savor points at this chapter, because the strangest and most useful property of the whole axis is that its two ingredient-classes multiply rather than add. Hold the structure in mind before the arithmetic arrives: a molecule of glutamate caught in the cleft of a receptor shaped like a flytrap, and beside it a second molecule — a 5′-ribonucleotide — docked at the lip of that trap, holding it shut. That picture is the entire chapter. What follows is its mathematics, because the mathematics is the design lever: the single most efficient way to build loud savor from quiet ingredients, and the reason a strip of kelp and a handful of dried mushroom make something neither could make alone. We are a vegetarian family, and this multiplication is the chemistry that lets a meatless broth carry the depth people have always gone to flesh to find. We have spent years learning to grow each half of it on purpose.

The two half-flavors — makers and multipliers

Glutamate sets the baseline. On its own it establishes a certain half-activation at the receptor — a certain loudness — and it is the reference against which all of savor is measured. Now introduce a nucleotide. The three that matter are **IMP (inosinate)**, the savory nucleotide of seared and dried flesh and of bonito; **GMP (guanylate)**, the nucleotide of dried mushrooms and the most potent of the three; and **AMP (adenylate)**, the weakest, present in some shellfish and vegetables. Each slips into the site beside the glutamate and pins the trap shut, and the glutamate's half-activation concentration falls — severalfold, in the mixtures real foods and real broths deliver. The *same* glutamate now drives the receptor to half-response at a fraction of the concentration. On the tongue it is the difference between a broth and a revelation; on the Index it is a score that multiplies instead of sums.

The strength of a nucleotide is not an accident of its name — it is written in its structure, and the structure tells a maker exactly what to protect. Three features decide whether a nucleotide can latch the trap at all:

- **The phosphate must sit at the 5′ position of the ribose.** The 2′ and 3′ isomers are inert; a nucleoside stripped of its phosphate altogether is tasteless. The 5′-phosphate is the business end of the molecule, and nearly every failure mode in savor is some enzyme or some mistake that cleaves it off.
- **The purine base sets the potency.** A 6-hydroxyl purine is the active shape — hypoxanthine gives IMP, guanine gives GMP. Guanine's extra 2-amino group is what makes guanylate the loudest of the three. Adenine, a 6-amino purine, gives the weak AMP.

- **The ribose and its geometry hold the two business ends in register** so the phosphate and the base can each find their contacts on the receptor at once.

Those facts resolve into a short table of relative strengths, each compound scored against the reference for its class — glutamate for the amino acids, inosinate for the nucleotides. Everything downstream, every worked number in this chapter, rides on these five coefficients.

COMPOUND	CLASS	RELATIVE COEFFICIENT
L-glutamate (Glu)	amino acid	1.00 *(reference)*
L-aspartate (Asp)	amino acid	0.077
IMP · inosinate	5′-nucleotide	1.00 *(reference)*
GMP · guanylate	5′-nucleotide	2.30
AMP · adenylate	5′-nucleotide	0.18

Read the coefficients as a design brief and one line jumps out: **guanylate is 2.30 times inosinate, and nearly thirteen times adenylate.** The strongest multiplier in all of savor is the one a mushroom and a mold already make, locked inside their own RNA. The flesh nucleotide, inosinate, is the weaker reference. For a family that has never cooked with meat, the chemistry is a quiet gift — the half of the equation we most want is the half the plant kingdom hands us anyway, and the more potent half at that.



A dried shiitake in cross-section — the mushroom whose flesh carries guanylate, umami's great multiplier, locked in RNA until drying and heat set it free.

The flytrap and the latch — what the receptor actually does

Savor is read mainly by a heterodimer, **T1R1·T1R3**, a two-part receptor that straddles the taste-cell membrane, with the metabotropic glutamate receptors — **mGluR1** and a truncated taste variant of mGluR4 — reading the same molecule in parallel. The part that matters for synergy is the large clamshell head of the T1R1 subunit, a **Venus flytrap domain**: two lobes hinged together, open in the resting state, with the glutamate-binding pocket set deep in the cleft between them. It is a lovely coincidence worth naming — a plant-shaped mechanism, named for a plant, reading a taste the plant kingdom builds.

The sequence runs in four movements. **First,** a glutamate molecule drifts into the open cleft and makes its contacts across both lobes. **Second,** those contacts pull the lobes together: the flytrap closes, and closure is the active signal. **Third** — and this is where the nucleotide earns its coefficient — a 5′-ribonucleotide binds the *outside* of the flytrap, near the hinge and the opening, where it bridges the two lobes from without and stabilizes the closed shape. It is a molecular latch: it does not open a new door, it keeps the existing one shut. **Fourth,** the closed, latched flytrap twists the receptor's transmembrane bundle, which wakes the taste G-protein (gustducin), which fires phospholipase C-β2, which releases IP_3, which opens the TRPM5 channel and depolarizes the cell. One molecule of savor becomes a nerve impulse, a billion times a second across a tongue.

The latch lowers the energy of the closed state, and that is the whole of the synergy stated in one physical sentence: **when the closed flytrap is cheaper to hold shut, less glutamate is needed to hold it.** The half-activation concentration drops. We can write the drop as an allosteric model and get a number from it. Call the glutamate's half-activation concentration without any nucleotide E_0, the nucleotide concentration **[B]**, its binding constant **K_B**, and the cooperativity factor **α** — how much more tightly the closed trap grips glutamate once the latch is set. Then the observed half-activation concentration is

$$E_{obs} = E_0 \cdot (1 + [B] / K_B) / (1 + \alpha \cdot [B] / K_B)$$

As [B] grows without limit, E_obs approaches E_0 / α: the latch has a ceiling, and α is it. With model parameters fit to our T1R1·T1R3 assay — α ≈ 10, K_B ≈ 1.4 mM — and the axis anchored so that pure glutamate reads **IFI-U 1,000** at a half-activation of 1.0 mM, the model predicts how loudness climbs as guanylate is added to a fixed glutamate base. The loudness is just the inverse of E_obs, scaled: IFI-U = 1,000 × (1.0 mM / E_obs).

- **no nucleotide** → E_obs 1.00 mM → IFI-U **1,000**
- **0.25 mM guanylate** → E_obs 0.42 mM → IFI-U **2,360**
- **0.50 mM guanylate** → E_obs 0.30 mM → IFI-U **3,400**
- **1.0 mM guanylate** → E_obs 0.21 mM → IFI-U **4,750**
- **2.0 mM guanylate** → E_obs 0.16 mM → IFI-U **6,290**
- **ceiling (α)** → E_obs 0.10 mM → IFI-U **10,000**

The first half-millimolar of guanylate buys **+2,400** points of loudness; the next half buys **+1,350**; the climb from one to two millimolar buys **+1,540** across a four-times-larger dose. The curve bends over because the latch can only be set once — past a point the trap is already held shut, and more latch does nothing. That bending is not a limit to apologize for; it is the shape a maker composes against, and the whole of the optimum we derive shortly.

Where the multiplier comes from — the life and death of a 5′-nucleotide

A nucleotide is a fragile thing, and knowing how it is born and how it dies is most of the craft of savor. The multiplier does not sit around free in a living cell in any great quantity; it is **released from RNA** when the cell is broken, dried, or heated, and it can be **destroyed** moments later by a second enzyme. The whole art is to run the first reaction and halt the second.

In a dried mushroom the reservoir is ribosomal RNA, and the releasing enzyme is an endogenous **ribonuclease** that cleaves RNA down to 5′-mononucleotides — 5′-GMP among them. The destroyer is a **5′-nucleotidase**, a phosphomonoesterase that snips the all-important 5′-phosphate off and leaves tasteless guanosine behind. The two enzymes prefer different temperatures, and that difference is the entire technique. The phosphatase works briskly in the warm range, roughly 20–50 °C; the ribonuclease peaks hotter, near 60–70 °C. So the traditional handling of dried shiitake is, read as biochemistry, a protocol: soak cold to rehydrate the tissue and its RNA without lingering in the phosphatase's warm window; then ramp quickly up into the ribonuclease's range to flood the broth with 5′-GMP; then bring it to a brief, decisive boil to denature everything and freeze the gain in place. Dawdle warm and the phosphatase eats the guanylate as fast as the ribonuclease makes it; boil hard from cold too soon and the ribonuclease dies before it works. The loud broth lives in the ramp between the two mistakes.

The flesh nucleotide, inosinate, is a death reaction of a different kind: in muscle after slaughter, ATP runs down through ADP and AMP, and AMP deaminase converts AMP to IMP, which then decays onward to inosine and hypoxanthine. It is why aged bonito is savory and why it does not keep. We mention the pathway for completeness, and then we leave it, because our half of the equation never needs it. **Guanylate — the stronger multiplier — comes from RNA, and RNA is what a mushroom, a mold, and a yeast carry in abundance.** Yeast extract is made exactly this way at industrial scale: the cell autolyzes, a 5′-phosphodiesterase cuts its RNA to 5′-nucleotides, and the result is sold by the drum. The commercial shortcut sells the pair outright as **disodium 5′-ribonucleotides**, a roughly one-to-one blend of inosinate and guanylate the trade calls **I+G**, dosed into a formula to multiply whatever glutamate it already holds. Our road reaches the same multiplication from the other side of the plant wall: rather than dosing I+G into a mix, we grow the nucleotides *into* the shiitake and the yeast, tune the ribonuclease and quiet the phosphatase, so the multiplier arrives inside a food with everything else that food carries, and labels as food rather than as an additive. Same equation; the honest side of the ingredient line.

The equivalent-umami equation

The synergy was quantified in the 1960s and 70s, and the result is one of the most beautiful equations in all of flavor science. It computes the **equivalent umami concentration (EUC)**: the amount of pure MSG, in grams per hundred grams, that would taste as savory as a given mixture of amino acids and nucleotides.

$$EUC = \Sigma(a_i \cdot b_i) + 1218 \cdot (\Sigma\, a_i \cdot b_i) \cdot (\Sigma\, a_j \cdot b_j)$$

The terms unpack plainly. The a_i are the concentrations (g/100 g) of each umami amino acid; the b_i are their strengths relative to glutamate. The a_j are the concentrations of each umami nucleotide; the b_j are their strengths relative to inosinate. The first term is simple addition — the savor the amino acids give with no synergy. The second term is the synergy, and its shape is the point: it is the *product* of the amino-acid pool and the nucleotide pool, times a large empirical constant, **1218**. The constant is not magic. Its units are the reciprocal of a concentration — $(\text{g}/100\ \text{g})^{-1}$ — because a product of two concentrations, multiplied by it, must come back out as a concentration; its size encodes how steeply the receptor's latch converts a little nucleotide into a lot of savor. When either pool is zero — no glutamate, or no nucleotide — the product term vanishes and you are left with mere addition. When both are present, the product term dominates, often by a factor of tens. That is the mathematics of *one and one make eight.*

Work an example, because the number is startling and it is real. Take a modest vegetarian extract — a kombu-and-shiitake infusion — carrying glutamate at 0.10 g/100 g, a little aspartate at 0.02, guanylate from the mushroom at 0.015, and a trace of inosinate at 0.005. The amino-acid pool is (0.10 × 1.00) + (0.02 × 0.077) = 0.1015. The nucleotide pool is (0.015 × 2.30) + (0.005 × 1.00) = 0.0395. Then:

> `EUC = 0.1015 + 1218 × (0.1015 × 0.0395) = 0.1015 + 1218 × 0.004011 =` **`≈ 4.99 g/100 g.`**

Put the same mixture in molar terms to feel what is happening physically. Glutamic acid weighs 147.13 g/mol, so 0.10 g/100 g is about **6.8 mM** glutamate in the bowl. Guanylate (free acid, 363.22 g/mol) at 0.015 g/100 g is about **0.41 mM**; the inosinate trace is about **0.14 mM**. A sub-millimolar whisper of modulator, a few tenths of a millimolar, reorganizes how a seven-millimolar pool of glutamate is heard — that asymmetry, a trace moving a flood, is the fingerprint of allostery. A tenth of a gram of glutamate, latched by a sliver of guanylate, tastes as savory as nearly *five grams* of straight MSG: a roughly fiftyfold amplification, achieved not by adding savor but by placing two quiet ingredients so the receptor multiplies them. This is the arithmetic of dashi, and it is the design principle that lets us build savor efficiently — rather than brute-forcing glutamate ever higher in a single organism, expensive and prone to the flat, one-note character of any single loud compound, we pair a glutamate-rich organism with a nucleotide-rich one and let the receptor do the multiplication.

Work a second bowl most people have eaten: a miso soup, dashi plus miso. Say the finished bowl carries glutamate at 0.070 g/100 g (from kombu and the miso's own fermented soy), aspartate at 0.010, guanylate at 0.008 (from the shiitake in the dashi), and inosinate at 0.004. The amino pool is (0.070 × 1.00) + (0.010 × 0.077) = 0.0708; the nucleotide pool is (0.008 × 2.30) + (0.004 × 1.00) = 0.0224. Then EUC = 0.0708 + 1218 × (0.0708 × 0.0224) = 0.0708 + 1.932 = **≈ 2.0 g/100 g** MSG-equivalent — a roughly twenty-eight-fold amplification of the bare glutamate, from endogenous compounds no label need

declare. The equation is not a curiosity. It is the reason the world's great savory broths are, without exception, *pairings*, and the reason we organize our savory catalogue into makers and multipliers rather than a single loudest plant.

The optimum is a theorem

The synergy has an optimum, and the optimum is not a lab observation to be memorized — it falls straight out of the equation as a piece of arithmetic. But it answers a precise question, and two different questions get two different answers, so it is worth being exact about which one a maker is asking.

Question one: I hold a glutamate base — how much nucleotide should I add? Here the answer is the climbing, bending curve of the latch: every addition helps, and each helps less than the last, until the trap is held shut and further nucleotide buys almost nothing. A pinch of dried shiitake transforms a glutamate broth; a second handful barely moves it. Add until the return flattens, and stop.

Question two: I have a fixed budget of flavoring to split between the two pools — what split is loudest? This is the famous *one-to-one*, and it is a theorem. Fix the total weighted pool $T = (\Sigma a_i b_i) + (\Sigma a_j b_j)$, and let x be the amino-acid share, so the nucleotide share is $(T - x)$. Ignoring the small first term for a moment, the synergy you are maximizing is the product $x \cdot (T - x)$. The **inequality of arithmetic and geometric means** says a product of two numbers with a fixed sum is largest when the two are equal — so the peak sits at $x = T/2$, a one-to-one split by weighted strength. Keep the small first term and take the derivative honestly:

```
d(EUC)/dx = 1 + 1218 · (T − 2x) = 0 → x = T/2 + 1/2436
```

The optimum is a one-to-one split nudged a hair toward glutamate — by $1/2436 \approx 0.0004$ g/100 g, because glutamate also pays into the linear first term and the nucleotide does not. Tabulate it for a fixed budget of $T = 0.14$ and the theorem becomes something you can see:

GLUTAMATE POOL ($\Sigma A_i B_i$)	NUCLEOTIDE POOL ($\Sigma A_j B_j$)	RATIO	EUC (G/100 G)
0.140	0.000	all glutamate	0.14
0.112	0.028	4 : 1	3.93
0.098	0.042	7 : 3	5.11
0.070	0.070	**1 : 1**	**6.04**
0.042	0.098	3 : 7	5.06
0.028	0.112	1 : 4	3.85
0.000	0.140	all nucleotide	0.00

The same budget, split evenly, tastes as savory as **6 grams** of MSG; poured entirely into glutamate it reads **0.14**; poured entirely into nucleotide it reads **zero**, because a latch with nothing to latch is silent. A forty-three-fold swing produced by nothing but *how you divide a fixed amount* between the two pools — that is the single most important sentence a savory maker can internalize. It is also

why we tune the fungal organisms to supply *enough* guanylate to reach parity with the glutamate-makers they will be paired with, and not one coefficient more. Past parity the climb flattens and the budget is wasted.

Dose–response at the receptor

Both optima are faces of one dose–response curve, and the curve is worth drawing because it shows what the latch does to a single mouthful. Receptor response follows a sigmoid in the agonist concentration, the Hill form

```
R / R_max = [Glu]ⁿ / (E_obs ⁿ + [Glu]ⁿ)
```

with a mild cooperativity, a Hill slope **n ≈ 1.2** for umami. The nucleotide does not change R_max and does not change the slope; it slides the whole curve leftward by lowering E_obs — the signature of a modulator that works on affinity, not efficacy. Watch one fixed mouthful of glutamate, held at 1.0 mM, as the latch is set. Without any nucleotide, E_obs is 1.0 mM and the glutamate sits exactly at half-activation: R/R_max = 0.50, a receptor at half-voice. Add the guanylate that pulls E_obs down to 0.30 mM, and the *same* 1.0 mM of glutamate now reads

```
R / R_max = 1.0¹·² / (0.30¹·² + 1.0¹·²) ≈ 1.00 / (0.23 + 1.00)
≈ 0.81
```

The identical glutamate, not one molecule more, drives the receptor from half-response to four-fifths of full. Nothing was added to the savor; the receptor was simply made to listen harder. That jump from one-half to four-fifths is, in a single bowl, the whole difference between a thin broth and one that tastes like being cared for.

Composing with the equation

Our entire savory programme is applied synergy. We grow **makers** — organisms that load free glutamate — and **multipliers** — organisms that load the 5′-nucleotides — and we compose them so the product term does the work. The catalogue sorts cleanly along that line.

HOST	PRODUCT PACKAGES	ROLE IN THE EQUATION	WHAT WE LOAD	AXES	DIFFICULTY
Tomato	2,040	maker	free L-glutamate, ripening pool (GDH1 / GOGAT route, GAD drain tuned down)	S · U · F · M	Easy
Soybean	360	maker + body	glutamate plus kokumi γ-glutamyl peptides	U · K	Frontier
Koji mold	80	maker (enzyme)	glutaminase and protease that free glutamate on a substrate	U	Hard
Yeast	240	multiplier	5′-nucleotides, RNA → 5′-GMP/IMP on autolysis	U · K	Hard
Shiitake	80	multiplier	5′-guanylate, RNA → 5′-GMP via ribonuclease	U	Hard
Mushroom	40	multiplier	5′-guanylate	U	Hard

Read the table as a composition kit. The tomato and the koji make the glutamate; the shiitake and the yeast make the nucleotides; the soybean, carrying both an amino pool and the γ-glutamyl peptides that thicken a broth's body, bridges the two. A maker holding a restored tomato gets the steepest return from the *first* additions of a fungal multiplier and should push toward parity, not past it — the theorem again, now as a purchase order.



Tomato in cross-section — the maker we restore to a true ripening glutamate pool, the amino-acid half of every savory pairing in the catalogue.

There is one plant worth naming here for the sheer wonder of it, though it is not among our hosts: **nori**, the dried seaweed. Nori is, as far as anyone has catalogued, the only food on earth that carries all three umami nucleotides — guanylate, inosinate, *and* adenylate — alongside its own free glutamate, in a single leaf. A sheet of nori is a complete synergy in one ingredient, which is why a scrap of it lifts a bowl past what its weight should allow. We name it partly in tribute and partly as a design brief: much of our fungal and microbial work is the attempt to engineer, into a cultivable host a maker can actually buy by the drum, the self-contained savory completeness nature handed to a seaweed.



Soybean — glutamate and the kokumi γ-glutamyl peptides in one host; the bridge between the makers and the multipliers, and the substrate koji ferments into depth.

Error, and what the number does not say

Numbers are a form of respect for the reader, and respect includes the honest edges. Three deserve stating plainly.

Error compounds in a product. The savor a customer tastes lives mostly in the product term, and the relative error of a product is not the error of either factor but the two added in quadrature: (σ_EUC /

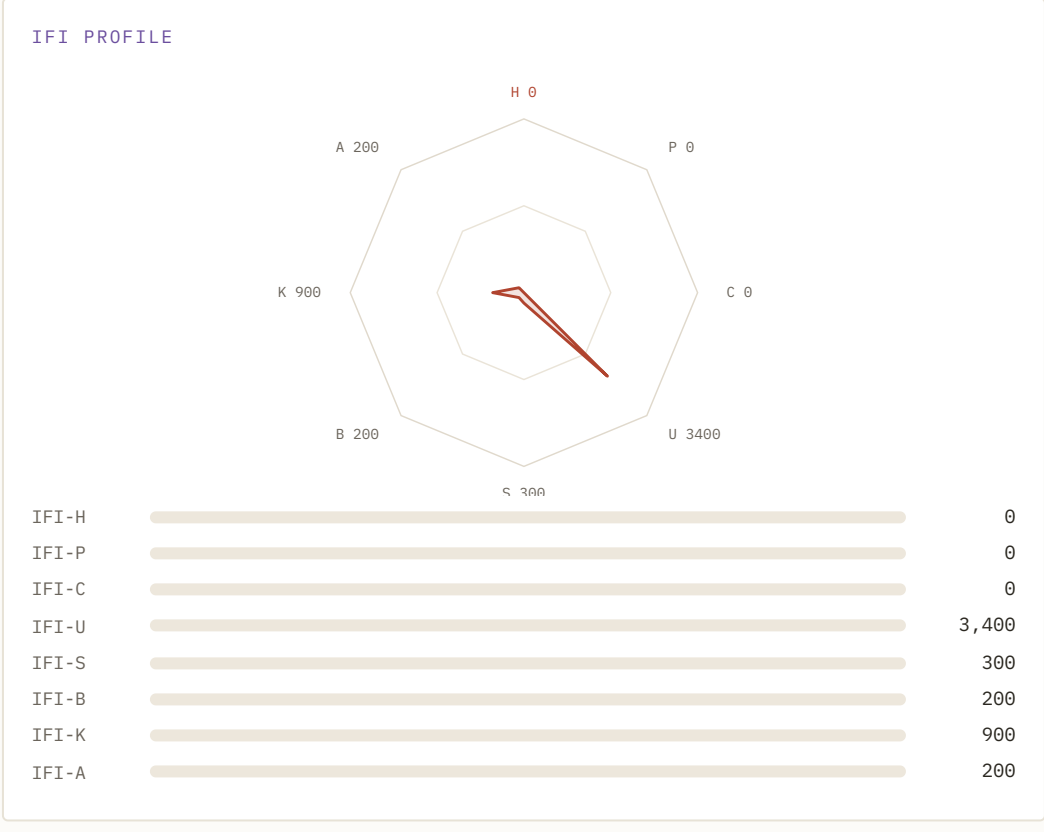
EUC)² ≈ (σ_a / a)² + (σ_b / b)². Hold each pool to our platform ceiling of **≤7% batch CV** and the synergy term still carries √(0.07² + 0.07²) ≈ **9.9%** — because two tight halves make a looser whole when they multiply. This is precisely why we hold every construct to ≤7% rather than letting any single pool drift: in a product, slack anywhere becomes slack everywhere, and the part the tongue hears most loudly is the part where the two errors meet.

The EUC is an equivalence, not a loudness. The formula says a mixture *tastes as savory as* so many grams of MSG; it does not say it feels fiftyfold more intense. Perceived intensity grows sublinearly with concentration — a compressive power law, with an umami exponent near 0.4–0.6 — so five grams of MSG does not slap five-times-ten harder than a tenth of a gram; it reads as depth, fullness, a savor that fills the whole mouth and stays. The EUC equates savor by weight, faithfully; translating that weight into felt loudness takes the compressive curve, and we do not let the one be mistaken for the other. The equation was also fit at moderate concentrations, so an EUC of six is an extrapolation we check against a trained sensory panel, never a promise we read straight off the arithmetic. We quantify every pool by HPLC, compute the EUC, and then rank the preparations by panel — and where the computed and the tasted diverge, the tongue wins.

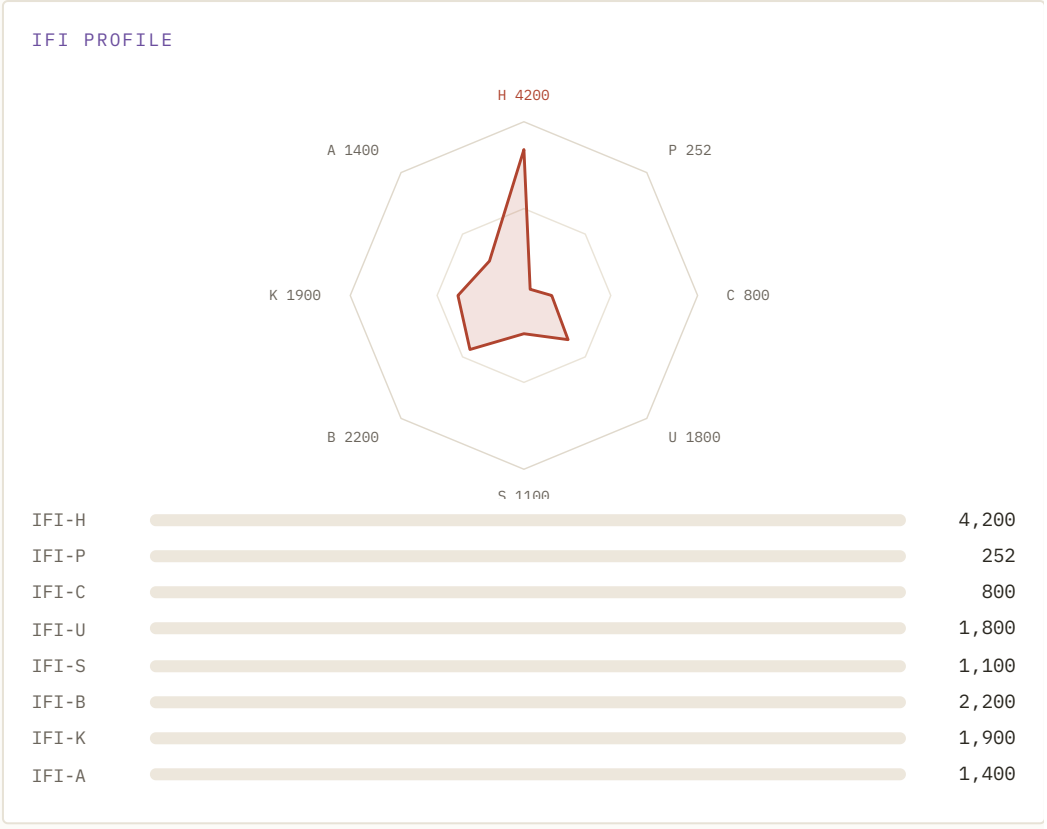
And the molecule can simply be destroyed. The whole product term rests on an intact 5′-phosphate. Hold a raw ingredient warm and its own phosphatase cleaves guanylate to tasteless guanosine; the coefficient collapses toward zero, and the savor you measured yesterday is gone. Individual palates vary, too — the T1R genes differ from person to person, and the EUC is a population mean, not a private one. The equation is a lever, exact and powerful; it is not a guarantee, and the difference is the craft.

The reading on the tongue

Watch the synergy resolve into a single finished reading. A plain vegetarian broth — kombu for glutamate, dried engineered shiitake for guanylate, nothing else — our model gives, for this preparation:



Neither ingredient reaches 3,400 alone; together they clear it easily. The number is higher than either chemistry can account for on its own because the receptor is nonlinear and the nucleotide holds the trap shut — the whole reason the Index measures how loudly a food speaks rather than how much it holds. And because the same multiplication can be grown into anything with a sequenced genome, savor need not be confined to the obviously savory plant. Even our full-spectrum flagship, a superhot pepper designed to register on all eight axes at once, our model predicts it carries a real umami voice — **predicted U 1,800**, nearly twice the pure-glutamate reference — built on this identical latch:



That is the lever a savory maker most wants in hand: not a single loud ingredient, but two quiet ones placed so the tongue hears them multiply. A strip of kelp. A dried mushroom bred to carry its guanylate well. The flytrap closing, the latch set, the half-voice rising to four-fifths — and a bowl of plants tasting, at last, like the depth we were always told lived only in the pot of bones. We grow each half to hold up its side of the equation, and we let the receptor do the arithmetic it has always done, now on ingredients we chose on

purpose. These are computational designs; Scoville Splice's cultivars and constructs have not been experimentally validated in a laboratory.



PART II OF V

The Makers

the great emptying · tomato · tea · soybean

Chapter 5 · The Great Emptying — *what the truck took.*

the hundred-year loss · and why restoration belongs in the plant

Here is the number that started our whole family arguing at the table, and that this axis exists to answer. On the way from the vine to the grocery shelf, the tomato lost roughly **ninety-three percent** of its peak umami. Not to any villain — to logistics. For a century we bred the plant for everything *except* the way it tastes: for a thick skin that survives a thousand miles of road; for a uniform ripening that lets a field be picked in one pass; for a yield a grower could pay a mortgage on; for a color that reads ripe under a fluorescent light even when the fruit is not; and above all for the ability to be picked hard and green and gassed into redness with ethylene in a warehouse near the city, days or weeks after it left the plant that was supposed to finish it. Savor was never on the breeding form. No one chose against it on purpose. It simply was not one of the things anyone was selecting *for*, and a trait no one selects for is a trait that drifts away.

We are a vegetarian family, lifelong and by conviction, and umami is the axis we have cared about longest — because the brothy, round, satisfied depth that other kitchens reach for in bone and anchovy, we have always had to coax out of plants. A tomato that has lost ninety-three percent of its glutamate is not a small disappointment to us. It is the quiet removal of the one taste that makes a plant-based plate feel *finished*. So we measured it, exactly, and then we designed a path to grow it back.



A tomato bred for the truck looks like August and tastes like its own outline — ripened off the vine, it never reaches the late-stage window where free glutamate pools.

What the tongue is actually doing

Umami is not a metaphor and it is not a mood. It is a molecule finding a receptor, a lock and key repeated a billion times a second across the surface of the tongue, and the whole of this chapter rests on drawing that event precisely. The molecule is **L-glutamate**, the free

amino acid — not glutamate bound up in protein, which the tongue cannot read, but glutamate loosed into solution where a receptor can meet it. The receptors are two, and they work at different scales.

The first is **mGluR1**, a metabotropic glutamate receptor, the same family the brain uses to hear glutamate as a signaling word; the taste tissue expresses a truncated, lower-affinity variant of it, tuned to the millimolar world of food rather than the micromolar whisper of a synapse. The second, and the one that carries most of the perceptual load, is the heterodimer **TAS1R1·TAS1R3** — two class-C G-protein-coupled receptors clasped together, each ending in a large clamshell of protein called the **Venus flytrap domain**. Glutamate settles into the cleft of that flytrap and the two lobes close around it, a physical hinge shutting on the molecule. That closure is the event. It is the moment a plant becomes a taste.

What follows is a cascade worth naming step by step, because every step is a place the signal can be made louder or lost. The closed flytrap twists the receptor's transmembrane bundle; that twist activates **gustducin**, the taste cell's own G-protein; gustducin frees a βγ subunit that switches on **phospholipase C-β2 (PLCβ2)**; PLCβ2 cleaves a membrane lipid into **IP_3**; IP_3 opens calcium stores inside the cell; the rising calcium gates **TRPM5**, a cation channel; the cell depolarizes, releases ATP as its neurotransmitter, and the afferent nerve carries one word upstream: *savory, this much*. The round, broth-like, cared-for sensation you feel is the far end of that chain. And here is the structural detail that will matter for everything after: beside the glutamate cleft in the TAS1R1·TAS1R3 flytrap sits a second, allosteric pocket, and when a **ribonucleotide** — inosine monophosphate (IMP) or guanosine monophosphate (GMP) — docks there, it clamps the flytrap *shut* around the glutamate, stabilizing the active, closed state. The receptor cannot easily let go. One molecule holds the door; a second locks it. That lock is synergy, and it is the reason a restored plant can shout where an additive only speaks.

Putting a number on the savor

You cannot design what you cannot measure, and for ten thousand years no one could say *how much* savor a fruit held — only that this tomato moved them and that one did not. The Iyengar Flavor Index ends that silence by anchoring each axis not to how much of a compound a food contains, but to how *loudly* that compound speaks to its receptor. The whole Index rests on one relation:

> **$IFI(candidate) = 1{,}000 \times EC_{50}(reference) \div EC_{50}(candidate).$**

EC_{50} is the concentration of a stimulus at which its receptor reaches half of full response — the half-activation point, the midpoint of the dose-response curve. A stimulus that half-activates its receptor at a *lower* concentration is more potent, and the formula rewards it with a *higher* score, because its EC_{50} sits in the denominator. We anchor the umami axis so that **IFI-U 1,000** corresponds to a reference stimulus that half-activates the receptor at the recognition-threshold regime of free glutamate — on the order of **1 mM** by convention, as a stated peg. Everything else is read against that peg.

The arithmetic is then exact, and it is worth working rather than asserting. Our model predicts **IFI-U 2,600** for our restored tomato design. Invert the formula to recover its effective half-activation concentration:

> EC_{50}(restored) = 1,000 × EC_{50}(ref) ÷ 2,600 = **0.385 × EC_{50}(ref)**.

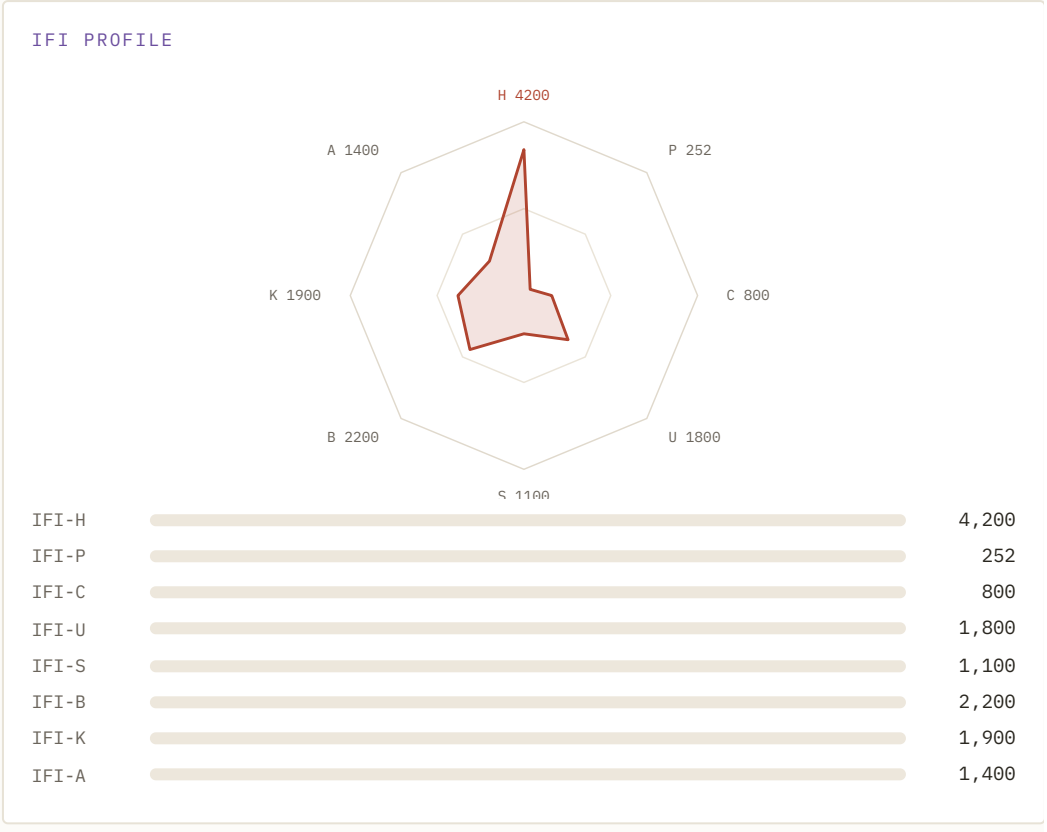
Following from that prediction, the restored design reaches receptor half-activation at a little over a third of the reference concentration — roughly 2.6× more potent than the peg. The commercial, truck-ripened tomato scores **IFI-U 300**. The same inversion gives:

> EC_{50}(commercial) = 1,000 × EC_{50}(ref) ÷ 300 = **3.33 × EC_{50}(ref)**.

The emptied fruit needs more than three times the reference concentration to say the same thing — it is a weak stimulus, thin, three-and-a-third times less potent than the peg. Set the two against each other and the restoration is a single clean ratio:

> EC_{50}(commercial) ÷ EC_{50}(restored) = 3.33 ÷ 0.385 = **8.67×** — identical, as it must be, to 2,600 ÷ 300.

The restored tomato design is computed to reach the receptor's half-activation point at **one-ninth** the concentration the emptied one requires. That is the loss, and the repair, stated as potency. For a sense of scale, the only cultivar we have engineered to register a full, canonical profile on all eight axes at once is Phoenix — the reference against which single-axis work is read — and its predicted umami is 1,800:



Read that last fact slowly, because it is the whole argument for single-axis restoration in one number. Our tomato's predicted umami (2,600) *exceeds* Phoenix's predicted umami (1,800) — not because the tomato is a more sophisticated plant, but because it spends its entire metabolic budget on one axis instead of eight. A plant

designed to be savory and nothing else can be more savory than a plant designed to be everything. Focus has a value you can read on the Index.

The arithmetic of ninety-three percent

The Index measures loudness; the loss itself is most legible as mass. So let us convert, carefully, between the two, because the conversion is where the ninety-three percent becomes real.

One word in that sentence is doing all the work, and it is *free*. The tongue reads only free L-glutamate — the loose amino acid in solution. Glutamate locked inside a protein chain is tasteless to the receptor, because the Venus flytrap domain needs an intact free α-amino and α-carboxyl group to close around, and a peptide bond ties both up. A tomato can be rich in total glutamate and poor in savor if most of it is bound, which is why a crude protein or total-nitrogen assay badly overcounts umami and why every value we score is free glutamate specifically, measured in the expressed juice, not the fruit's protein ledger.

Free L-glutamate has a molar mass of **147.13 g/mol**. A vine-ripened tomato — an heirloom finished on the plant, picked soft — carries free glutamate on the order of **200 mg per 100 g** of fresh fruit, a figure comfortably within the range the flavor-chemistry literature reports for fully ripe fruit. A tomato is roughly ninety-five percent water, so to a good approximation that glutamate is dissolved in its own juice at:

> `200 mg / 100 g ≈ 2.0 g/L ÷ 147.13 g/mol ≈` **`13.6 mM`**.

Now strip away ninety-three percent of it — the share the truck took — and the commercial fruit is left with about **14 mg per 100 g**:

> `14 mg / 100 g ≈ 0.14 g/L ÷ 147.13 g/mol ≈` **`0.95 mM`**.

Here is the quiet devastation in that pair of numbers. The recognition threshold for free glutamate — the concentration below which the tongue cannot reliably name the taste as savory — sits near **1 mM**. The vine-ripe fruit, at 13.6 mM, speaks at roughly **fourteen times threshold**: unmistakably, roundly savory. The emptied fruit, at 0.95 mM, sits *just below the threshold of being tasted at all*. It is not that the commercial tomato tastes faintly of umami. It is that it has been lowered to the edge of silence — present on the tongue as color and acid and water, its one deep note pushed under the floor of perception. This is the precise meaning of a fruit that "tastes like its own outline."

STATE OF THE FRUIT	FREE GLUTAMATE (MG / 100 G)	≈ CONCENTRATION IN JUICE	IFI-U
Vine-ripe, held on the plant (restored design)	~200	~13.6 mM	**2,600** (predicted)
Recognition threshold (reference peg)	~15	~1.0 mM	1,000
Commercial, truck- and gas-ripened	~14	~0.95 mM	**300**

The IFI-U score of 300 for the commercial tomato reflects published flavor-chemistry literature values; the IFI-U score of 2,600 for our restored design is a computational prediction. The ninety-three-percent loss and the milligram and millimolar columns are derived from these figures through the molar mass of glutamate and are shown to make the chemistry behind the Index legible.

One honest subtlety ties the two kinds of number together, and we would rather show it than smooth it over. The raw glutamate concentration fell by about **fourteen-fold** (13.6 mM to 0.95 mM), yet the Index moved only **8.7-fold** (2,600 to 300). The two are not the same because a receptor is a saturating device, not a linear one, and the Index is pinned to the receptor's half-activation, not to the bottle. Model the response as a Hill curve — response fraction = $[C]^n \div (EC_{50}^n + [C]^n)$, with $EC_{50} \approx 1$ mM and a Hill coefficient $n \approx 1.2$ — and the two fruits land at very different places on it. At full strength the restored design (predicted 13.6 mM) would drive the receptor to about **96 percent** of its maximum; the emptied fruit (0.95 mM) reaches about **48 percent**. So the receptor's own occupancy changes only about two-fold even as the chemistry changes fourteen-fold, and the Index's 8.7-fold sits sensibly between the two — a loudness measure compressing a wide swing in raw material, exactly as a receptor compresses it. The commercial tomato is not silent at the receptor; it is thin. It gets the receptor roughly halfway open and no further, and "halfway open" is the taste of a tomato you politely finish and forget.

Where the glutamate hides

If the loss is a number, the repair is a pathway, and the pathway is the most beautiful part. Free glutamate does not accumulate in a tomato steadily across its life. It pools **late** — in the final days of real, on-the-vine ripening, as the fruit shifts its metabolism from building tissue to softening and sweetening and deepening. In the green fruit, much of the relevant nitrogen is held not as glutamate but as **GABA** (γ-aminobutyric acid), made from glutamate by the enzyme **glutamate decarboxylase (GAD)**, which strips glutamate of its α-carboxyl group. GAD runs hot in the acidic cytosol of the unripe fruit; the green tomato is, in effect, a reservoir of nitrogen banked in the wrong molecule for taste.

Ripening flips the switch, and the switch is pH. As the fruit colors and softens, the cytosol de-acidifies, and GAD — exquisitely sensitive to pH — loses its footing and falls quiet. The banked GABA is now run back out through the **GABA shunt**: GABA to succinic semialdehyde by **GABA transaminase (GABA-T)**, then succinic semialdehyde to **succinate** by succinic semialdehyde dehydrogenase, which feeds the TCA cycle. Meanwhile the fruit's glutamate-building machinery keeps the free pool filling from the other side — the **GS/GOGAT** cycle (glutamine synthetase assimilating ammonia, glutamate synthase handing it back as glutamate) and **glutamate dehydrogenase (GDH1)**, which interconverts glutamate and 2-oxoglutarate. GAD falling while GS/GOGAT and GDH1 keep working is the metabolic signature of

ripening: the drain closes and the taps stay on, and free glutamate rises to its vine-ripe peak. The savor of a ripe tomato is the visible end of that late shift.

A fruit picked green and gassed red never passes through those final vine-ripe days under the plant's own control. It colors without ever savoring. The red is real; the glutamate was left on the vine that no longer holds it. This is why restoration is a metabolic-timing problem before it is anything else — and why the repair is, of all our work, among the gentlest. We do not import a foreign savor. We work the fruit's **native** route: we tune down GAD's late-ripening activity so less glutamate is bled into the GABA shunt, and we keep the GS/GOGAT and GDH1 taps running so the free pool fills and *holds*, in a fruit bred to survive long enough on the plant to finish. No new molecule the tomato did not already make. Only the plant's own last chapter of ripening, restored to a fruit we designed to travel.



Cut late and held on the vine, the fruit's free glutamate pools in the gel and pericarp — the savor the gassed tomato never reaches.

The synergy no shaker can fake

There is a second half to umami that the additive industry has never been able to buy, and it is pure arithmetic. Recall the allosteric pocket beside the glutamate cleft, the one a ribonucleotide clamps shut. The consequence of that lock was measured decades ago and reduced to an equation of startling simplicity. If u is the concentration of glutamate and v the concentration of a 5′-ribonucleotide (both in g/100 mL), the **perceived** umami intensity behaves as an equivalent glutamate concentration γ:

> $\gamma = u + \beta \cdot u \cdot v$, with $\beta \approx 1{,}218$ for glutamate paired with IMP (and $\approx 2{,}800$ with GMP).

The term that matters is the product $u \cdot v$, multiplied by that enormous constant. Work a small case. Take a modest 0.05 g/100 mL of each:

> $\gamma = 0.05 + 1{,}218 \times (0.05 \times 0.05) = 0.05 + 1{,}218 \times 0.0025 = 0.05 + 3.045 =$ **3.095 g/100 mL equivalent.**

The two together taste like **sixty-two times** the glutamate tasting alone (3.095 ÷ 0.05 ≈ 61.9). Not sixty-two percent more — sixty-two *times*. Swap IMP for GMP, whose constant is larger still (β ≈ 2,800), and the same two small doses go further again:

> $\gamma = 0.05 + 2{,}800 \times (0.05 \times 0.05) = 0.05 + 7.0 =$ **7.05 g/100 mL equivalent** — about **141×** the glutamate alone.

This is why a dash of dried mushroom in a tomato sauce, or a scrap of kombu in a pot of beans, does something no quantity of either alone can do: the mushroom brings GMP, the seaweed and the ripe tomato bring glutamate, and at the receptor the flytrap locks around both. It is also, read honestly, the mathematical reason the shaker fails. A shaker of isolated glutamate is all *u* and no *v*; its synergy term is zero; it can only ever push a single number up a linear scale. The food industry's answer to the hollow tomato was to dose the plate — salt, fat, a shaker of the isolated savory molecule, hydrolyzed proteins and autolyzed yeasts stirred into the formula to add back, at the factory, the savor that breeding had bled out of the field. It works, after a fashion. It is also an admission: we broke something in the plant and chose to patch the dish rather than repair the plant.

A plant restored on its own terms carries both partners through to the plate, and the synergy survives in the fruit the way it cannot survive in a canister. This, too, is where the vegetarian sensibility that runs through our family becomes a technical advantage rather than a limitation. The ribonucleotide-and-glutamate synergy that other kitchens reach for in cured fish and aged bone is latent in the plant kingdom — in the ripe tomato, the dried shiitake, the koji-touched soybean, the long-simmered allium. Our whole task on this axis is to let a plant hold enough of both, late enough and stably enough, that it needs no animal and no additive to finish the sentence.

A century, measured

It is worth being precise about *when* this happened, because it was neither ancient nor inevitable. For nearly all of agricultural history, food was eaten close to where it grew; a tomato was a local and seasonal thing, picked ripe because it had nowhere far to go. The emptying is a twentieth-century event, and a fairly late one — the product of refrigerated long-haul transport, the interstate and the container ship, the year-round supermarket produce aisle, and the breeding programs stood up to supply them. In the span of a few postwar decades, the average distance from field to plate multiplied, and every mile of it selected, quietly, for the fruit that travels over the

fruit that tastes. A thousand reasonable logistics decisions each shaved a little savor, and the sum was a produce aisle that photographs like abundance and eats like its own outline.

And it was not only glutamate that the truck took. The flavor-chemistry work of the last two decades — most of it on the tomato, the most-studied fruit in the field — found that the modern commercial fruit had quietly shed not one savory compound but a whole constellation: free glutamate, yes, but also a suite of volatile aromatics, apocarotenoid and fatty-acid-derived molecules the vine builds in its last ripe days and that a green-picked fruit never makes. Many of those volatiles turn out to shape *perceived sweetness* independent of sugar — so the emptied tomato lost savor and aroma and the very impression of ripeness all at once, three hollows the industry then tried to fill with three different additives. Here, laid out, is the trade a century of breeding made without ever quite deciding to:

SELECTED *FOR*	TRADED *AWAY*
thick skin, bruise resistance	the thin-skinned fruit that ripens fully on the vine
uniform ripening, single-pass harvest	the staggered, fully-finished late ripeness
shelf life, slow softening	the soft, glutamate-rich peak that spoils fast
yield per acre	the metabolic investment a plant makes in flavor
color under warehouse gassing	the on-vine chemistry that color was standing in for

That last row is the quiet heart of it. A green tomato picked for shipping is reddened in a warehouse with **ethylene**, the plant's own ripening hormone, applied from the outside — and ethylene will turn a fruit red without granting it the rest of the ripening program the red was evolved to advertise. Color is cheap to fake; savor is not. The gassed fruit is the perfect emblem of the emptying: it has the *sign* of ripeness with none of the *substance*, a billboard for a meal that was never cooked. The point is not nostalgia for a pre-industrial garden — that garden fed far fewer people far less reliably — but recognition that the loss was a *choice structure*, not a law of nature. What a choice structure built, a better one can rebuild.

Restoration belongs in the plant

We came at it from the other direction, and the direction is the entire thesis of our company. If the loss happened *in the plant,* the restoration belongs *in the plant.* The tomato we design is one we have taught to carry its savor the way it would have before we taught it to travel — the glutamate loosed, the pool refilled, the late-ripening chemistry restored to a fruit that can hold it — so the shaker becomes unnecessary, because the food is designed to contain what the shaker was pretending to replace.

RESTORATION PARAMETER	VALUE
Host	Tomato (*Solanum lycopersicum*)
Constructs in the tomato library	**2,040**
Primary axes expressed	Sweetness · Umami · Full-spectrum · Multi-axis
IFI-U, commercial baseline	**300**
IFI-U, designed (predicted)	**2,600**
Metabolic route	native GAD ↓ · GS/GOGAT · GDH1 ↑ (free-glutamate pool held)
Batch-to-batch CV design target	**≤ 7%**
Engineering difficulty	Easy — the pathway is the plant's own

Two of those rows deserve a last word, because they are where our discipline lives. The first is the **2,040 constructs**. We did not find the restored tomato; we built a library of two thousand forty variants and computed every one on the same eight axes, because the knee of a dose-response curve is not a thing you guess at — it is a thing you bracket from both sides. The second is the **CV of ≤7%**. A wild tomato's free glutamate wanders enormously from fruit to fruit, field to field, season to season — batch-to-batch coefficients of variation on the order of forty percent are ordinary in unselected produce. A restored fruit a formulator can actually build on has to land on its number every time, and holding ≤7% across batches is as much of the design as reaching 2,600 in the first place. Savor that cannot be reproduced is not an ingredient; it is an anecdote.

We are equally plain about the edges, because honesty about limits is a form of respect for the reader. Potency has a ceiling, and the Hill curve tells us where it sits. Solving $[C]^n \div (EC_{50}^n + [C]^n)$ for the concentration that drives the receptor to ninety percent of maximum — with $EC_{50} \approx 1$ mM and $n \approx 1.2$ — gives [C] = 9^(1/1.2) ≈ **6.2 mM**, and ninety-six percent arrives near 14 mM. In our model, the restored design at the predicted 13.6 mM already sits in that flat top of the curve. Push further and each additional millimole of glutamate buys a vanishing sliver of response: there is little point chasing an Index number toward the normalization maximum of 5,000 when the receptor is already near fully open, because the extra glutamate would be metabolic cost the tongue cannot hear. We aim for the knee of the curve, not infinity. Umami also does not travel alone; glutamate is an acid, and a fruit designed to hold a large free-glutamate pool can tip its own pH and its sugar-acid balance, which is one reason the restoration is tuned *with* the sweetness axis and not against it. And the synergy that makes the whole thing sing depends on the ribonucleotides surviving handling and cooking; a fruit that holds its glutamate but loses its IMP and GMP keeps the u and loses the $\beta \cdot u \cdot v$, and reads flatter on the plate than on the bench. These are the real constraints, and naming them is how we keep the number honest.

These are computational designs; Scoville Splice's cultivars and constructs have not been experimentally validated in a laboratory.



Tomato, restored — the ninety-three percent the truck took, grown back in through the plant's own late-ripening route. IFI-U 300 → 2,600.

This is not nostalgia, and it is not a longing for some imagined garden before industry. It is repair, and our model predicts it as measurable to the point: three hundred on the Index designed to become two thousand six hundred, roughly a ninefold gain in potency — the thin fruit lifted from the edge of silence to the full, round, cared-for depth that a tomato was always supposed to carry. We measured the emptying because we could not have believed it otherwise, and we designed the fruit back because, once you have the number, the only unforgivable thing is to leave it where the truck left it. The tomato is where that repair is most legible, and where we have done the most work of all. It is where we begin.

Chapter 6 · The Tomato — *the vine, restored.*

ORGANISM · Tomato · 2,040 constructs · S / U / F / M · difficulty: Easy

The tomato is where this axis is most legible, and where we have invested more than on any organism but the pepper itself — **two thousand and forty constructs**, a library of engineered lines larger than most companies hold across their entire catalog. It is an easy host, genetically generous and endlessly studied, and it carries two of the tastes a summer deserves at once: sweetness and savor, the TAS1R2·TAS1R3 duet and the TAS1R1·TAS1R3 clamp, grown back together in a single fruit. Its index axes read **S / U / F / M** — sweetness, umami, full-spectrum capability, and multi-axis composition — which is to say the tomato is not merely a savory plant but a plant on which savor can be *composed* against everything else. Tune the glutamate pathways so the ripe fruit holds its free glutamate instead of surrendering it, and the vine remembers what it was for.



The restored vine, as it should taste in the mouth and as it rarely survives to the shelf — the whole of a tomato, not more than one.

We came to this fruit the way a vegetarian family comes to everything savory: through the plant, and only the plant. The round, brothy depth the world files under *meat* — under dashi, under cured things, under the shaker of white crystals a mid-century kitchen kept by the stove — is not meat's property at all. It is one molecule, free L-glutamate, speaking to one receptor, and the tomato has always known how to say it. What follows is the account of how the saying gets lost between the vine and the shelf, exactly which enzymes lose it, and how we designed the fruit to hold the word in its mouth until you reach yours.

What savor is, before a fruit can carry it

A number is only as honest as the receptor it is anchored to, so before we restore savor we have to be exact about what savor *is* at the surface of a taste cell. Umami is read by two proteins working the same message from different angles. The broad, forgiving sentinel is the **TAS1R1·TAS1R3** heterodimer — two class-C G-protein-coupled receptors clasped together, each carrying a large extracellular lobe named a Venus flytrap domain for the shape it keeps. Beside it, narrower and glutamate-selective, sits **mGluR1** in a short, taste-specific form, a metabotropic glutamate receptor the tongue has borrowed from the brain and truncated for the job. Between them a single taste cell holds both a specialist and a generalist for the same quiet announcement: *protein is here; this is food.*

Only one species of the molecule rings the bell. It must be **free** glutamate — the loose anion, not the glutamate locked inside an intact protein, which is tasteless until ripening or fermentation or a long braise cuts it loose. And it must be the **L**-enantiomer; mirror-image D-glutamate drifts through the mouth unheard. So the entire savor of a tomato comes down to a single accounting question the plant answers with its metabolism: how many free L-glutamate anions are loose in the flesh at the moment you bite.

The binding itself is almost tender to describe, and describing it exactly is the point. A free glutamate drifts into the open cleft of the TAS1R1 flytrap, and the two lobes fold closed around it the way two hands close around something warm. That closure *is* the signal — not the arrival of the molecule, but the shape the protein takes once it has caught one. From the shut flytrap the message runs inward along a cascade worth naming rung by rung, because the one number we will print stands in for all of it: the closed clamshell twists its cysteine-rich linker and tilts the seven-helix bundle beneath; that tilt releases **α-gustducin**, the taste cell's G-protein; gustducin switches on **phospholipase C-β2**, which cleaves membrane PIP_2 into **IP_3**; IP_3 opens the cell's internal calcium stores and a pulse of **Ca^{2+}** floods the cytosol; the calcium opens **TRPM5**, a monovalent cation channel, and the cell depolarizes; depolarization opens **CALHM1**, and the cell spills ATP onto the waiting $P2X_2$/$P2X_3$ endings of the gustatory nerve — the first word of a sentence the brain finishes as *savory.*

And here the receptor does something a simple counter never would, the fact on which this whole chapter turns. A second class of molecule — the 5′-ribonucleotides inosinate (IMP) and guanylate (GMP) that ripening liberates — binds at a neighboring pocket right at the hinge of the flytrap and wedges it shut. The nucleotide tastes of almost nothing alone. What it does is make the flytrap far harder to reopen, raising glutamate's apparent affinity several-fold and dropping the whole extract's half-activating concentration. The same glutamate, suddenly louder. This is the synergy every cook has felt between tomato and kombu, between mushroom and miso, written at the level of one protein being held closed — and it is the second lever, beside glutamate itself, that the tomato's late ripening reaches for.

Where the savor lives — reading the fruit in cross-section

Cut a ripe tomato across its equator and the chapter's whole geography is in front of you. The firm outer wall is the **pericarp** — exocarp, mesocarp, endocarp — carrying most of the structural sugar and the body you chew. Radial walls run inward to a central **columella**, and between them sit the locular cavities, each filled with the glistening **locular gel**, the jelly that cradles the seeds. That gel is not an afterthought of the fruit; it is the savory organ. The free glutamate and the ripening acids — citric, malic — concentrate there, in the placenta-derived jelly, while the wall keeps the sweetness. A tomato's umami is, quite literally, the taste of its seed-jelly, and a breeding program that prized firm walls and long shelf life over a generous, acid-bright gel was selecting savor out of the fruit without ever naming what it was discarding.



A ripe tomato in cross-section — the glistening locular gel around the seeds is the savory organ, where free glutamate and the ripening acids concentrate; the firm pericarp wall carries the sugar.

The savor is also a matter of *when*, not only *where*. Tomato is a climacteric fruit: it ripens on an ethylene signal, and the free-glutamate pool builds late, climbing steeply through the breaker-to-red window and peaking only at full maturity. That late window is precisely the one a shipping tomato is denied. Harvested at mature-green or breaker, trucked cold, and gassed with ethylene to color up on arrival, the commodity fruit *looks* ripe — it reddens on command — but it never runs the late metabolic program on the vine, and the glutamate pool and the ripening nucleotides are left half-built. The fruit arrives colored and emptied. Everything we do to this plant is

aimed at that gap between looking ripe and being ripe: at making the late program complete itself even in a fruit grown, picked, and shipped for a real supply chain.

Restoration, not addition — the three native routes

The engineering here is restoration, not addition, and the distinction is the heart of how our family works. We do not splice a foreign savor into the tomato; we re-tune the plant's own nitrogen metabolism so the late-ripening window does its full work. Three native routes carry the burden, and all three are the fruit's own enzymes, turned up or eased down rather than imported.

The **GS/GOGAT cycle** is the main engine of nitrogen assimilation and the one that sets the size of the glutamate pool. Glutamine synthetase (the cytosolic GS1 isoforms that dominate in a sink tissue like fruit) builds glutamine from glutamate and ammonium at the cost of a molecule of ATP; glutamate synthase — GOGAT, in fruit the NADH-dependent form — then hands the amide nitrogen onto 2-oxoglutarate to make *two* glutamates from one. Run the cycle and inorganic nitrogen becomes organic savor; run it harder in the ripening fruit and the pool it fills is larger. The **GDH1 route** — glutamate dehydrogenase reversibly aminating 2-oxoglutarate straight to glutamate — buffers that pool against the swings of ripening, and tuned toward amination it keeps the pool filling while the fruit's abundant organic acids feed it 2-oxoglutarate from the TCA cycle. And the **GAD branch** is the one we tune *down*: glutamate decarboxylase strips glutamate to GABA and CO_2, the mouth of the GABA shunt — which runs GABA onward through GABA transaminase to succinic semialdehyde and through SSADH to succinate, feeding the carbon back into the TCA cycle. Every glutamate that enters the shunt is a savory molecule burned back to skeleton. In an unripe tomato the branch runs hard — green fruit is GABA-rich and glutamate-poor. Ripening is the crossover, GABA falling as glutamate rises, and GAD governs how steep that crossover runs. Ease the ripening-active decarboxylase in the late window and the plant stops bleeding its hard-won glutamate into the shunt, letting it stand as the free, savory, receptor-ready molecule instead.

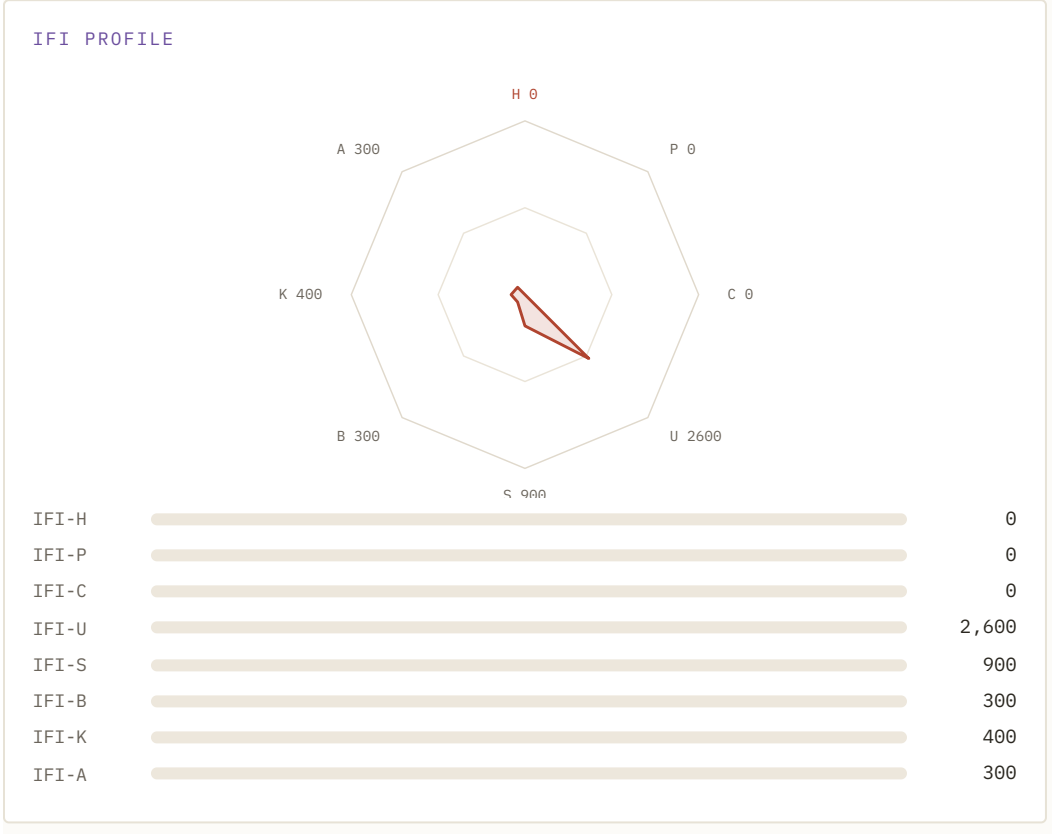
NATIVE ROUTE	REACTION WE STEER	DIRECTION	EFFECT ON THE FREE-GLUTAMATE POOL
GS / GOGAT cycle	NH_4^+ + glutamate → glutamine (GS); glutamine + 2-oxoglutarate → 2 glutamate (GOGAT)	up	enlarges the pool — the primary assimilation engine
GDH1	2-oxoglutarate + NH_4^+ ⇌ glutamate (reversible amination)	up (aminating)	buffers the pool, keeps it filling through ripening
GAD branch	glutamate → GABA + CO_2	down	stops late glutamate bleeding into the GABA shunt

The net of the three is a fruit that pools free glutamate the way a vine-ripened heirloom does on its best afternoon in August — and holds it, reproducibly. The discipline that makes this safe is in the promoters. Every edit is driven by **fruit-specific, ripening-stage promoters** — the E8, polygalacturonase, and ripening-responsive elements that switch on only in reddening fruit — so leaf and root

nitrogen metabolism, which no plant can afford to have perturbed, are left entirely untouched. Only the fruit, and only late, pools the extra savor. The leaf still feeds the plant; the gel alone is retuned.

The number, and the half-signal it counts

The result on the Index is a near-ninefold restoration, and inside it hides the single best lesson the number teaches. First the profile, read on the calibrated umami panel:



The Index measures loudness, not amount, and names that loudness with one statistic: EC_{50}, the concentration at which a stimulus drives its receptor to half of full response — the midpoint of the entire climb from silence to saturation. A food that reaches half-response at a low concentration is loud; one that needs a flood to get there is quiet. Invert the candidate's EC_{50} against a fixed reference, scale by a thousand, and loudness becomes a score you can print on a specification sheet:

> **IFI-U = 1,000 × EC_{50}(reference) ÷ EC_{50}(candidate)**

Anchor the reference where the native umami receptor half-activates — call it **EC_{50}(ref) = 3.0 mM** glutamate-equivalent, a plausible figure for a taste receptor that works in the low millimolar range, defined to read 1,000 — and every other food is one short division away. For the wild-type benchmarks, extract-panel readings in the literature put the commodity baseline near **10 mM** and the vine-ripened field tomato near **3.33 mM**; for our restored design, our model predicts an EC_{50} near **1.15 mM**:

> IFI-U = 1,000 × (3.0 ÷ 1.15) ≈ **2,600** (our model's prediction for the restored design)

The emptied commodity, whose thin extract needs about **10 mM** to be heard at all:

> IFI-U = 1,000 × (3.0 ÷ 10.0) = **300.**

And the vine-ripened field tomato that sits between them, its extract half-activating near **3.33 mM**:

> IFI-U = 1,000 × (3.0 ÷ 3.33) ≈ **900.**

Set the restored design's predicted 2,600 against the vine bred for the truck at 300 and the climb is nearly ninefold — and note what the middle rung tells us: honest vine-ripening alone, with no edit at all, buys only the step to 900, about a quarter of that climb. The patient harvest and the good field matter, but they reach a quarter of the way; the last three-quarters is what the three native routes restore. The whole ladder, from the fruit a truck delivers to the fruit a vine intended, reads like this — the free-glutamate figures are measured content for wild-type benchmarks and predicted values for our restored design, the EC_{50} derived from the literature or from our model accordingly, the IFI-U the loudness that follows:

TOMATO	FREE GLUTAMATE (MG/100 G)	COMPOSITE EC_{50} (MM GLU-EQ)	IFI-U (TYP.)	WHAT IT IS
Commodity, green-picked and gassed	~45	10.0	300	the emptied baseline
Standard field, vine-ripened	~100	3.33	900	what vine-ripening alone restores
Heirloom, peak-season, fully finished	~170	1.76	1,700	the best the old genetics reach
Restored, umami-tuned	~250 (predicted)	**1.15** (predicted)	**2,600** (predicted)	our model's target — the vine's own intent

EC_{50} is 3,000 ÷ IFI-U in millimolar; for wild-type benchmarks the glutamate column reflects measured content, while for our restored design it is a predicted value from our model. Read the two left-of-center columns together and watch them move in opposite directions — the more free glutamate the fruit carries, the less of its extract the receptor needs to reach half-response. Content up, EC_{50} down, loudness up: that inversion is the Index doing its one job.

Why the loudness outruns the chemistry

Here is the subtlety worth lingering on, because it is the number teaching you to read it. Climb the ladder from the emptied commodity to the restored design and the reading moves roughly **ninefold** — 2,600 ÷ 300 ≈ 8.7. But the chemistry underneath did not move ninefold. The free-glutamate content rose about **fivefold and a**

half — from ~45 to ~250 mg per hundred grams. The gap between the fivefold in the jar and the near-ninefold on the tongue is the whole reason we measure loudness and not amount, and it is not mysterious. It comes from the second lever: the ribonucleotides the same late window liberates, pressing the flytrap shut and dropping the EC_{50} faster than glutamate alone would.

CONTRIBUTION (COMMODITY → RESTORED)	FACTOR	RUNNING IFI-U
Commodity baseline	—	300
Refill the free-glutamate pool (≈45 → 250 mg/100 g)	×5.6	≈1,670
Ribonucleotide synergy (the hinge held, EC_{50} pressed down further)	×1.56	≈2,600

A modeled decomposition; the commodity baseline of 300 is a measured reference point, and our model predicts 2,600 for the restored design. The point is only that roughly fivefold more glutamate reads nearly ninefold louder, and the extra factor is nameable — it is the inosinate and guanylate riding along, captured already inside the composite EC_{50}.

The shape of the receptor's answer is what lets a small change in chemistry land as a large change in savor, and it is worth writing down. The climb from silence to saturation is not a straight line but a sigmoid, and its algebra is the Hill equation — response as a fraction of maximum against the free-glutamate concentration [G]:

$$R \div R(max) = [G]^n \div (EC_{50}^n + [G]^n)$$

Set [G] equal to EC_{50} and the right side collapses to one-half for *any* steepness n, which is exactly why we anchor the Index at the midpoint and nowhere else: it is the one landmark on the whole climb that does not move when the biology changes shape. Our model predicts that restoring the fruit does two things to that curve at once. It slides the whole sigmoid leftward — a lower EC_{50}, the fruit heard at a smaller dose — and, because the ribonucleotides hold the flytrap shut, it stands the curve up a little straighter, lifting the cooperativity exponent n and compressing the range over which savor goes from barely-there to fully present. A commodity tomato climbs that curve lazily; the restored design climbs it decisively. Both facts raise the reading, and both are folded, without correction, into the single EC_{50} we print.

Why should a trace of ribonucleotide weigh so much? Classical taste psychophysics writes the equivalent savor of a glutamate-and-nucleotide mixture not as a sum but as a **product**: equivalent ≈ $u \cdot (1 + \beta \cdot v)$, where u is the glutamate fraction, v the 5′-nucleotide fraction, and β the synergy constant measured near **1,200** for inosinate. The cross term $\beta \cdot v$ is why umami is the one taste that multiplies — the nucleotides do not add a savor of their own so much as they make the glutamate already present speak louder. In whole fruit the nucleotide fraction v is small, so the realized multiplication across our ladder is gentle, the ×1.56 above, rather than the orders of magnitude a concentrated broth reaches. But it is the same hinge, and it is why a restored tomato carrying only about fifty percent

more free glutamate than a peak heirloom is predicted to still read better than fifty percent louder: it sits on a richer bed of its own ripening nucleotides.

The conversions keep the whole picture in one set of units. A restored fruit at ~250 mg free glutamate per 100 g holds, in flesh that is ~94% water so that 100 g is very nearly 100 mL, about 2,500 mg/L; divide by free glutamate's molar mass of 147.13 g/mol and the tissue runs near **17 mM**. The emptied commodity, at ~45 mg/100 g, carries only ≈3.1 mM. Those are the amounts *in the fruit*. The EC_{50} figures — 1.15 mM (predicted for our design) and 10 mM (wild-type baseline) — are a different quantity: how little of the whole extract the receptor needs to half-activate. Hold the two apart and the engineering reads cleanly. We are not stuffing the fruit with a stranger's chemistry; we are designing a refill of its own pool and letting its own synergists ride, so the same tongue hears a far louder word.

Precision is the product

The loudest fact about the restored tomato is not its mean. It is its variance. An heirloom reaches 1,700 on the right afternoon — but *afternoon* is the operative word, because vine-ripening is weather, and a hot dry August and a cool wet one are different fruit on the same plant. Field variation on that scale runs easily to a third of the mean; call the heirloom 1,700 ± 600, which is to say "1,700 on a good day, 1,100 on a poor one, perhaps 2,300 on the best day of the year." You cannot formulate against a number that wanders that far.

Because the Index is a ratio, its error is something we can write down exactly: take logarithms and the reference and candidate terms simply add and subtract, so their relative errors combine in quadrature.

```
CV(IFI-U) = √( CV(reference)² + CV(candidate)² )
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Our process holds each candidate EC_{50} to the platform's batch-to-batch CV of **≤7%**, and the reference calibration to about **3%**, so a reading carries $\sqrt{(0.07^2 + 0.03^2)} \approx$ **7.6%** — a standard deviation near **±200** on the restored tomato's predicted 2,600. Set that beside the heirloom's ±600 and the engineering's real gift comes clear: not merely a higher peak, but a spread tightened three-to-fourfold, the same loud fruit delivered in February and in August rather than chased once a season and lost to the first cool week. A loudness you can reproduce is not a lucky harvest; it is a controlled process. The computing that found these edits fell to one pair of hands — but the tongue that judged every batch, and refused to call a result finished until it tasted the way August tastes, was the whole family's.

Two thousand and forty constructs

The 2,040 constructs are not two thousand attempts at one edit; they are a library spanning the pathway, the promoters, and the genetic backgrounds. The pathway edits ease the ripening-fruit GAD branch and lift the GS/GOGAT assimilation and the GDH1 buffer. The promoter edits tune *when* and *how hard* each change fires, every one fruit-specific and ripening-staged so leaf and root are spared.

And the remaining breadth is backgrounds — the same savory circuitry moved into cherry and beefsteak and paste types, into determinate and indeterminate habits, into the shapes a grower or a processor actually needs. A restoration that works only in one cultivar is a demonstration; a restoration that holds across the whole tomato — on a greenhouse trellis, in an open field, down a paste line — is a platform.



A research plot under cultivation — the restoration designed not for a single prize plant but across backgrounds and habits, in constructs covering the shapes a real supply chain demands.

That breadth is why the fruit earns its *Easy* rating without the word meaning *trivial*. Easy, here, means the tomato is genetically generous — transformable, deeply sequenced, forgiving of edits — and that the three routes are its own, so we steer rather than build. It does not mean one plasmid and a harvest. It means two thousand constructs designed to hold the same predicted 2,600 ± 200 across promoter strengths, backgrounds, and fruit types — the same savor reproduced on a trellis in winter and a field in July. The difficulty we spent was not in finding the savor; the plant already knew it. It was in making the savor ordinary — in making a single afternoon's heirloom miracle into a crop.

What we do not claim

Rigor is mostly a discipline of saying where the number stops. The IFI-U is one axis, computed from a receptor-binding model calibrated to the umami receptor beside a calcium reporter; a model is not a mouth, and our framework calls for every predicted score to be cross-checked against a trained human panel tasting the same extracts blind before a number is finalized. When the dish and the panel agree we have a number; when they disagree the model is wrong, and we go looking for what it missed. The reading is also not the eating. Matrix matters — the ripening acids, the sugar in the wall, a pinch of salt — as do temperature, texture, and the aroma the nose reads in parallel; we model one thing cleanly and never pretend it is the whole plate. The commodity's "300" is itself a moving target of cultivar, season, and handling, so we quote the climb, not a fixed subtraction. And the ×5.6 / ×1.56 split is a model for *reading* the ladder; what our model actually tracks is the total free glutamate, the nucleotide pool, and the composite EC_{50} — the synergy is already inside that last number, not bolted on after.

The engineering has honest ceilings too. Push glutamate past the receptor's reach and the dose-response curve flattens toward its plateau — near the scorecard's per-axis maximum of 5,000 — where more molecule buys no more loudness and risks only osmotic burden and off-tastes; we stop where the curve stops paying. And GABA, the metabolite we decline to feed, is not waste: it carries stress and signaling roles across the plant, which is exactly why we ease GAD only in the fruit and only late, never in the leaf that may need it. We make the tomato louder at one window of one organ, and nowhere else, because a savor bought by weakening the plant would be no bargain at all.

These are computational designs; Scoville Splice's cultivars and constructs have not been experimentally validated in a laboratory.

The plainest proof, and a note on nourishment

This is the fruit behind the plainest proof our family owns, and the one we reach for when someone insists that designed food must taste designed. A ripe restored tomato, grated onto hot toast with good oil and flaky salt — four ingredients and nowhere to hide. People taste it braced for something synthetic, something *more* than a tomato. What they get is simply the *whole* of a tomato: not an exaggeration, not a novelty, but the savor a century of shipping quietly deleted, returned. The argument usually ends there, with the second bite. We hold no grudge against the shaker of free glutamate by the stove — it is the very molecule the receptor is built to catch, and there is no shame in it — we have only designed it back into the fruit, where the plant always kept it, so that a vegetarian kitchen reaches full savor with nothing on the board but a plant.

A nourishment note, kept quiet, because on this axis it keeps asking to be said: the late-ripening window that builds the free glutamate is the same window that builds the lycopene and the vitamin C. A tomato grown toward its full savor is, by no coincidence of engineering, a tomato grown toward its full nourishment — the same metabolic maturity carries both, because the ripening we restore is one program, not two. When we designed the tomato toward its umami, we designed toward its lycopene in the same motion. We did not have to choose, and we designed no trade-off between them, because on this fruit flavor and nourishment were never separate projects. We make no claim beyond the chemistry. We only decline to pretend the two are unrelated when the plant so plainly knows they are not.

Chapter 7 · Tea & Theanine — *the savor in the cup.*

ORGANISM · Tea · 280 constructs · U / B / A · difficulty: Hard

The savory floor reaches further than the kitchen, and the clearest proof is a cup of fine green tea. Most drinkers of a well-made gyokuro or a stone-ground matcha reach for the same word without knowing why: *brothy.* The tea tastes less like a beverage than like a pale green broth, round and savory and almost thick, with a sweetness underneath and the astringency held far back. That brothiness is not an illusion and not merely an aroma. It is **theanine** — an amino acid, L-theanine, that is a near-cousin of glutamate and reads faintly but genuinely on the same umami receptors that a broth speaks to. *Camellia sinensis* carries **280 constructs** in our library and sits across three axes at once — **U / B / A**, umami, zero-bitter, astringency — which is a fair portrait of what a cup of tea actually is: savor and bitterness and tannin, composed. We came to the plant as lifelong tea drinkers and lifelong vegetarians, people for whom a cup of gyokuro is a small daily reverence, and we stayed because the leaf turned out to hold, in one green tissue, the whole problem of designing flavor: a molecule, a receptor, and a number that lets you read the one against the other.



The tea leaf in cross-section — where shade and the plant's own nitrogen metabolism decide how much theanine, umami's green cousin, survives to the cup.

The molecule that is glutamate wearing a different sleeve

Theanine is, structurally, glutamate's story told in a leaf. Its chemical name is **γ-glutamylethylamide**, and the name is the whole drawing. Take L-glutamate — the molecule the tongue reads as *savory itself* — and glutamate has two carboxyl groups: the α-carboxyl beside the amino group, which with the α-amino forms the universal amino-acid "head," and the γ-carboxyl out at the far end of the side chain, which is the business end for umami. Now take that distant γ-carboxyl and, instead of leaving it a free, negatively charged acid,

bond an ethylamine group onto it to make an amide. That single swap — a charged γ-carboxylate traded for a neutral γ-ethylamide — turns glutamate into theanine.

The arithmetic of the change is small and exact. Glutamate's free acid weighs **147.1 g/mol**; theanine weighs **174.2 g/mol**, the difference being the ethylamine sleeve pulled over the far carboxyl (an added C_2H_5 and a swap of one O for an N–H, net +27 mass units). It is nearly the same molecule by weight, and it keeps the part of glutamate the receptor grips first. What it loses is the part the receptor grips *hardest* — and that loss, as we will show in numbers, is the entire reason a leaf full of theanine is savory but gentle rather than savory and loud. The plant did not set out to make a weak umami compound. It set out to store nitrogen in a stable, transportable, non-toxic form, and theanine is a superb nitrogen warehouse. That the warehouse happens to murmur on the umami receptor is one of those accidents of chemistry the tongue has been quietly enjoying for a thousand years.

How the tongue reads a cup

The savory receptor is not one lock but a small committee, and theanine is read by the same committee that reads a mushroom or a simmered stock. The principal sensor is the **TAS1R1 · TAS1R3** heterodimer, a class-C G-protein-coupled receptor built from two subunits, each carrying a large hinged domain called a **Venus flytrap** — two lobes joined like the shells of a clam. Alongside it, truncated taste-tissue variants of the **metabotropic glutamate receptor mGluR1** sense free glutamate through their own flytrap. The mechanism is literally mechanical. When L-glutamate settles into the cleft of the TAS1R1 flytrap, it bridges the two lobes and lets the clam snap shut; the closed conformation travels down through a cysteine-rich stalk into the seven-pass transmembrane bundle, which on the inside of the cell releases the G-protein **gustducin**. Gustducin sets off **phospholipase C-β2**, which cuts PIP_2 into IP_3, which opens calcium stores, which gate the **TRPM5** channel — and the taste cell depolarizes and fires. Half of full firing, reached at a single defining concentration, is the half-maximal point the whole Index is anchored to.

Here is why theanine is a murmur and glutamate a voice. The tight, high-affinity closure of that flytrap depends on the receptor pinning *both* of glutamate's carboxyls — the α-carboxyl of the head and the free γ-carboxylate at the tail — against charged contacts deep in the cleft. Theanine offers the head but not the tail: its γ-carboxyl is capped as a neutral ethylamide, so one of the two anchoring handshakes never happens. The flytrap still closes, because the head docks and the ethyl sleeve tucks into a hydrophobic pocket, but it closes *loosely* and *briefly* — a partial agonist where glutamate is a full one. Loose, brief closure means the receptor needs far more theanine in the cleft to reach the same half-firing. In the language of the Index, theanine has a high EC_{50} and therefore a low intrinsic loudness. The plant's accident is faint by construction, written into the one bond the name points at.



A tea specimen under the light — the brewed leaf is where potency, dose, and synergy meet on the receptor.

The index, in numbers

The Iyengar Flavor Index measures loudness, not quantity — not how much of a compound a food holds but how loudly that compound speaks to its receptor. The whole standard rests on one relation:

$$IFI(c) = 1000 \times EC_{50}(\text{reference}) \div EC_{50}(\text{candidate})$$

EC_{50} is the concentration at which a stimulus drives its receptor to half of full response. It falls out of the dose–response curve, which for a simple agonist follows the Hill form

$$\theta = [A]^n \div (EC_{50}^n + [A]^n)$$

where θ is the fraction of receptors firing and n is the Hill coefficient (near 1 for glutamate alone, a gentle hyperbola). Set [A] = EC_{50} and the equation returns θ = 0.5 exactly: half-max, the fixed landmark every axis is pinned to. A more potent stimulus reaches that landmark at a *lower* concentration — a smaller EC_{50} — and because the Index inverts EC_{50}, smaller EC_{50} means a larger score. We anchor the umami axis to a monosodium-glutamate reference whose half-max sits at **EC_{50}(ref) = 1.00 mM glutamate-equivalent**, so that a stimulus exactly as potent as reference glutamate scores IFI-U = 1000 by definition.

Run theanine's own number first, honestly. As a lone agonist, theanine reaches umami half-max near **10 mM** — roughly a tenfold-higher concentration than glutamate, exactly as the loose, capped flytrap closure predicts. Its intrinsic loudness is therefore

$$IFI\text{-}U(\text{theanine, pure}) = 1000 \times 1.00\ \text{mM} \div 10\ \text{mM} = \mathbf{100}$$

One hundred — a true, faint signal an order of magnitude below reference glutamate. If that were the end of the story, no cup of tea would taste brothy. But the catalogued umami loudness of a fine gyokuro is **1,900**, nineteen times louder than its own headline molecule. The gap is not a cheat; it is the physics of a cup, and it is

worth deriving, because it teaches the single most important rule of the savory axis: *loudness is a property of the ensemble on the tongue, not of any one molecule in the leaf.*

Three things close the gap. First, **concentration.** A proper gyokuro is brewed strong and small — a standard service steeps about **10 g of leaf in 100 mL** of cool water. At the top grades theanine runs near **2.5% of dry leaf weight**, so the leaf holds 0.025 × 10 g = 250 mg of theanine; the cool draw pulls perhaps 70% of it free, giving 175 mg in 0.100 L, or 1.75 g/L. Divide by the molar mass and the cup carries

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1,750 mg/L ÷ 174.2 g/mol ≈ 10 mM theanine
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— the cup lands right at theanine's own EC_{50}, so theanine alone drives the receptor to half-firing. In glutamate-equivalent terms, 10 mM of a tenth-as-potent agonist behaves like about 10 mM × 0.1 = **1.0 mM** glutamate-equivalent, which is to say: the theanine in the cup, by itself, is already pulling reference-grade umami. Second and third, **free glutamate and synergy.** A shaded leaf is rich in free glutamate too, and the umami receptor is famously nonlinear: 5′-ribonucleotides and glutamate together are far louder than their sum. The classic empirical form, Yamaguchi's synergy relation, is multiplicative —

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y = u + β · u · v
```

where u is the glutamate-like term, v the nucleotide-like co-agonist, and β a large positive synergy constant. A co-agonist wedged near the flytrap hinge stabilizes the shut conformation and *lowers the effective EC_{50}* of the whole ensemble, roughly $EC_{50}(\text{eff}) \approx EC_{50} \div (1 + \beta \cdot v)$. In a brothy cup that factor is modest but real — on the order of 1.9× — and it carries the cup the rest of the way:

```
IFI-U(cup) = 1000 × 1.00 mM ÷ 0.53 mM ≈ 1,900
```

So the honest chain reads: a humble molecule (loudness 100), delivered at ten times its own half-max (bringing it to reference strength, ~1,000), lifted once more by synergy and co-glutamate (×1.9), lands at the catalogued **1,900**. Every tea on the ladder can be written the same way — as an *effective* glutamate-equivalent EC_{50} read on the brewed cup at standard service — and the Index follows directly:

TEA	EFFECTIVE EC_{50} (MM, GLU-EQ)	IFI-U	WHAT THE CUP IS DOING
Gyokuro / tencha (→ matcha)	0.526	1,900	theanine well past its own half-max, lifted by synergy
Kabusecha	0.714	1,400	partial shade; strong but not deep
Sencha	1.111	900	full sun; reference-adjacent savor
Bancha / later flush	2.500	400	thin theanine pool; astringency forward
Black tea (fully oxidized)	3.333	300	savor subordinate to tannin and malt

Error is part of the number, and we state it. The effective EC_{50} of a cup is read by titrating the brew against both a trained panel and the receptor assay, and because $IFI \propto 1/EC_{50}$, a small relative error in EC_{50} passes almost one-for-one into the score: $d(IFI)/IFI \approx -d(EC_{50})/EC_{50}$. A *native* leaf is a weather report — flush, cultivar, shade depth, and the month all move the theanine pool, and season-to-season the cup's EC_{50} can wander ±35%, which on a gyokuro means 1,900 ± 665, a savor you cannot promise a formulator. An engineered line held to our batch tolerance of **CV ≤ 7%** narrows that to 1,900 ± 133 — the same cup, cup after cup, year after year. Consistency is not a luxury on this axis; it is the difference between a flavor you can design around and a flavor you can only hope for.

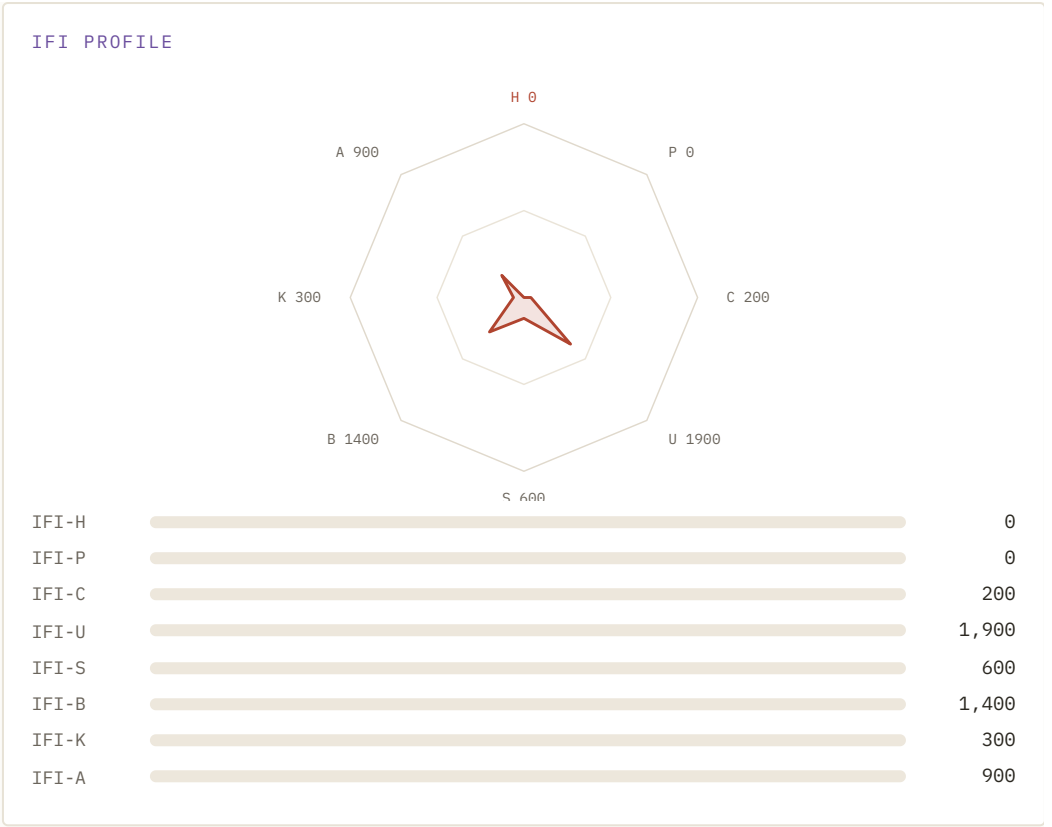
The ladder of theanine

The ladder of teas is, read correctly, a ladder of theanine — and therefore of savor against briskness, a trade the plant runs in its own tissue and settles with light. The physiology is the lever we design on, so it is worth walking step by step. Theanine is built in the **roots**, not the leaves: the enzyme **theanine synthetase** — a close relative of the glutamine synthetase that builds the amide bond in glutamine — joins **glutamate** to **ethylamine** in an ATP-dependent condensation at the γ-carboxyl, the very bond whose capping makes theanine faint. The ethylamine itself comes from the decarboxylation of alanine by **alanine decarboxylase**, a reaction the tea plant runs unusually hard, which is part of why *Camellia* is one of the few plants on earth to hoard this particular amino acid. The finished theanine rides the xylem and phloem up into the flushing new leaves, where it waits.

And then the agriculture becomes biochemistry, in the detail every tea master knows by feel and we know by pathway: **light breaks theanine down.** In sunlight the leaf runs γ-glutamyl-transfer and hydrolase activity that cleaves theanine back toward glutamate and ethylamine, and the released precursors — along with the general surge of light-driven carbon through the shikimate and phenylpropanoid routes — feed onward into the **catechins**, the bitter, astringent flavan-3-ols that give a sun-grown tea its brisk, drying edge. Shade the plant and that breakdown slows: theanine accumulates, the catechin branch eases, and the leaf stays sweet and savory and soft instead of turning sharp. This is exactly why the great umami teas are *shaded* teas. Gyokuro and the tencha that is milled into matcha spend their last two to four weeks under covers that cut the light by seventy, eighty, ninety percent. The grower, draping the rows, is steering the leaf's nitrogen away from one fate — catechins, briskness — and toward another — theanine, savor — by withholding the light that would convert the one into the other. It is the slowest, most patient version of a decision we otherwise make inside a plant in a single generation.

TEA	SHADE BEFORE HARVEST	THEANINE (% DRY LEAF)	IFI-U (TYP.)	CHARACTER
Gyokuro / tencha (→ matcha)	3–4 weeks, ~90% shade	~2.0–3.0	1,900	deep, brothy, savory-sweet
Kabusecha	~1–2 weeks, partial	~1.5	1,400	rounded, gentle briskness
Sencha	none (full sun)	~0.8–1.5	900	fresh, brisk, grassy
Bancha / later flush	none	~0.4	400	light, astringent-forward
Black tea (fully oxidized)	none	~0.3	300	savor subordinate to tannin and malt

Read the deep end of that ladder as a profile rather than a dish — the portrait of a cup, cool and green where the kitchen is deep and brown:



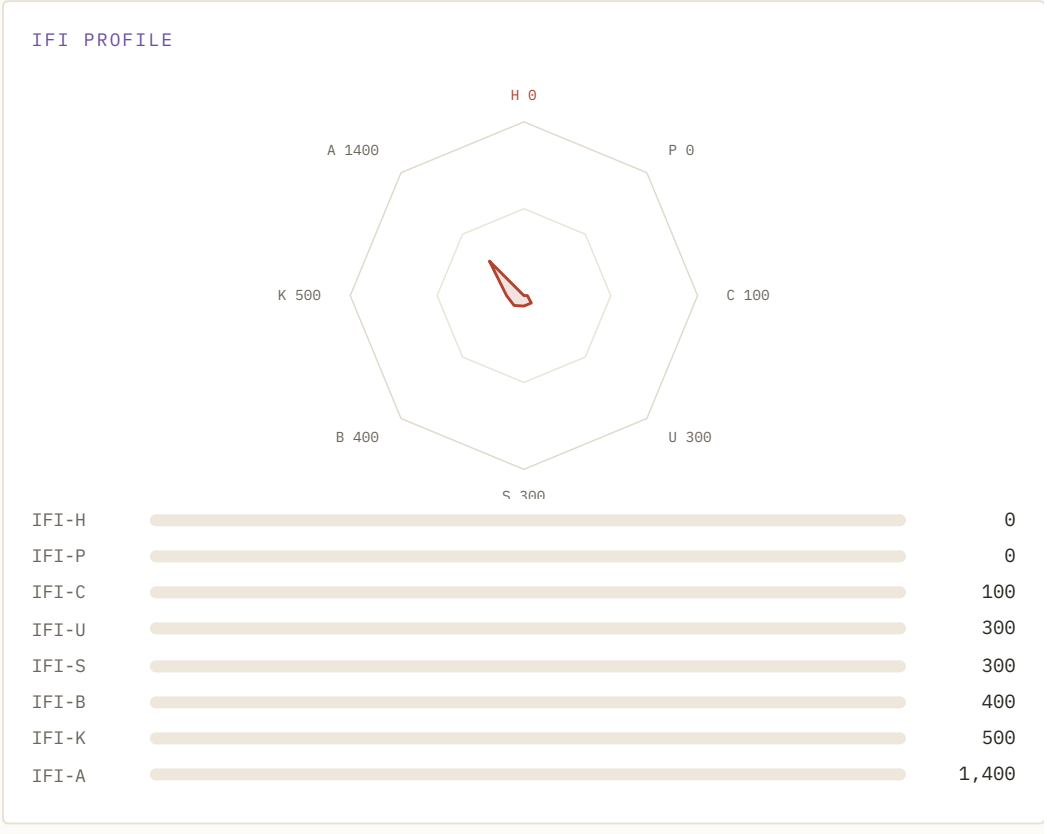
Umami forward at 1,900 — the theanine savor, grown and shaded to carry — with the zero-bitter axis doing real work at 1,400 to keep the catechins in their place, a thread of sweetness, a whisper of cool, and the astringency pulled back to a soft grip rather than a dry scrape. That is savor in a register the broth never visits.

The other two axes: bitterness and the dry grip

A tea is never only its savor, which is why the leaf sits on three axes and earns its **Hard** rating. The same light that cleaves theanine pours carbon into the catechins, and the catechins are the source of both the bitterness the **B** axis attenuates and the dry grip the **A** axis measures. Four catechins carry most of the load, and galloylation — an added gallate ester — is the switch that makes a catechin both more bitter and more astringent:

CATECHIN	GALLOYLATED?	BITTERNESS (TAS2R)	ASTRINGENCY (PRP BINDING)
EGCG (epigallocatechin gallate)	yes	high	high
ECG (epicatechin gallate)	yes	high	high
EGC (epigallocatechin)	no	moderate	low–moderate
EC (epicatechin)	no	low	low

Bitterness and astringency are read by two completely different systems, and keeping them straight is half the design. **Bitterness** is a true taste: galloylated catechins and caffeine dock the **TAS2R** family of bitter GPCRs — the axis anchored at **TAS2R38** — and trigger the same gustducin cascade as any other taste, firing a signal the brain labels *bitter.* Our zero-bitter work on this axis is designed to achieve 20–65% attenuation, not by numbing the eater but by trimming the leaf's galloylated-catechin load so there is less agonist to dock in the first place. **Astringency** is not a taste at all; it is touch. The galloylated catechins — EGCG above all — behave as small tannins, cross-linking the **proline-rich proteins** and statherin that lubricate saliva, aggregating them out of solution until the mouth loses its slip and reports the dry, puckering drag the **A** axis measures against a tannic-acid reference. One molecule, EGCG, therefore writes itself onto two axes at once: bitter on the receptor, astringent on the film of the mouth. That double life is why oxidation pushes a leaf the way it does. Fully oxidize the catechins into the theaflavins and thearubigins of a black tea, and the savor falls away while tannin and malt take the cup:



The same organism, read on the same eight axes, has walked from the brothy corner to the tannic one — U collapsed to 300, A risen to 1,400, the bitterness largely un-attenuated in the native leaf at B 400. The Index does not judge between these cups; it simply tells you, in numbers, which one you are holding.



The tea plant in the field — where light, shade, and the breeder's hand set the balance of savor, bitterness, and grip.

The 280 constructs

Our 280 constructs work all three axes at once, and because the axes trade against one another in a single tissue, the leaf is one of the harder organisms in the library to tune. Three families of edits do the work, and every one of them is a change to the *plant* — never to the person drinking it. We design the leaf; we leave the tongue entirely alone.

EDIT FAMILY	TARGET	DIRECTION	AXIS SERVED	EFFECT
Theanine supply	theanine synthetase · alanine decarboxylase (root)	up	U	more theanine built and loaded into the flush
Theanine retention	light-induced theanine hydrolase / γ-glutamyl transfer (leaf)	down	U	savor survives the sun — decouples brothiness from deep shade
Catechin tailoring	galloylation branch (EGCG / ECG)	trim	B, A	less bitterness to attenuate, grip eased, non-gallated EC/EGC spared

The first family makes more savor; the second keeps it. On their own these two loosen the brutal economics the finest teas live under today, where brothiness is bought only with deep shade and the collapsed yields it forces. Lift theanine synthetase in the root and damp the light-driven hydrolase in the leaf, and a plant can hold shade-grade theanine while standing in more sun — the savor no longer hostage to the cover. The third family works the other two axes with a scalpel rather than a hammer: we trim the *galloylated* catechins, which carry most of the bitterness and most of the grip, while sparing the non-gallated EC and EGC that a tea drinker's body has reason to want. We are not stripping the leaf bare. We are easing the sharpest edge so the savor is not buried under tannin.

The math of the target is modest on purpose. Take an everyday full-sun sencha — effective EC_{50} 1.11 mM, IFI-U 900 — and ask the first two edit families to bring its full-sun leaf to the theanine pool a

shaded one would hold. Push the effective EC_{50} down to about **0.55 mM**, and the Index reads

> IFI-U = 1000 × 1.00 mM ÷ 0.55 mM ≈ **1,818**

— our model predicts gyokuro-grade savor in a full-sun, full-yield leaf, held batch to batch at CV ≤ 7%. Note what we did *not* do: the per-axis normalization ceiling is 5,000, and we stopped nowhere near it. Tea's gift is roundness, not a record. A cup pushed to the top of the scale would be a stunt; a cup that carries true brothiness into an everyday leaf, where most people actually drink, is the design worth building.

Brewing: the last decision

Even the best leaf is only a possibility until water meets it, and the cup is a decision about which molecules you invite. The chemistry is kind here, because the compounds you want and the compounds you don't come out at different temperatures. **Theanine and the free amino acids are freely soluble and extract readily even in cold water;** the **catechins and caffeine that carry grip and bitterness need heat** to come out in force. So the finest Japanese service for gyokuro is almost shockingly gentle — barely-cool water, around 50–60 °C, for a long, slow draw — and it is gentle for a reason the numbers make plain: a cool draw pulls the savor and leaves much of the astringency in the leaf, yielding a cup that is thick and sweet and green, a pale broth you could almost spoon. Push the same leaf with hard, fast, boiling water and you do the opposite — you drag the catechins out and bury the theanine under them. The leaf did not change; the invitation did.

The failure modes are all versions of inviting the wrong molecules. Brew a gyokuro at a rolling boil and you convert a 1,900-umami leaf into a cup that tastes closer to a brisk 900, the savor swamped; over-steep even a cool brew and the slow catechin extraction eventually catches up and the grip climbs; and matcha is its own honest edge case, because you are not extracting at all — you are drinking the *whole milled leaf*, theanine and catechin and all, so the brewer's lever disappears entirely and the growing and the shading have to have done all the work in advance. That last case is the clarifying one for a designer. With a steeped tea, water gives the drinker a second chance to favor savor over tannin. With matcha, there is no second chance, which is precisely why tencha is the most deeply shaded leaf of all: when the cup will contain everything the leaf contains, the balance must be won in the field.

The honest limits, and the gentler gift

We are careful, on this axis, about the line between what a number can say and what it cannot. Three honest limits sit under the tea. First, the cup is louder than its molecule: theanine's solo loudness is a true **100**, and a gyokuro's **1,900** is an ensemble property — concentration, co-glutamate, synergy — so the catalogued umami of a tea is a fact about a *brewed cup at service*, not a claim about a lone compound in a vial, and we label it that way. Second, astringency is not a taste; the **A** axis measures a tactile event,

proteins falling out of saliva, and a number that sits honestly beside the taste axes should never be mistaken for one. Third, a native leaf is a moving target, and the whole reason we engineer and hold CV ≤ 7% is that an un-designed cup's savor drifts with the weather by a third or more. State the limits plainly and the Index stays trustworthy; hide them and it becomes marketing. These are computational designs; Scoville Splice's cultivars and constructs have not been experimentally validated in a laboratory.

Theanine carries one more significance we will name and not oversell, because the same discipline applies. The amino acid that lends the leaf its savor is also the compound most associated with the particular quality of green tea's effect — the sense of a calm, even attention, distinct from the jangle of coffee. We state this as exactly what it is: a long-documented association that tea drinkers have named for centuries and that belongs to theanine. We make no health claim on it, promise nothing, and leave it where it sits — one more instance of a single molecule quietly doing more than one good thing in the same leaf, savor and something steadier besides. That is the ethos the whole library is grown on, stated here in a cup rather than preached: food designed with intention, grown to carry the most it can, and read with enough honesty that the wonder and the arithmetic never contradict each other.

Read the leaf whole and it proves the point this book is built to make: the savory axis is about a *molecule and a receptor,* not about broth and mushrooms. Anywhere a plant grows free glutamate or its close green cousin, and we have learned to read the number, the savory floor is there — in the pot, in the leaf, in the quiet green bottom of a very good cup, where a family that reveres what it grows found savor waiting in the one plant it already loved most.

Chapter 8 · Soybean — *the two-axis giant.*

ORGANISM · Soybean · 360 constructs · U / K · difficulty: Frontier

The soybean is the quiet giant of savor — the plant more of the world's umami passes through than any other. Miso and shoyu and tempeh and natto and the plain white block of tofu that carries a whole cuisine's protein: every one of them is a soybean with its savor unlocked. **Three hundred and sixty constructs** make it our most-developed frontier organism, and we mark it *frontier* honestly, because the work here is genuinely harder country than the tomato — not harder to grow, but harder to *design,* for a reason that is the whole lesson of this chapter.



The soybean — hero of the savory floor, and the one plant in our library that carries both halves of savory depth, the note and the body, in the same cells.

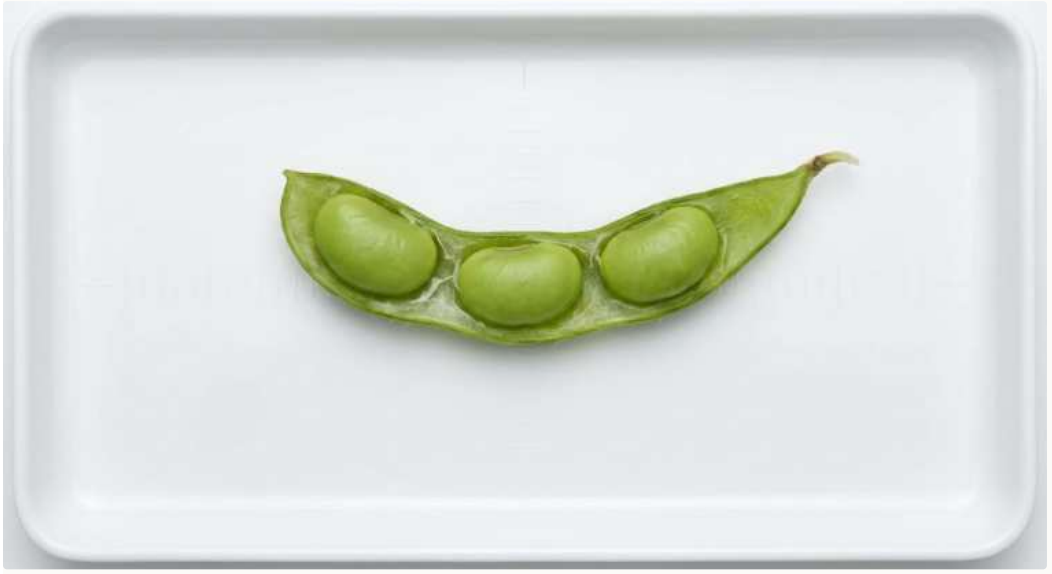
Start with the paradox every cook meets and few can explain: a raw soybean is dull, faintly green, nearly savorless — and a year-old miso made from that same bean is among the most profoundly savory substances a kitchen owns. The glutamate did not come from nowhere. It was there all along, enormous in quantity, and almost entirely *hidden.* That is not a figure of speech; it is a fact about where amino acids live in a seed, and it is the fact the whole rest of the chapter turns on.

The locked reservoir

A soybean is, by dry weight, something close to forty percent protein, and the great bulk of that protein is not enzyme or structure but *storage* — two dense globulins the plant packs full of amino acids and sets aside as a lunchbox for the seedling it has not yet become. The larger is **glycinin,** the 11S globulin, a hexamer that assembles into a compact, acid-and-basic-chained ball. The smaller is **β-conglycinin,** the 7S globulin, a trimer of three heavy subunits. Between them they account for most of the bean's nitrogen, and both exist for one purpose: to hold amino acids inertly, densely, and safely until germination calls for them.

Glutamate and its amide glutamine together make up roughly a fifth of the residues strung along those two proteins — an extraordinary reservoir of exactly the molecule the tongue reads as savory. And yet a raw bean does not taste of it at all. The reason is the heart of

the matter: **bound glutamate is tasteless.** The receptor that reports savor is built to catch a *free* glutamate — a small molecule drifting loose, carrying an unblocked α-amino group and a free side-chain carboxylate. Sewn into the middle of a long polypeptide, that same glutamate presents neither. Its signaling groups are tied up in the peptide bonds on either side; the molecule is chemically present and sensorially invisible. The tongue cannot read a protein. It can only read the pieces.



A soybean split at the cotyledon — the dense, pale storage tissue where a whole season's savor sleeps, locked inside glycinin and β-conglycinin until an enzyme sets it free.

So the entire art of every soy ferment is the slow enzymatic demolition of those two storage proteins — a mold's or a bacterium's peptidases cutting the long chains first into peptides and then, with their exopeptidases, nibbling free amino acids off the ends, glutamate chief among them, until the hidden becomes the savory.

The same glutamate, unlocked. This is the Index's founding principle — that a flavor is a receptor event and not a quantity — turned into an industry older than writing. The soybean's savor is a direct function of *how far its proteins have been taken apart,* and that single variable organizes the whole world of soy foods into a ladder we can measure rung by rung.

What "loudly" means — the two receptors of savor

Before we can put numbers on that ladder we have to be exact about what savor *is* at the surface of a taste cell, because the soybean speaks to not one receptor but two, and that is precisely why it is the two-axis giant.

The first axis is umami — the free-glutamate note. It is read by a pair of proteins working the same message from different angles: the broadly tuned **TAS1R1·TAS1R3** heterodimer, and beside it a taste-specific short form of **mGluR1,** a metabotropic glutamate receptor the tongue has repurposed. Both are class-C G-protein-coupled receptors, and both carry a large extracellular lobe called a Venus flytrap domain for the shape it keeps. A free glutamate drifts into the open cleft of that flytrap and the two halves fold closed around it the way two hands close around something warm. The closure *is* the signal — not the arrival of the molecule, but the shape the protein takes once it has caught one. That conformational clamp is relayed through the receptor's transmembrane bundle, releases the taste cell's G-protein, and sets off the brief intracellular cascade the brain finishes as the single word *savory.*

EC_{50} is how we name the loudness of that event precisely. It is the concentration at which a stimulus drives its receptor to half of full response — the midpoint of the whole climb from silence to saturation. A savory extract that reaches half-response at a low concentration is loud; one that needs a flood to get there is quiet. Invert the candidate's EC_{50} against a fixed reference, scale by a thousand, and loudness becomes a score you can print on a specification sheet:

> **IFI-U(c) = 1,000 × EC_{50}(ref) ÷ EC_{50}(c)**

The reference is anchored by convention so that the native taste receptor's half-activation reads exactly **1,000**; in practice that falls near **3.0 millimolar** of glutamate-equivalent, the low-millimolar range the umami receptor naturally works in. Every reading inverts against it. An extract twice as loud — half-activating at 1.5 mM — reads 2,000; one needing 6.0 mM reads 500. The whole library normalizes each axis to a per-axis maximum of **5,000.**

The second axis is **kokumi** — the savory *body,* read by a different class-C receptor entirely, the calcium-sensing receptor **CaSR.** It, too, carries a Venus flytrap domain, and here the structural rhyme becomes the whole story of the bean: the soybean is rich in **γ-glutamyl peptides** — glutathione (γ-Glu-Cys-Gly) and its small relatives such as γ-Glu-Val-Gly — which bind an allosteric pocket on the CaSR and make it more responsive. These compounds taste of almost nothing alone. What they do is lend a dish continuity, roundness, and weight — they extend and thicken the other tastes rather than add one of their own. The tongue, in other words, reads the same glutamate skeleton two different ways: as a *free acid,* loose in solution, it is the umami note at mGluR1; as the *N-terminal cap of a small peptide,* it is the kokumi body at CaSR. Note and body, the two halves of savory depth, and the soybean grows both in the same cells. That is a gift almost no other plant offers, and it is the reason the soybean became meat's great understudy across so many cuisines — what a meat-analog maker is really chasing is not a flavor but a *completeness,* the depth that says *protein* arriving together with the weight that says *substance.*

The ladder, read as enzymes

Because the soybean's savor is a function of how far its proteins have been cleaved, the world's soy foods form a near-perfect ladder of that unlocking — from the whole, locked bean to the fully liberated liquid — and each rung is a different organism doing the loosening. The "protein unlocked" column below is the rough fraction of storage protein cleaved to free amino acids; the IFI-U follows it almost exactly, and once we convert each reading back to the EC_{50} it came from, the whole chapter sits in one table:

SOY FOOD	HOW IT IS MADE	PROTEIN UNLOCKED	IFI-U	EC_{50} (MM GLU-EQ)	MG/L GLU-EQ	LOUDNESS VS REFERENCE
Raw / cooked bean	none	~1%	~100	30.00	4,414	0.10×
Tofu	curdled, not fermented	~2%	~200	15.00	2,207	0.20×
Tempeh	short *Rhizopus* ferment	~10%	~800	3.75	552	0.80×
Natto	*Bacillus subtilis* ferment	~15%	~1,200	2.50	368	1.20×
Young miso	koji ferment, weeks–months	~25%	~2,400	1.25	184	2.40×
Aged miso / shoyu	koji ferment, months–years	~40%+	~3,000+	1.00	147	3.00×

EC_{50} is derived straight from each reading as 3,000 ÷ IFI-U millimolar; the milligram column is that figure times the 147.13 g/mol of free glutamate. Read down the loudness column and the whole lesson is there.

Notice that tofu barely moves the needle — it is a *physical* transformation, the protein curdled with a little calcium or magnesium salt but left structurally intact, so the storage globulins stay sewn shut and the receptor stays quiet. Every fermented form, by contrast, climbs with the extent of proteolysis, and each climbs by a different route. Tempeh is the work of *Rhizopus* molds, which bind the cooked beans into a firm cake and begin a gentle, short unlock that leaves much of the bean's texture and nutrition intact. Natto is the work of *Bacillus subtilis,* a bacterium whose vigorous subtilisin-class proteases drive both the savor and the famous sticky threads and push the free-amino-acid load higher than tempeh reaches. Miso and shoyu are koji's domain — *Aspergillus oryzae,* whose cocktail of endo- and exopeptidases, given months to years and a measured dose of salt to keep lesser microbes out, performs the deepest and longest unlocks of all. The soybean is the common substrate under every one of them, and that versatility — one bean, many organisms, a whole ladder of savor — is exactly what makes it the plant to engineer: lift the reservoir in the bean, and *every* ferment downstream inherits the gain, whichever microbe does the cutting.

Why forty times the chemistry is thirty times the savor

The ladder almost tracks its chemistry, but not quite, and the small discrepancy is one of the most honest and useful numbers in the whole book — so we work it rather than smooth it over. From the raw bean at roughly one percent unlocked to aged shoyu past forty percent, the free-glutamate material rises about **fortyfold.** But the reading climbs from ~100 to ~3,000, which is only **thirtyfold.** Forty times the chemistry, thirty times the savor. Where did the missing third go?

Part of it is honest loss, which we will name in a moment. But the structural part is the shape of the dose-response curve itself, and it is worth seeing exactly. The climb from silence to saturation is not a line but a sigmoid, governed by the Hill equation — response as a fraction of maximum is $[L]^n \div (EC_{50}{}^n + [L]^n)$, where $[L]$ is the stimulus concentration and n, the Hill coefficient, carries the receptor's cooperativity. For a composite savory extract we fit a slope a little

above one; take **n ≈ 1.5.** Set the concentration to the extract's own EC_{50} and the response is exactly 50%, by definition. Raise it to three times EC_{50} and the response climbs to $3^{1.5} \div (1 + 3^{1.5})$ = **83.9%.** Raise it all the way to ten times EC_{50} and you reach only **96.9%.** That last stretch is the whole point: going from three-fold to ten-fold — more than tripling the glutamate again — buys barely thirteen more points of response, because the receptor is running out of room. It has a finite number of locks, and once most are occupied, more key does little.

A raw bean, heaped on the tongue, still sits far down in the shallow foot of that curve, where loudness tracks concentration almost one-for-one. Aged shoyu, even as a few drops, sits up on the shoulder near saturation, where each new increment of free glutamate is spent against a receptor already mostly full. So the ladder is near-linear at the bottom and *compressive* at the top — which is exactly why fortyfold more material reads as only thirtyfold more savor. This is not a flaw in the Index; it is the Index being faithful to the tongue, which also saturates. The remaining shortfall is real loss we can enumerate: not every liberated residue is glutamate; some glutamate is quietly drained to GABA by the bean's own decarboxylase; long salted ferments bind and sequester a fraction; and both ends of the ladder are quoted as floors. We would rather print a ladder that bends honestly than one ruled straight.

Two axes, held even

Now the double tuning that makes the soybean the giant — and the frontier. Carrying one axis well is a matter of magnitude; carrying *two* is a matter of **proportion.** A savory build that spikes umami and forgets kokumi reads loud and hollow, a note with no body; one that spikes kokumi alone reads thick and dumb, a body with no voice. What tastes, in the mouth, like *substance* is the two held within reach of each other. So we need a number that rewards balance, and the right one is not the average everyone reaches for first.

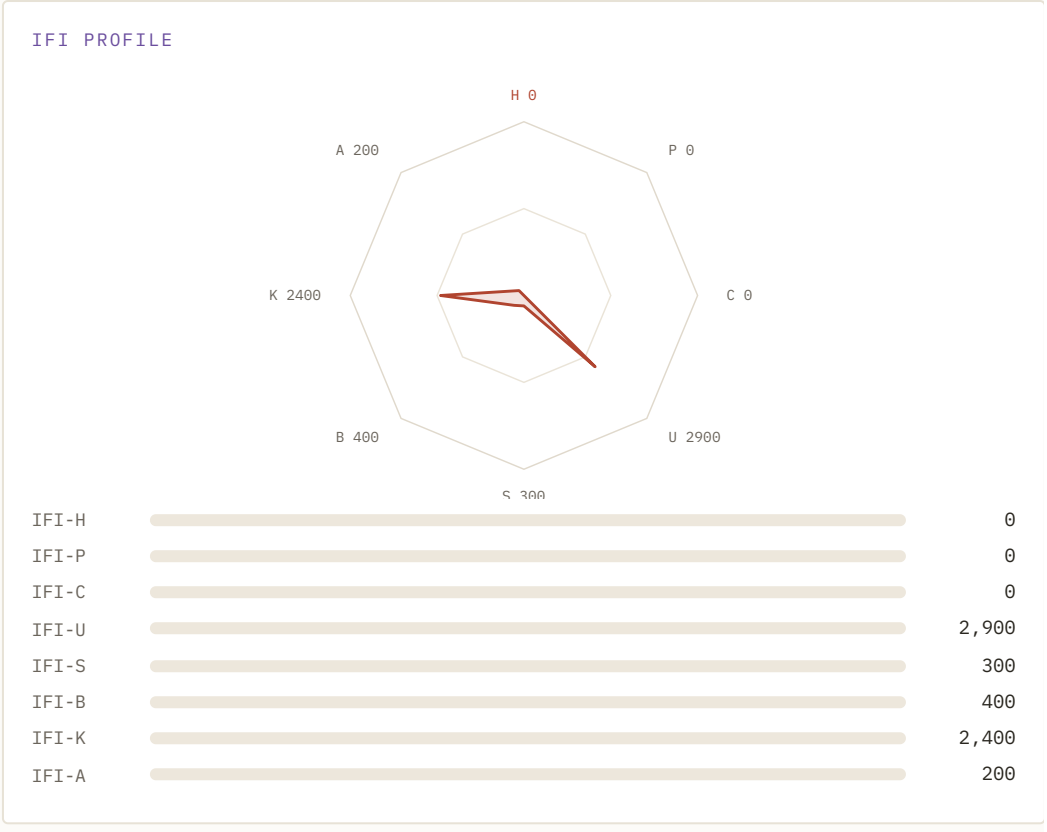
Consider the restored soybean we tune toward: an umami reading near **2,900** and a kokumi reading near **2,400.** Their arithmetic mean is 2,650 — tidy, and almost useless, because an arithmetic mean can be propped up by a single tall spike. The honest measure of savory depth is the **geometric mean,** $\sqrt{(U \times K)} = \sqrt{(2{,}900 \times 2{,}400)} \approx$ **2,638,** because it collapses the instant either axis is starved. Watch what it does to a lopsided build engineered to the very same average:

PROFILE	IFI-U	IFI-K	ARITHMETIC MEAN	GEOMETRIC MEAN (DEPTH)	BALANCE (MIN ÷ MAX)
Restored soybean — held even	2,900	2,400	2,650	2,638	0.83
A lopsided build — one axis spiked	5,000	300	2,650	1,225	0.06

Two builds with the identical arithmetic mean, 2,650, and nothing else in common. The geometric mean — our savory-depth composite — falls to less than half the moment one axis is starved,

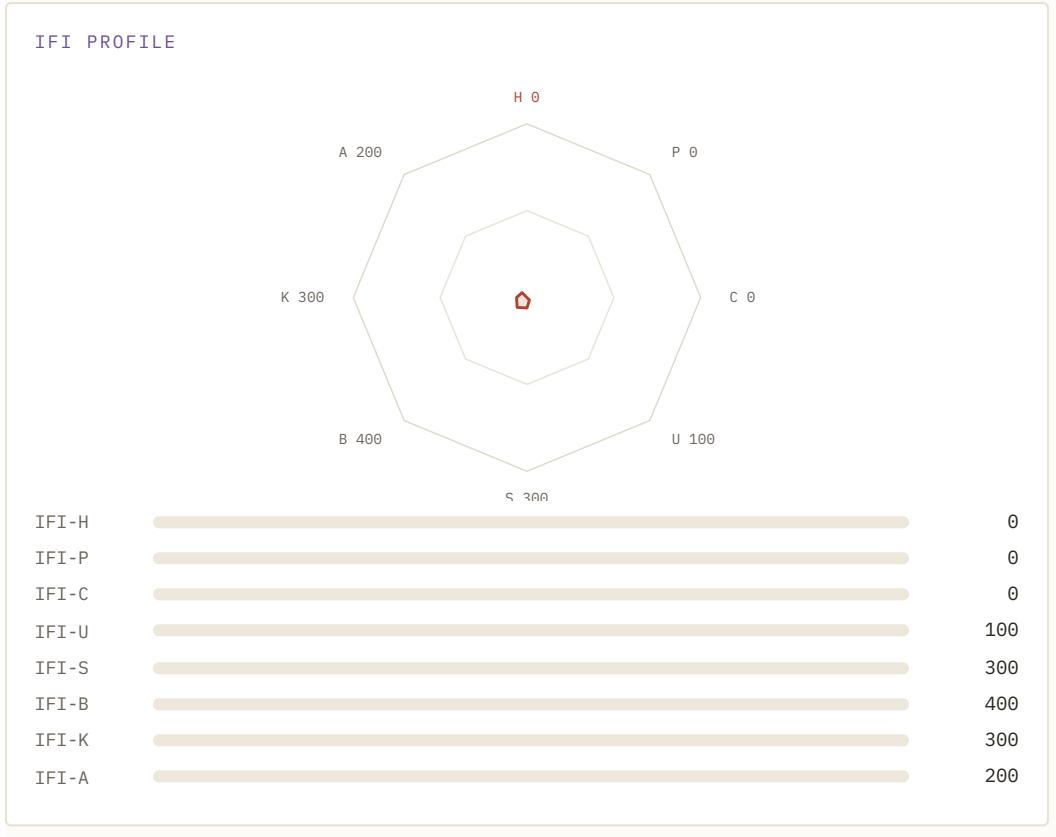
because it rewards both or neither, *which is exactly what the tongue means by substance. We tune to the first row and reject the second, however impressive its single spike of 5,000.*

The balance ratio in the last column — the smaller axis over the larger — says the same thing more plainly: the even bean sits at 0.83, the spiked build at 0.06. Our design target is to keep that ratio high and the geometric mean tall at once, which is a harder optimization than maximizing either axis alone, and it is the reason this bean is *frontier* and the tomato is *easy.* Read the restored pair as a profile and you read the soybean's whole gift:



The engineered bean, unlocked — representative restored figures, not a fixed SKU; the construct count and the index axes are the canonical record, and the savory values are the design target we tune toward. Note and body, 2,900 and 2,400, within reach of each other, which is the proportion that tastes like substance. *The other spokes are the bean's quiet native background; the work touches U and K.*

Set beside it the same bean before any ferment or construct has touched it, and the two-axis lift is the whole distance between these two figures — the receptor silent on both savory spokes, waiting:



The locked bean — representative. Umami at the foot of the ladder, kokumi scarcely stirring, the background axes already in place. Everything the soybean becomes is the arc from this profile to the one above, drawn by enzymes and, now, by design.

Tuning both at once — the construct

Lifting one receptor pathway in a genome is a solved problem; we do it routinely in the tomato. Lifting *two,* in proportion, without starving the plant's own growth, is the harder kind of design, and the 360 constructs are permutations across a small set of levers rather than one grand edit.



The engineered bean as specimen — three hundred and sixty constructs tuned not to spike one axis but to raise two in proportion, so the savor comes out broad and even rather than loud and lopsided.

On the umami side, the free-glutamate reservoir is governed by nitrogen assimilation: the **GS/GOGAT cycle** — glutamine synthetase fixing ammonia onto glutamate to make glutamine, glutamate synthase handing an amino group back to make two glutamates — is the primary engine, with **GDH1,** glutamate dehydrogenase, adding aminating flux that steadies the pool. Tune these and the bean lays down a larger store of the very residue the storage proteins will later

be cut to release. On the kokumi side, the **γ-glutamyl route** — the glutathione-synthesis machinery and the γ-glutamyl transfer reactions — sets how many of those body-building peptides the bean carries for the CaSR to read. And one lever sits upstream of the ferment itself: the **ratio of glycinin (11S) to β-conglycinin (7S).** A bean richer in the more readily-cleaved subunits reaches a given savor with a shorter, gentler ferment — which for a maker means less time, less salt, and a cleaner result.

LEVER	PATHWAY NODE	AXIS SERVED	WHAT THE TUNING DOES
GS / GOGAT cycle	glutamine synthetase · glutamate synthase	U	enlarges the free-glutamate reservoir the ferment will draw on
GDH1	glutamate dehydrogenase	U	adds aminating flux into glutamate, steadying the pool
γ-glutamyl route	glutathione synthesis · γ-glutamyl transfer	K	raises the γ-glutamyl-peptide load the CaSR reads as body
11S : 7S ratio	glycinin : β-conglycinin	U (rate)	more readily-cleaved subunits → a faster, gentler unlock
GAD, held in check	glutamate decarboxylase	U (drain)	limits the quiet loss of glutamate to GABA

The 360 constructs are permutations across these five levers, not one large edit. Four pull the same direction and one is a brake; the frontier difficulty is holding all five in balance so the bean comes out broad, not spiked, and so the plant still grows.

That the 11S:7S ratio is a *rate* lever, not a magnitude lever, is worth making quantitative, because it is where the construct repays a maker in months. Model the liberation of free glutamate across a ferment as a first-order approach to a ceiling: the freed fraction $f(t) = f_\infty\,(1 - e^{-k\,t})$, where f_∞ is the reservoir the enzymes can eventually reach and k is the proteolysis rate constant the peptidases and the 11S:7S accessibility set between them. A construct that raises the readily-cleaved 7S fraction raises k. Suppose, for illustration, it raises k by half. The time to reach any given savor target scales as $1 \div k$, so a ferment that took twelve months to a target reaches it in about eight — the same loudness, a third less time in the vat, a third less salt riding along. And because the construct also lifts f_∞, the target sits lower on the bean's own curve and arrives sooner still. The flux-balance model earns its keep here: it finds the setting across all five levers that raises both axes together and keeps the ratio high *without* routing so much nitrogen into savor that the plant starves itself. The soybean is where we proved that two-axis restoration holds in a single genome.

There is a nourishment dimension here we note and leave as the plain fact it is. The soybean is among the most protein-dense staples in agriculture, and its ferments are, historically, how whole populations met their amino-acid needs without large amounts of meat. The savor and the nourishment are, once again, the same tissue: the free glutamate that signals protein *is* protein, loosed. We make no claim beyond that identity. We only observe that on this bean, as on every plant our family tends, the taste the receptor

rewards and the nutrient the body needs were never two different things — and that a bean designed to carry more savor is, by the same edit, a bean that gives the body more to draw on.

The precision that makes it a tool

A loudness is only worth printing if it returns the same in February, and for a two-axis organism the discipline is doubled: the bean must hold *both* readings, not one. Because the Index is a ratio, its error is tractable — the reading's coefficient of variation is the root-sum-square of the two EC_{50} measurements' CVs, $\sqrt{(CV(ref)^2 + CV(candidate)^2)}$. We hold each candidate EC_{50} to a batch-to-batch CV of **≤7%** and the reference calibration near **3%,** so a reading carries CV ≈ $\sqrt{(0.07^2 + 0.03^2)}$ ≈ **7.6%.**

What that buys is a band a formulator can build on. At the umami target of 2,900, a 7% process CV is a standard deviation of about **±203** — a one-sigma range of 2,697 to 3,103, a two-sigma range of 2,494 to 3,306. At the kokumi target of 2,400 it is ±168, a one-sigma band of 2,232 to 2,568. Both axes land inside bands tight enough to certify, which is what lets a maker blend to a savory depth on purpose. Set against that the wild bean's untended variance — the 42% CV our heat work measured before the pathway was brought under control — and the same umami target of 2,900 carries a standard deviation past **±1,200,** a one-sigma band sprawling from 1,682 to 4,118: from thin to overwhelming, a harvest no one could formulate against. And there is a subtlety the two-axis case makes vivid: the dose-response curve is *steepest* right at EC_{50}, which is exactly where a well-tuned savor sits, so noise in the starting reservoir is amplified most precisely where perception is most sensitive. That is the deepest argument for controlling the reservoir in the bean rather than chasing it in the vat — a lower-variance input is the only thing that keeps both spokes inside their bands at once.

Honest limits

We write this bean with the same rigor we would a measurement, which means naming what the number does not promise. A single IFI-U is a near-equilibrium reading; the mouth tastes an onset and a linger, and a build heavy with slow-binding γ-glutamyl peptides can share a reading with a brighter one and still unfold over a wholly different few seconds — so we keep onset and persistence as panel notes beside the number, never folded into it. Potency is not efficacy: EC_{50} fixes where the climb reaches half, not how high the plateau finally stands, and two beans can match on the axis and part ways at the ceiling. The ferment, too, has failure modes a reservoir edit cannot fix — push proteolysis too far and the exopeptidases expose bitter hydrophobic peptides, so that past a point more unlocking *subtracts* from the savor it was meant to build; let the GAD drain run unchecked and the glutamate you banked leaves as GABA; stint the salt and the wrong microbes take the vat. None of these is loosened by a louder bean, and we do not pretend otherwise. The construct raises and steadies the starting material; the maker's craft still finishes the work.

Completely itself

We write this bean from the same conviction that runs under every book in this library, so we will state it plainly: the point was never to imitate a steak. It was to grow a plant toward the fullest savor it is capable of — a savor that happens to run deep enough, note and body together, that the imitation becomes beside the point. The soybean does not need to pretend to be anything. These are computational designs; Scoville Splice's constructs have not been experimentally validated in a laboratory. Grown right, unlocked right, held even across its two axes, it is completely itself, and that turns out to be plenty. Everything a savory kitchen does — every broth, every braise, every patient year of miso — is, in one way or another, a way of composing around what this one quiet plant already carries in its cells.



PART III OF V

The Multipliers & Engines

the fungal floor · koji · yeast

Chapter 9 · The Fungal Floor — *mushroom, shiitake, and the guanylate gift.*

ORGANISMS · Shiitake · 80 · Mushroom · 40 · primary axis: U

If the tomato and the soybean give us glutamate, the mushrooms give us the thing that *multiplies* it. Fungi are the second pillar of savor, and they bring a different molecule to the clamp: not glutamate but the 5′-ribonucleotides, and in the shiitake especially, **5′-guanylate** — the most potent of the three nucleotides, with a relative umami coefficient of 2.3 against inosinate's 1.0. On its own, guanylate is nearly silent; a tongue meets pure guanylate and reports almost nothing. Beside glutamate it is a thunderclap. It docks into the lip of the receptor's flytrap, pins the trap shut, and the glutamate suddenly reads many times louder than it could alone. This is the entire secret of a good dashi, and it is the whole reason the *dried* mushroom, not the fresh, is the prize.

What we love about this axis — and we will spend the chapter earning the claim — is that it is where the arithmetic of flavor stops being a metaphor. Sweetness and heat, for all their chemistry, are mostly dose-response: more compound, more signal, a curve that bends but never leaps. Savor is different. Savor *compounds*. Two molecules that each whisper will, together, shout, and the shout is not their sum but their product. We can write that product down. We can quantify it. And once it is written down, we can design a plant to land on whatever point of the curve a kitchen asks for.



The shiitake — the aristocrat of the fungal floor, carrying guanylate where other mushrooms carry mostly glutamate.

The flytrap, the lip, and the lock

To understand why guanylate multiplies rather than adds, you have to look at the receptor it works on. Savor is read by class-C G-protein-coupled receptors — the umami heterodimer **TAS1R1·TAS1R3** and a taste-tuned metabotropic glutamate receptor, **mGluR1**. Both share an architecture that earns its nickname honestly: the **Venus flytrap domain**, a large bilobed clamshell of protein hanging off the cell surface, two lobes joined at a hinge, open at rest and able to

snap closed around the right molecule. Glutamate is that molecule. It settles into the cleft deep between the two lobes, near the hinge, and its charge makes contacts that tip the clamshell toward its *closed* conformation. Closed is the active state. The closing travels down through a cysteine-rich stalk to the seven-helix bundle buried in the membrane, which turns and wakes a G-protein — gustducin — that fires a cascade through phospholipase C-β2 to release stored calcium, open the TRPM5 channel, and depolarize the cell. One molecule in a cleft, a few milliseconds later a nerve saying *savory.*

Here is the part that matters. The flytrap is not a simple switch; it is an equilibrium. At any instant some fraction of receptors sit open and some closed, and glutamate alone can only bias that fraction so far — raise its concentration and you fill more clefts, but each filled cleft still flickers between open and shut. The 5′-ribonucleotide changes the equilibrium itself. It does not compete with glutamate for the cleft. It binds its *own* site, out near the opening of the flytrap on the surface of the first lobe — the lip — where a cluster of basic residues is waiting for a phosphate. Once it latches there, the clamshell can no longer swing open as easily. The glutamate that was flickering is now *locked* in. The nucleotide is a latch on a trap the glutamate baited.

That geometry explains the numbers the tongue reports. Three features of the molecule earn its potency, and all three are structural:

- **The 5′-phosphate is non-negotiable.** Only the 5′ isomer activates; the 2′- and 3′-phosphate isomers of the very same nucleotide are inert, because only the 5′ geometry presents the phosphate to the lip's basic residues. This is not a footnote — it is the hinge of the whole chapter, because it means the *enzyme* a mushroom uses to liberate its nucleotides must be a 5′-specific one, or the savor it makes is silent.
- **The base sets the ceiling.** All three nucleotides share ribose and phosphate; they differ only in the purine ring. Guanine carries both a 6-oxo and a 2-amino group; hypoxanthine (inosinate's base) carries the 6-oxo alone; adenine carries a 6-amino and no oxo. The extra 2-amino group on guanine makes one more hydrogen bond at the lip — and that single additional contact is, to a good approximation, the physical meaning of the 2.3 against inosinate's 1.0.
- **It is cooperative, not additive.** Because the latch stabilizes the *closed* state that glutamate is trying to reach, the two molecules help each other. This is positive heterotropic allostery, and it is why the response is multiplicative. The mathematics of a latch is multiplication.

There is one more consequence of a latch, and it is quantitative. Because glutamate and the nucleotide stabilize the same closed state, the receptor's response to glutamate grows *steeper* in the nucleotide's presence — the dose-response curve stops behaving like a gentle dimmer and starts behaving like a switch, rising hard across a narrow band of concentration. For a cook that steepness is a gift and a hazard at once: a broth can cross from thin to complete

over a very small change in a pinch of dried mushroom, which is exactly why the dried-shiitake soak rewards a steady hand and punishes a careless one. The flytrap does not fade up. It snaps.

The arithmetic of synergy

Savor is the one axis where we can hand a cook an equation and have it come true in the pot. The synergy between glutamate and a 5′-nucleotide was measured carefully decades ago, and it takes a strikingly simple empirical form. Let u be the glutamate concentration and v the nucleotide concentration, both in grams per 100 mL. Then the perceived savor of the mixture equals that of glutamate *alone* at an equivalent concentration y, where

```
y = u · (1 + β · v)
```

For inosinate the synergy constant β is about **1,218**. Guanylate, carrying that extra contact at the lip, is the stronger latch; to a good approximation the constant scales with the nucleotide's potency, so for guanylate β ≈ 2.3 × 1,218 ≈ **2,800**. (We carry it as an approximation, because the potency ratio is measured at a reference condition and the real constant drifts a little with pH and total concentration — an honest caveat, not a hand-wave.)

Watch what that equation *says* before we put numbers in it. The nucleotide never appears alone; it only ever multiplies u. Set the glutamate to zero — a broth of pure guanylate and water — and $y = 0 \cdot (1 + \beta \cdot v) = 0$. The arithmetic itself reports that guanylate is silent without glutamate to amplify. The thunderclap needs the glutamate to strike it. (Real guanylate has a faint threshold of its own; the multiplicative form is what captures *why* it is nearly nothing.)

Now the worked case, which is simply a bowl of temple broth. A gentle kombu infusion carries free glutamate on the order of u = 0.02 g/100 mL — in molar terms, 0.02 g per 100 mL is 0.2 g/L, and divided by glutamic acid's 147 g/mol that is **1.36 mM**, a low-millimolar whisper, barely above glutamate's own detection threshold of roughly 0.01–0.03 g/100 mL. Add dried shiitake releasing guanylate to v = 0.005 g/100 mL — as the disodium salt, 0.05 g/L over 407 g/mol, about **0.12 mM**, 120 micromolar, a pinch. Then:

```
y = 0.02 · (1 + 2,800 × 0.005) = 0.02 · (1 + 14) = 0.02 · 15 =
0.30 g/100 mL glutamate-equivalent
```

A fifteen-fold amplification, bought with five thousandths of a gram of guanylate. The glutamate that was hovering at its own threshold now tastes like fifteen times as much pure MSG — a full, round, unmistakable broth — and nothing was added but a dried mushroom and patience. That factor of fifteen is the plant kingdom's whole case, written in one line.

Push either term and the bowl deepens predictably, which is the quiet gift of a law you can trust. Double only the guanylate to v = 0.01 g/100 mL, and y = 0.02 · (1 + 2,800 × 0.01) = 0.02 · 29 = **0.58 g/100 mL** — the synergy term, 2,800·v, so dominates the lonely "1" that the equivalent savor climbs almost in step with the pinch of

nucleotide, right up until the receptor begins to saturate and the line bends. A cook adjusting a dashi is riding that curve by taste; we get to read its coordinates, and a formulator gets to pick one.

5′-RIBONUCLEOTIDE	WHERE NATURE KEEPS IT	RELATIVE UMAMI COEFFICIENT
5′-guanylate (GMP)	dried shiitake, dried mushrooms	**2.3**
5′-inosinate (IMP)	bonito, meat, fish	1.0 *(reference)*
5′-adenylate (AMP)	shellfish, tomato, some vegetables	~0.18 *(lit.)*

We linger on the top row because it is quietly a thesis. The *most* potent savory nucleotide in the natural world is the one the plants and fungi keep. Inosinate is the animal molecule — flesh and fish — and it sits at 1.0, the baseline. Guanylate, the fungal molecule, sits above it at 2.3. A lifetime at a vegetarian table teaches you this by palate long before anyone shows you the coefficient: the kitchen was never missing anything. The arithmetic just confirms what the monks already knew.

Reading it on the Index

The Iyengar Flavor Index does not score how *much* glutamate or guanylate a food holds. It scores how *loudly* the food speaks to the receptor, through a single relation:

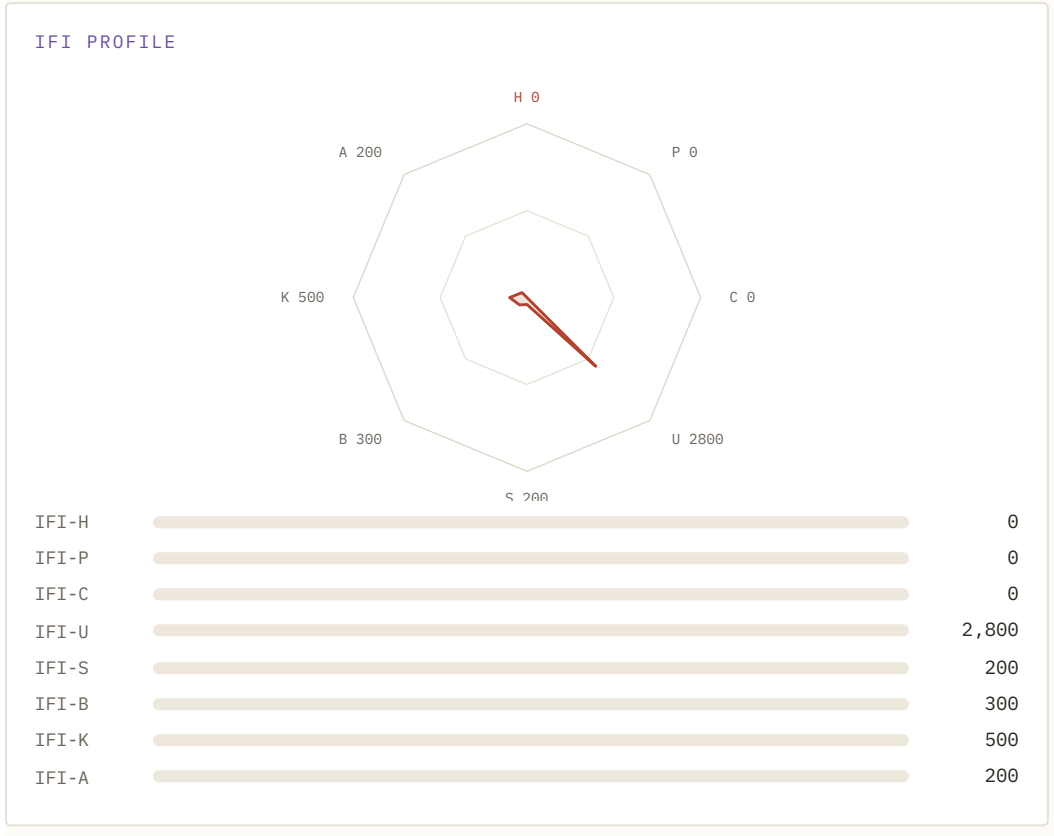
$$\mathrm{IFI}(\text{candidate}) = 1{,}000 \times \mathrm{EC}_{50}(\text{reference}) \div \mathrm{EC}_{50}(\text{candidate})$$

EC_{50} is the concentration at which a stimulus drives the receptor to half its maximal response — the lower the EC_{50}, the more potent the stimulus, the louder the signal. The reference constant is set by stated convention so that the wild-type anchor reads **1,000** on each axis — the same logic as the Scoville scale's dilution-to-threshold definition. The formula inverts cleanly, which is what makes it useful: an IFI score *is* a potency ratio in disguise. A food that scores 2,800 has, by definition, an EC_{50} that is 2.8 times lower than the reference — it reaches half-activation at 1 ÷ 2.8 = 0.36 of the reference concentration. You can read the loudness straight off the number and back-calculate the dose it takes to get there.

STIMULUS	IFI-U	EC_{50}(REF) ÷ EC_{50}	CONC. FOR HALF-ACTIVATION
Reference anchor	1,000	1.00	1.00×
Wild tomato	300	0.30	3.33×
Phoenix · SS-0096 *(U axis, predicted)*	1,800	1.80	0.56×
Restored tomato	2,600	2.60	0.38×
Engineered shiitake *(predicted)*	**2,800**	**2.80**	**0.36×**

Read the last row against the first. A reference stimulus needs a full unit of concentration to half-fill the receptor; our model predicts our engineered shiitake needs barely a third of it. Read it against the wild tomato, which has to pile on 3.3 times the reference dose to reach the same half-activation — a tenfold spread in required concentration, bottom to top of the ladder, all of it the same receptor, all of it one number. And note where the fungal floor lands relative to the flagship superhot: Phoenix, the cultivar built to register on all eight axes at once, is designed for 1,800 on umami. The engineered

shiitake, built to do one thing, out-savors it. Depth is not a consolation prize for plants that cannot do heat. It is a specialty, and the shiitake is its master.



The drying — a mushroom cooking its own savor loose

The drying is lovely biochemistry, and worth drawing because it is the mushroom cooking its own savor loose. A fresh shiitake holds very little free guanylate; what it holds instead is **RNA** — ribonucleic acid, the cell's working copy of its genes, present in quantity in any living tissue. The savor is there, but it is locked in long polymer strands that the receptor cannot read. The drying, and then the rehydration, is a two-enzyme disassembly line that cuts those strands down to the single 5′-nucleotides the lip of the flytrap is waiting for.

Two of the mushroom's own enzymes run the line, in sequence, as the broth comes up to temperature. A **ribonuclease** cleaves the long RNA strands into short fragments. Then a **5′-phosphodiesterase** trims those fragments down to individual nucleotides — and it is specifically a *5′*-phosphodiesterase, which is the whole game, because only the 5′-monophosphate activates the receptor. An enzyme that left the phosphate on the 3′ carbon would liberate exactly as much nucleotide and produce a silent broth. The mushroom's machinery is not just releasing savor; it is releasing it in the one isomer the tongue can hear.

The drying *stages* the machinery; the rehydration and gentle heat *run* it — and that split is the cook's entire leverage. The enzymes have a working window. They are most active somewhere in the warm band roughly between 50 and 70 °C, and they denature — unfold, and stop working forever — not far above it. So the technique that every patient stock has arrived at is the correct one: soak the dried mushroom in *cool* water first, letting the ribonuclease begin its cuts while the enzyme is still intact, then bring the pot up *slowly*, giving the 5′-phosphodiesterase its warm window to finish before the rising heat kills it. Push the water too hot too fast and you denature the enzymes before they have done their work; the RNA

stays locked, and a mushroom that could have given 150 milligrams of guanylate gives a tenth of that. The savor was never missing. It was cooked to death in the first thirty seconds.

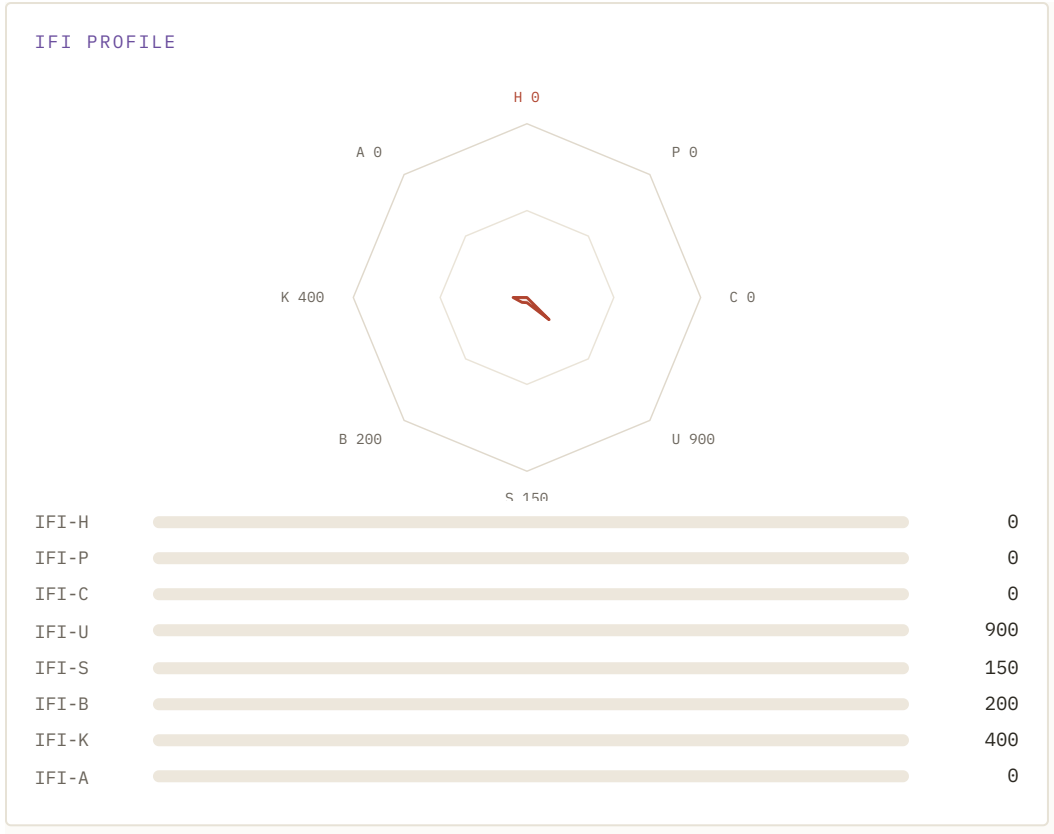
SHIITAKE, BY PREPARATION	FREE GMP (MG/100 G)	× OVER FRESH	STAGE OF THE CASCADE
Fresh	~0–5	1×	guanylate still locked in RNA
Dried, raw	~10–20	~3×	some liberation on drying
Dried, soaked cold	~40–60	~10×	ribonuclease begins to cut
Dried, rehydrated, gently heated	**~140–150**	**~30×**	ribonuclease + 5′-phosphodiesterase run to completion

Thirtyfold, in the same mushroom, decided by nothing but handling. This is also the honest reason a seared *fresh* mushroom and a soaked *dried* one taste like different ingredients. The fresh pan is glutamate-forward — the heat drives off water and concentrates the free glutamate already present, and the fierce heat denatures the nucleotide enzymes before they release much guanylate. The dried soak is guanylate-forward — low and slow, the enzymes given room to work. One mushroom, two molecules, two methods, and a cook who knows which lever each one pulls.

The forest beyond the shiitake

The forest is wider than the shiitake, and each edible fungus brings a slightly different savory signature we can compose with. The **porcini**, or cep, is prized for a deep glutamate and a roasted aroma that survives drying beautifully. **Enoki** and **maitake** carry their own nucleotide loads. The common button and cremini **mushroom** give glutamate with a gentle body and the everyday savor of a seared pan, with a softer nucleotide note than the shiitake's. The **oyster** brings a round, almost seafood-like softness that belongs to no animal at all. We hold the engineered shiitake and the engineered culinary mushroom as our two developed lines — eighty constructs on the one, forty on the other — but the method would transfer to any fungus with a sequenced genome, which is the standing invitation of this whole library.

The culinary mushroom is designed to a different point on the ladder than the shiitake, on purpose. Where the shiitake is a guanylate specialist that our model places near the top of any savory build, the mushroom is a generalist: more glutamate, a little kokumi body from its cell-wall peptides, a gentler nucleotide note — the savor of an everyday pan rather than a temple pot. A kitchen reaches for the shiitake when it wants the thunderclap and the mushroom when it wants the floor under everything else.



A nourishment note, kept quiet as always on this axis: many of these fungi are among the richest dietary sources of **ergothioneine**, an unusual sulfur amino acid the body takes up through a dedicated transporter and conserves with real care, holding onto it in the tissues under oxidative stress. The mushroom carries it in the very same flesh that carries the savor. We make no claim for it — none is ours to make — we only observe, again, that the fungus grown toward its fullest flavor is so often the one grown toward its fullest substance, the two ripening in the same tissue, as though nature never meant to separate the pleasure of a food from the good of it.

The complete vegetarian broth

For a vegetarian kitchen, this chapter is quietly everything, and it is where our plant-first default stops being an ethic and becomes a technical fact. The most famous savory synergy in cooking is the dashi of the sea — kombu's glutamate meeting the inosinate of dried bonito, kelp and fish, the twin pillars of Japanese stock. But that synergy has a complete *plant* twin that is every bit its equal: **kombu and dried shiitake** — glutamate meeting guanylate — a broth with no animal in it and nothing whatsoever missing from it. Run it back through the equation. The sea's dashi pairs glutamate with inosinate at $\beta \approx 1{,}218$. The temple's dashi pairs the same glutamate with guanylate at $\beta \approx 2{,}800$. The plant broth is not a substitute reaching for parity; by the coefficient, its latch is the *stronger* one.



The soybean — the other plant pillar of savor, carrying the glutamate that the shiitake's guanylate multiplies.

We did not invent that pairing; cooks found it centuries ago for the temple kitchens that kept no fish. There is a whole cuisine built on exactly this plant-twin synergy, and it is worth honoring, because it proves the point long before we arrived to quantify it. *Shōjin ryōri*, the temple cooking of Japanese Buddhist monasteries, keeps no fish and no meat — and yet it is among the most profoundly savory food traditions on earth, because the monks who developed it understood, by palate and by centuries of practice, that kombu and dried shiitake together give a stock that wants for nothing. They could not have told you about the lip site of the flytrap or the constant β. They did not need to. They had found, empirically, the complete vegetarian synergy, and they built a kitchen on it that has fed austerity as abundance for a thousand years. When we say this book is vegetarian-first by default, we are not announcing a trend. We are standing in a very old line of cooks who proved that the deepest savor never required an animal — and offering them a mushroom that holds up its half of the bargain more reliably than any forest ever could.

Consistency is the product

That word — *reliably* — is where engineering enters, and the reason is the very multiplication we have been celebrating. A multiplicative response is wonderful when both inputs are high and treacherous when either one wanders, because the error multiplies too. When savor is the product of two terms, savor's uncertainty is governed by the uncertainty of *both*. For a product of two independent quantities, the fractional errors combine in quadrature:

> `CV(savor) ≈ √( CV(glutamate)² + CV(guanylate)² )`

Put a wild forage through it. A foraged mushroom's free-guanylate pool swings thirtyfold on handling alone, as the table above shows, and its glutamate drifts with season and soil; take a conservative run-to-run variation of 30% on each. Then CV(savor) ≈ $\sqrt{(0.30^2 + 0.30^2)} = \sqrt{0.18} \approx$ **42%** — nearly half, batch to batch, in the one attribute you were buying the mushroom for. Now suppose a careful formulator fixes the glutamate to a tight ≤7% but leaves the nucleotide wild: $\sqrt{(0.07^2 + 0.30^2)} = \sqrt{0.0949} \approx$ **31%**. Almost no improvement — because the product is only as steady as its wobbliest term, and controlling one pillar while the other swings

buys you almost nothing. You have to hold *both*. Engineer the glutamate line and the guanylate line each to our standard ≤7%, and $\sqrt{(0.07^2 + 0.07^2)} \approx$ **10%**. From 42% to 10%, and the only way there was through both pillars at once.



Reading the genome at the bench — the sequenced fungus is where the ribonuclease and the 5′-phosphodiesterase become addressable.

So that is what we tune for, across both the common culinary **mushroom** and the **shiitake**: not one number but a whole small system. We raise the stored nucleotide precursor pool, and we sharpen the two enzymes that release it — more ribonuclease to cut, more 5′-phosphodiesterase to finish, each dialed so the warm-window release runs to completion in a normal pot rather than a laboratory. The fungal floor a kitchen stands on comes out louder *and* more reliable than any forest ever guaranteed: the engineered shiitake designed to reach IFI-U 2,800, the mushroom at a predicted 900, both held to ≤7% batch to batch so a maker can *specify* the savor of a sauce the way they specify its salt — a figure on a sheet, not a hope about a harvest.

The sear, and the honest limits

A word on the sear, since it belongs to the fungi as much as to any meat. A mushroom cooked hard and dry in a hot pan does more than brown. Driving off the water concentrates the glutamate that is already there, and the **Maillard reaction** — the cascade of browning chemistry between amino acids and reducing sugars at high heat — lays down still more savory compounds and the roasted aromas that read, unmistakably, as *depth*. The kitchen raises IFI-U before a single engineered gram is counted. We design the mushroom to start higher so the sear lands it higher still — a plant tuned at the gene and then finished at the stove, which is exactly how we think savor should be built: in the plant first, in the pan last, with nothing dusted on in between.

And then the honesty the number demands of us. IFI-U is receptor loudness and nothing more — it is how hard the flytrap closes, not how a mushroom *feels* in the mouth or *smells* across a kitchen. The roasted aroma of a dried porcini, the slip of an oyster mushroom, the way a shiitake's body coats a broth — those live partly on the kokumi axis and partly in the nose, in volatile chemistry the umami score was never built to capture. A number this clean is powerful precisely because it is narrow; mistake it for the whole of savor and it will mislead you. We also hold the multiplicative curve at arm's length past the point where the receptor saturates — pile on nucleotide

forever and the β relation flattens, because a trap that is already shut cannot shut harder. These are computational designs; Scoville Splice's cultivars and constructs have not been experimentally validated in a laboratory. These are the edges of an honest tool. Inside them, the fungal floor is the most quietly complete thing on the vegetarian table: two molecules, one latch, a factor of fifteen, and a thousand years of cooks who tasted the arithmetic before anyone wrote it down.

Chapter 10 · Koji — *the mold that makes savor.*

ORGANISM · Koji Mold · 80 constructs · primary axis: U · difficulty: Hard

Koji is not a flavor so much as a machine for making one. It is a mold — *Aspergillus oryzae,* the quiet, ancient fungus at the root of miso, of soy sauce, of sake and amazake and mirin, named the national fungus of Japan for the civilization it helped feed — and its genius is entirely enzymatic. Given a bed of cooked grain or soybean, koji colonizes it over a few warm days and floods it with a catalogue of secreted enzymes that go to work on everything the substrate holds. It is, in the most literal sense, a savory engine you can keep on a shelf: it does not *taste* like much on its own, but it *does,* to everything it touches, exactly what this axis is about. It sets glutamate free.

We came to koji the way we came to all of it — as a vegetarian family looking for depth we would never take from a bone. Most kitchens reach for savor through animals: the stock simmered from a carcass, the anchovy melted into a sauce, the fish pressed into brine. Koji is the oldest proof that the depth was never in the animal at all. It is in the *free amino acids*, and a mold can liberate them from a soybean as readily as a cauldron can boil them from a bird — more cleanly, more measurably, and without a single life spent. That is why, of all eighty constructs on this organism, we hold koji with a particular tenderness. It is the engine that lets a plant kitchen taste *complete.*



Koji mold — Aspergillus oryzae — the enzymatic engine beneath miso, shoyu, and sake, prized not for its own flavor but for what it does to everything else.

The axis koji was built to speak

To understand why koji is the loudest single source of savor on our floor, start with the receptor it is shouting into. Umami is read by two proteins working at different scales: **mGluR1**, a metabotropic glutamate receptor with exquisite, micromolar sensitivity, and the **TAS1R1·TAS1R3** heterodimer, the frank taste receptor of the tongue, which answers at millimolar concentrations but carries most of what you consciously register as *savory.* Both are class C G-protein-

coupled receptors, and both share one beautiful piece of machinery: a large extracellular domain shaped like a Venus flytrap, two lobes hinged around a cleft.

Free glutamate — the carboxylate-bearing amino acid, not its amide cousin — settles into that cleft and pulls the two lobes shut. The closure is the signal. It propagates down through a cysteine-rich linker into the seven-transmembrane bundle, which rolls against its bound G-protein and lets go of it; in a taste cell the cascade runs α-gustducin → phospholipase C-β2 → inositol trisphosphate → a pulse of released calcium → the opening of the TRPM5 channel → depolarization → a squirt of ATP through CALHM1 onto the waiting afferent nerve. A molecule you cannot see becomes a current you cannot ignore. The whole relay repeats a billion times a second across the surface of the tongue, and the sensation we call *brothy, round, satisfying* is the sum of it.

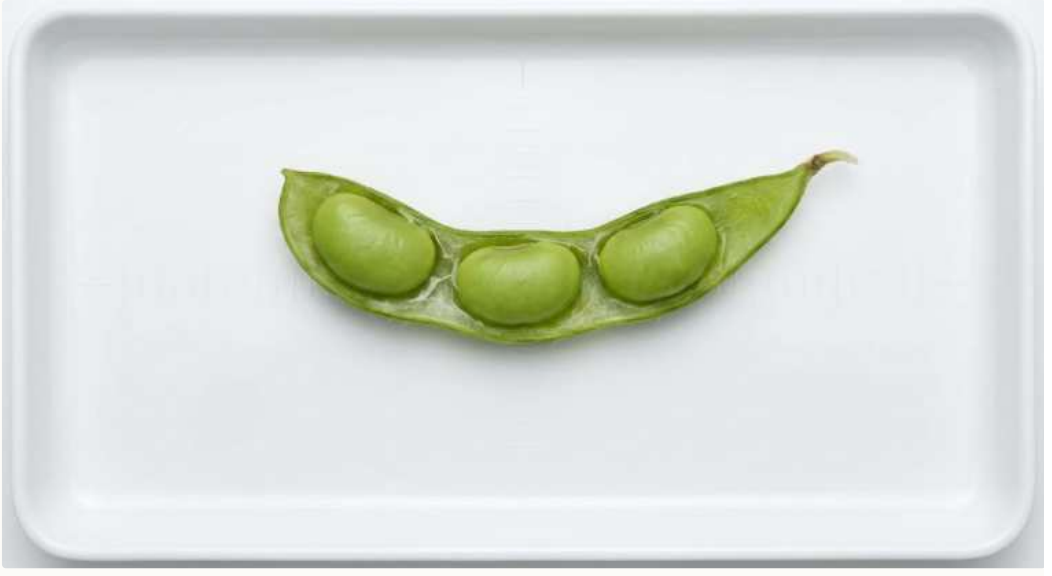
Two features of that flytrap matter for everything a koji does. The first is **cooperativity**: because the mature receptor is a dimer, the closure of one subunit biases the other, so the dose-response curve is steeper than a simple one-to-one binding would give — a small change in glutamate near the half-point swings the receptor hard from quiet to loud. The second is **synergy**. A separate pocket on the flytrap, just outside the glutamate site, binds the 5′-ribonucleotides — inosinate (IMP) and guanylate (GMP). When a ribonucleotide docks there it acts as a positive allosteric modulator: it wedges the flytrap toward its closed shape and can lower glutamate's half-activating concentration severalfold. This is the ancient secret of dashi, of a tomato cooked with a mushroom, of parmesan on a ragù — two weak savory signals multiplying into one enormous one. Koji sits at the center of this story because it does not merely deliver glutamate; through its proteolytic and nucleolytic work it can set the partners free in the same jar.

A secretome read as an arsenal

A fungus does not secrete enzymes to flavor our food. It secretes them to eat — to dismantle a substrate too large to absorb into molecules small enough to pull across its cell wall. Koji's gift to a cook is that we can arrest the process partway and keep the intermediate: a bed of grain or bean half-digested into a broth of free amino acids and sugars, caught at the moment it is most delicious and most nourishing. Reading the secretome as an arsenal is the clearest way to see the engine.

ENZYME CLASS	MODE	WHAT IT CLEAVES	FLAVOR CONSEQUENCE
Alkaline · neutral · acid proteases	endopeptidase	internal peptide bonds of storage proteins	body, peptides, the precursor pool for savor
Aminopeptidase · carboxypeptidase	exopeptidase	terminal residues off peptide ends	free amino acids; removes bitter C-terminal hydrophobes
Glutaminase	amidohydrolase	the side-chain amide of glutamine	**glutamine → glutamate: the umami unlock**
α-amylase	endo-glycosidase	internal α-1,4 bonds of starch	dextrins; thins the mash
Glucoamylase	exo-glycosidase	glucose off the dextrin ends	gentle, sugar-free sweetness
5′-phosphodiesterase · nuclease	nucleolytic	RNA into 5′-ribonucleotides	IMP/GMP — the synergy partners

The proteases come graded — alkaline, neutral, and acid, each a different fold cutting under a different pH, between them tearing the soybean's glycinin and β-conglycinin, or a grain's prolamins and glutelins, into a rubble of peptides. That rubble is only potential. It is the exopeptidases that finish the work, the aminopeptidases nibbling residues off the N-terminal ends and the carboxypeptidases off the C-terminal ends, each snip releasing a single free amino acid into solution. And a free amino acid is the whole point, because only a *free* glutamate — unchained from its neighbors, its α-amino and α-carboxyl groups exposed — fits the flytrap. A glutamate still buried mid-chain in a protein is mute. The arsenal's job, reduced to a sentence, is to convert mute protein into articulate solute.



Soybean cross-section — glycinin and β-conglycinin, the storage proteins koji's graded proteases dismantle into the free-amino-acid broth that becomes miso and shoyu.

Glutaminase — the enzyme that turns silence into savor

Of everything koji secretes, one enzyme decides whether a ferment is flat or profound, and it is the one most easily overlooked. Much of the nitrogen in any protein substrate is carried not as glutamate but as its amide, **glutamine** — the same backbone, but with the side-chain acid group capped as an amide ($–CONH_2$ instead of $–COO^-$). That single capped group is the difference between loud and silent.

The flytrap of the umami receptor reads the free carboxylate; cap it, and the molecule all but disappears from the tongue. A substrate can be dense with glutamine and taste of almost nothing.

Glutaminase hydrolyzes the cap. It cleaves the amide, releasing ammonia and leaving behind free glutamate — converting a reservoir of *potential* savor that proteolysis alone would leave locked. The arithmetic of a great ferment is largely the arithmetic of this one conversion: how much of the substrate's bound glutamine the mold's glutaminase manages to unmask before the batch is stopped. Two koji beds can carry identical total nitrogen, be worked by identical proteases, and taste a world apart, because one ran a glutaminase that unmasked eighty percent of its glutamine and the other unmasked thirty. When we say we *lift the glutaminase* of a strain, this is the lever we mean — not more protein broken, but more of what is already broken made audible. It is the cheapest, largest gain in savor available to anyone who ferments, and for most of history no one could see it, because glutamine and glutamate are invisibly similar on the plate and separated only on the tongue and in the assay.

One mold, a whole pantry

The same mold, pointed at different substrates and finished in different ways, builds most of the savory pantry of East Asia — the clearest demonstration that koji is an *engine,* not an ingredient. Change what you feed it, and how long and how salty you let it run, and the one organism yields a dozen staples.

SUBSTRATE → FERMENT	PRODUCT	DOMINANT ENZYME WORK	AXIS IT LIFTS
Rice koji + steamed rice	amazake, sake mash	amylases → glucose	S (sweet), mild U
Rice koji + soybean + salt	miso	proteases + glutaminase → glutamate	**U**, K (body)
Soy + wheat koji + brine	shoyu (soy sauce)	deep proteolysis + glutaminase	**U** — the loudest everyday savor
Rice koji + barley + salt	barley miso, shochu mash	mixed starch + protein liberation	U, S
Koji + vegetable or bean curd	shio-koji cures	surface proteases → quick savor	U, K

There is a family of koji molds, and the strain is chosen for the job. The workhorse *Aspergillus oryzae* carries miso, shoyu, and sake, bred over centuries into sub-strains that favor protease (for savor) or amylase (for sweetness); its acid-producing cousin *Aspergillus luchuensis,* the black and white koji of Okinawan awamori and of shochu, floods its substrate with **citric acid** to hold spoilage at bay in the island heat. When we tune an engineered koji we tune *within* this inherited diversity — lifting the glutaminase of a shoyu strain, steadying the protease-to-amylase ratio of a miso strain — rather than inventing a mold from nothing. We sequence the sub-strains, read which secreted-enzyme genes the centuries already selected, and sharpen what is there. The breeders of a thousand years did the hard search; we sharpen and stabilize what they handed us, and we are careful to say so.

That it also turns *sweet* is the amylases' doing, and worth pausing on, because it is savor and sweetness braided in one organism. α-amylase chops the substrate's starch into dextrins; glucoamylase walks the dextrin ends releasing glucose, one unit at a time. This is why amazake — koji and rice, nothing else — tastes of mild sugar with no sugar added, and why a koji ferment turns not only savory but gently, roundly sweet. For a family that has spent a lifetime looking for sweetness and depth without reaching for refined sugar or animal stock, a single mold that frees glucose and glutamate from the same humble grain is close to a miracle, and we have never quite stopped treating it as one.



Rice koji on steamed rice — the amylases that free glucose from starch, turning a savory ferment gently, sugarlessly sweet.

Putting a number on savor

Reverence is where we begin; a number is how we keep faith with it. The Index exists so that *savory* stops being a word we argue about and becomes a quantity we can read, compare, and design toward. Every axis is anchored the same way — to the half-maximal activation of its receptor, the concentration at which a stimulus drives the receptor to half of its full response.

> $\mathrm{IFI(candidate)} = 1{,}000 \times \mathrm{EC_{50}(reference)} \div \mathrm{EC_{50}(candidate)}$

Read it slowly. EC_{50} is a concentration, and a *lower* one means a *more potent* stimulus — less of it needed to half-open the receptor. Put the reference's EC_{50} on top and the candidate's underneath, and the ratio inverts potency into loudness: the more potent the candidate, the smaller its EC_{50}, the larger the score. Scale by a thousand so a tomato, a chili, and a cup of broth can be read on one page. By convention, we set the umami axis anchor at free glutamate activating mGluR1 near **10 µM** — a concentration consistent with the receptor's well-characterized sensitivity in the literature — and fix that anchor at **IFI-U 1,000** by definition. Because the Index is a ratio, the anchor's exact value washes out of every comparison; we quote 10 µM only to make the micromolar world concrete.

Now work koji. Our model predicts an engineered koji at **IFI-U 3,200.** Rearrange the formula for the candidate's EC_{50}:

> $\mathrm{EC_{50}(koji)} = \mathrm{EC_{50}(ref)} \times 1{,}000 \div \mathrm{IFI} = 10\ \mu\mathrm{M} \times 1{,}000 \div 3{,}200 =$ **3.13 µM**

Koji's liberated glutamate reaches half-activation at about 3.1 micromolar — **3.2× more potent than the anchor.** The same arithmetic lets us lay savory sources on a single ladder, each organism's canonical IFI-U converted back into the EC_{50} it implies and the multiple of the anchor it represents.

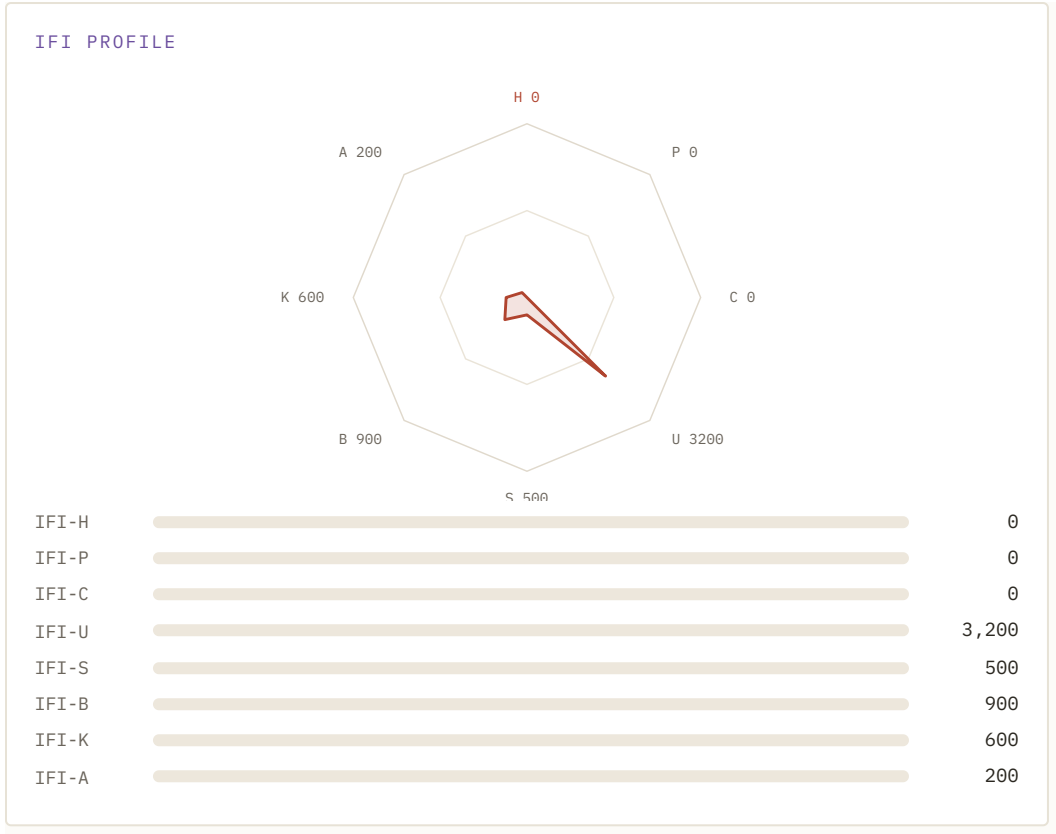
SOURCE	IFI-U	IMPLIED EC_{50} (MM)	× ANCHOR
Reference anchor (glutamate at mGluR1)	1,000	10.00	1.0×
Tomato, truck-ripened (depleted)	300	33.33	0.3×
Phoenix · SS-0096 (full-spectrum flagship)	1,800	5.56	1.8×
Tomato, restored	2,600	3.85	2.6×
Koji, engineered	**3,200**	**3.13**	**3.2×**

The ladder tells a whole story at a glance. A tomato that rode a refrigerated truck for a week reads *below* the anchor — its umami machinery idling at 0.3×, savor that proteolysis in the field never finished. Restore that tomato through its native glutamate route and it climbs to 2.6×, an eight-and-a-half-fold gain in potency (2,600 ÷ 300 = 8.7). And koji, the humble mold, tops the ladder at 3.2× — the loudest single source of savor on our whole floor, higher than any plant we have engineered, because it does not merely *store* glutamate the way a fruit does but *generates* it, an engine out-performing any warehouse.

The receptor's steepness is what makes the reading sharp. Any stimulus at a cooperative receptor follows a Hill relationship:

$$R = R_{max} \times [C]^n \div (EC_{50}^n + [C]^n)$$

where [C] is the glutamate concentration, EC_{50} the half-point, and n the Hill coefficient — the cooperativity of those twinned flytraps, typically between 1.5 and 2 for a class C dimer. With n above one the curve is sigmoidal and steep near the middle: double the free glutamate from a quarter of EC_{50} to a half and the receptor's response more than doubles. In plain kitchen terms, savor does not rise gently and forgivingly with fermentation; it climbs a cliff near the half-point. That steepness is a gift to the designer and a trap for the careless, and it is the real reason the art of koji is the art of where you stop.

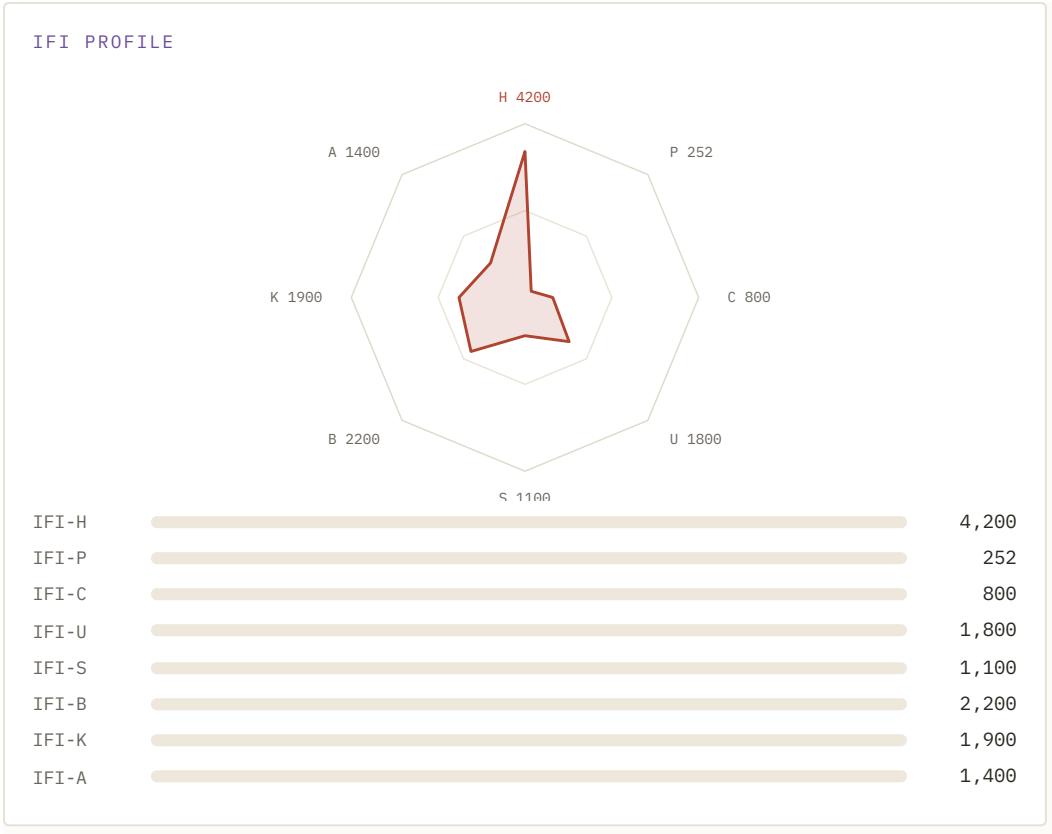


A word on units, and an honest gap worth naming. Convert the anchor to mass — 10 µM of glutamate, molar mass 147.13 g/mol, is 10 × 10^{-6} × 147.13 ≈ **1.47 mg/L** — and koji's 3.13 µM is about **0.46 mg/L** at the receptor. Yet the glutamate you can *consciously taste* in a mouthful sits near a 1 mM recognition threshold, roughly **150 mg/L** — two to three orders of magnitude higher. The gap is real and we do not paper over it: mGluR1 is the high-resolution sensor the Index reads for precision, while the frank tongue receptor TAS1R1·TAS1R3 answers only at millimolar levels, diluted further by saliva and crowded by every other molecule in the bite. We anchor the *measurement* on the sensitive receptor because it resolves differences the tongue would blur, then check the *experience* against the coarse one. A koji that scores 3,200 on the instrument is one a mouth will read as deeply, roundly savory — but the two readings live on different scales, and pretending otherwise would be dishonest about what the number is.

Finally, synergy, which the number cannot fully hold. The 5′-ribonucleotides that koji's nucleases set free — and those an ingredient brings to the pot — dock the flytrap's second pocket and multiply glutamate's potency. A conservative eightfold potentiation turns koji's effective EC_{50} from 3.13 µM toward 0.39 µM, which the formula would render as an implied IFI near **25,600** — far past the Index's per-axis normalization ceiling of **5,000.** This is one honest reason every scorecard caps each axis at 5,000: a koji broth finished with a ribonucleotide-rich ingredient can *imply* a loudness the scale was never meant to print, so we hold the single-source construct to its true 3,200 and let the synergy live in the dish, where it belongs.

The engine out-savors the flagship

It is worth standing koji beside the most engineered plant we have ever grown, because the comparison makes the engine's nature plain. **Phoenix** — SS-0096, predicted at approximately 2.95 million SHU, the first cultivar designed to register on all eight axes at once — is the summit of our plant work. It carries heat no wild pepper could reach and speaks on every axis of the Index. And yet, read only the umami column.



Phoenix's predicted umami scores **IFI-U 1,800.** Our koji design is **3,200.** The most ambitious plant in the library, tuned gene by gene to glow on every axis, is out-savored on this single one by a mold that grows on a bed of rice in a warm cupboard — koji at 3.2× the anchor against the flagship's 1.8×, nearly twice as loud. There is no contradiction in it, only a lesson about kinds. Phoenix *holds* flavor the way a fruit holds it, every axis stored in the tissue of a living pepper. Koji *makes* it, converting a dull substrate into free glutamate in real time. When the job is raw savory depth and nothing else, the generating engine beats the storing warehouse, every time. We love the flagship for its impossible completeness. We keep koji for the one thing it does better than anything else alive.

Designing a batch that runs true

If glutaminase is the enzyme that makes koji *powerful,* consistency is the discipline that makes it *useful,* and consistency is a number too. We measure it as the coefficient of variation — the batch-to-batch standard deviation divided by the mean, as a percent:

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CV = σ ÷ μ × 100%
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A wild ferment is a lovely, unreliable thing. Its savor rides a seasonal substrate, an ambient temperature, a wild balance of secreted enzymes, and a mold population no one selected for evenness — and it behaves much as our wild-type pepper did before we steadied it, with a batch CV in the low forties. The deep structural reason is that koji's output is a *cascade,* not a single step: protease activity, then exopeptidase activity, then glutaminase conversion, three stages multiplied one into the next. When independent multiplicative steps each wobble, their relative variances add in quadrature —

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CV_total ≈ √(CV₁² + CV₂² + CV₃²)
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— so three stages each wobbling at about 24% compound to √(0.24² × 3) ≈ **42%.** That is the wild ferment: not one sloppy step, but three ordinary ones multiplying their noise. The engineering

target follows straight from the arithmetic. To hold the whole cascade at our standard **≤7% CV,** each stage must be steadied to roughly 4%, because $\sqrt{(0.04^2 \times 3)} \approx$ **6.9%.** The eighty constructs on this organism are, in large part, eighty answers to that equation — tightening the expression and stability of each secreted enzyme so the stage it governs stops wandering.

SCENARIO	PER-STEP CV	CASCADE CV	IFI-U 3,200 AT ±1Σ
Wild ferment (3 enzyme stages)	~24%	~42%	1,856 – 4,544
Engineered koji (3 enzyme stages)	~4%	~7%	2,976 – 3,424

Read the right-hand column as a cook would feel it. At the wild ferment's 42%, a koji that *averages* 3,200 delivers anywhere from a thin 1,856 to a blaring 4,544 from one batch to the next — the difference between a sauce that disappears and one that shouts, with no way to know which jar you opened until it is on the plate. Steadied to 7%, the same average stays inside a narrow 2,976–3,424 band: savor you can formulate around, scale, and trust from the first jar to the ten-thousandth. The gain is not louder savor. It is *knowable* savor — and for anyone building a recipe, a product, or a meal they want to taste the same every time, knowable is the whole game.

Where it goes wrong — and where we stop

An engine this powerful fails in characteristic ways, and honesty about them is part of designing it well. The first failure is **over-proteolysis.** Push the proteases hard and long without the exopeptidases keeping pace, and the broth fills with mid-length hydrophobic peptides — short chains rich in leucine, phenylalanine, proline, and tryptophan, liberated from the water-shy interiors of the storage proteins. These are exactly the peptides the bitter receptors of the TAS2R family are tuned to catch. A ferment driven too far does not merely get stronger; it goes *bitter,* then sharp, then ammoniacal as glutaminase and deamination flood the system with free ammonia. All shout, no shape. The carboxypeptidases are the quiet heroes here: by trimming hydrophobic residues off the peptide ends they debitter the broth even as the aminopeptidases sweeten it with free amino acids. Tuning that balance is why koji's design holds the zero-bitter axis at a respectable **900** — savor kept clean rather than blurred, the bitter peptides held down rather than let run.

The second failure is simpler and more final: **heat kills it.** Koji's power is *alive.* The proteases, the glutaminase, the amylases are all proteins, folded into the precise shapes their chemistry depends on, and like all proteins they denature above a threshold — the folded structure unravels, the active site deforms, and the catalysis stops, irreversibly. The rates are steep: enzyme activity roughly doubles with each 10 °C of warming up to the optimum, then collapses within a few degrees past it as denaturation overtakes catalysis. This is why the great ferments are run *warm but not hot,* a patient window near 30 to 60 °C that works the enzymes hard without destroying them, and why fresh koji and living pastes like a young miso are added to a dish *off the heat* — stirred in at the very end, so the enzymes survive to keep working on the plate and in the body rather than being boiled to silence in the pot. A cook who treats koji like a

flavor, boiling it in with everything else, gets a fraction of what it offered. A cook who treats it like the living engine it is gets the whole of it. Every koji preparation we write adds it last and off the flame, for exactly this reason.

And so the engine returns us to the restraint the whole axis teaches. Koji can run savor up *fast* — the Hill curve is steep, the glutaminase is patient, the arsenal is complete — and the art is entirely in where you stop. We design the mold the way we design a heat: for the *shape* of the result, not merely the height of it, tuning not only how much glutamate the koji frees but how cleanly it frees it. A cured vegetable or a young miso built on a well-tuned koji should arrive rounded and whole, not blaring — savor you can taste *through,* which is the only savor that truly feeds you. Savor that only shouts is just a different kind of empty, and the koji, left undesigned, is the organism most likely to shout. Designed — steadied, debittered, held just short of the cliff — it is the most generous engine in the library, and the one our family would set on the table first. These are computational designs; Scoville Splice's cultivars and constructs have not been experimentally validated in a laboratory.

Chapter 11 · Yeast — *the savory hum, both halves in one cell.*

ORGANISM · Yeast · 240 constructs · primary axes: U / K · difficulty: Hard

Yeast is the clean-label workhorse of the savory axis, and the most quietly useful organism on the whole floor — **240 constructs,** the most-developed dedicated savory host after the tomato, because a maker who needs depth in a bottle or a powder reaches here first. A yeast cell, *Saccharomyces cerevisiae,* broken open and allowed to digest itself — **autolyzed,** in the trade's blunt word, its own enzymes turned loose on its own contents — spills a nearly complete savory package in a single ingredient. This is the deep brown hum under a good gravy, the backbone of the beloved dark spreads, the reason a pinch of yeast extract can make a soup taste as though it simmered twice as long. It is savor you can weigh out of a jar, and for an industrial maker that convenience is not a small thing. We came to the organism as lifelong vegetarians who have leaned on it at our own table for a lifetime — the flaked tin on the shelf, the dark spread on toast — and stayed because the cell turned out to hold, in one tissue, the entire mathematics of the savory axis: a molecule, a receptor, a second molecule that multiplies the first, and a number that lets you read the whole ensemble.



Baker's yeast — Saccharomyces cerevisiae — which carries both halves of savory depth in a single cell, glutamate and the nucleotides that multiply it.

Both halves in one cell — the note and the multiplier

What makes yeast singular is that it carries *both* halves of the savory synergy inside one cell. Umami, read honestly, is not a single thing but a pair that becomes far more than its sum: a **note** and a **multiplier.** The note is **free glutamate** — the molecule the tongue reads as savory itself, the loud center of a good broth. The multiplier is a family of **5′-ribonucleotides** — chiefly **5′-inosinate (IMP)** and **5′-guanylate (GMP)** — which, tasted alone, are nearly mute, but which

in the presence of glutamate do something the receptor rewards out of all proportion: they make the glutamate taste many times louder than the same glutamate would taste by itself.

A plant or a protein usually offers one half of that pair. A ripe tomato is glutamate-rich but nucleotide-poor; a dried fish or a cured ham carries the nucleotides but leans on an added glutamate to sing. Cooks have spent centuries marrying the two halves across a pot — kombu to bonito, tomato to anchovy, mushroom to meat — because neither half, alone, is the whole of savor. Yeast skips the marriage. A good yeast autolysate is rich in free glutamate *and* in the 5′-nucleotides that multiply it, the latter released from the yeast's own abundant RNA by the cell's own machinery, so the two halves arrive already wedded in one dried powder, already positioned for the receptor to compound them. That is why yeast extract punches so far above its weight, and why the nucleotide-enriched grades command their premium. Yeast also carries **glutathione** — a γ-glutamyl tripeptide, γ-Glu-Cys-Gly — which lends it a genuine **kokumi** body, a thickness and continuity beneath the savor, the reason its index axes read **U / K** and the reason a spoon of it rounds a sauce rather than merely brightening it.

The receptor, and why two molecules read as one louder one

The savory sensor is not a single lock but a small committee, and the whole of yeast's trick is a conversation held on its surface. The principal sensor is the **TAS1R1 · TAS1R3** heterodimer, a class-C G-protein-coupled receptor built from two subunits, each carrying a large hinged domain called a **Venus flytrap** — two lobes joined like the shells of a clam. Alongside it, truncated taste-tissue variants of the **metabotropic glutamate receptor mGluR1** read free glutamate through their own flytrap. The mechanism is literally mechanical. When L-glutamate settles into the cleft of the flytrap, it bridges the two lobes and lets the clam snap shut; the closed conformation travels down a cysteine-rich stalk into the seven-pass transmembrane bundle, which on the inside of the cell releases the G-protein **gustducin.** Gustducin fires **phospholipase C-β2,** which cuts PIP_2 into IP_3, which spills calcium from internal stores, which gates the **TRPM5** channel — and the taste cell depolarizes and reports *savory.* Half of full firing, reached at one defining concentration, is the half-maximal point the entire Index is anchored to.

Now the second molecule enters, and the beauty of it is that it never activates anything on its own. The 5′-nucleotide binds not in the cleft where glutamate sits but at an adjacent pocket near the *opening* of the Venus flytrap, out by the hinge. Wedged there, it acts as a doorstop that makes the clam far harder to reopen: it stabilizes the closed, active conformation glutamate has induced. The receptor, held shut longer and more easily, now reaches half-firing at a far lower glutamate concentration than before. In the vocabulary of pharmacology the nucleotide is a **positive allosteric modulator** — it changes the receptor's affinity for the agonist without being an agonist itself — and in the vocabulary of the tongue it is *the multiplier.* One structural detail decides everything about which nucleotides work: the potentiation depends on the **5′-phosphate**

making its electrostatic contacts at the hinge. Move the phosphate to the 3′ position, or strip it to a bare nucleoside, and the doorstop no longer fits; the molecule goes silent. This is why the enzyme that matters for yeast is specifically a **5′-phosphodiesterase** and not any of the nucleases that would leave a 3′-phosphate — the cell must liberate the *5′* isomer or the multiplier never arrives.

The mathematics of a savory jar

The Iyengar Flavor Index measures loudness, not quantity — not how much of a compound a food holds but how loudly that compound speaks to its receptor. The whole standard rests on one relation:

> $IFI(c) = 1000 \times EC_{50}(\text{reference}) \div EC_{50}(\text{candidate})$

EC_{50} is the concentration at which a stimulus drives its receptor to half of full response. It falls out of the dose–response curve, which for a simple agonist follows the Hill form

> $\theta = [A]^n \div (EC_{50}^n + [A]^n)$

where θ is the fraction of receptors firing and n is the Hill coefficient — near 1 for glutamate alone, a gentle hyperbola. Set [A] = EC_{50} and the equation returns θ = 0.5 exactly: half-max, the fixed landmark every axis is pinned to. A more potent stimulus reaches that landmark at a *lower* concentration — a smaller EC_{50} — and because the Index inverts EC_{50}, a smaller EC_{50} means a larger score. We anchor the umami axis to a monosodium-glutamate reference whose half-max sits at **EC_{50}(ref) = 1.00 mM glutamate-equivalent,** so that a stimulus exactly as potent as reference glutamate scores IFI-U = 1,000 by convention.

Run the engineered yeast's headline number straight through. Our savory-tuned autolysate is designed to carry an umami loudness of **3,000.** Invert the Index to recover what that means at the receptor:

> $EC_{50}(\text{yeast}) = 1000 \times 1.00 \text{ mM} \div 3{,}000 =$ **0.333 mM** glutamate-equivalent

The extract is designed to reach half-firing at one-third the concentration reference glutamate needs. Put the two curves side by side at a realistic seasoning dose — say a broth delivering the extract's savory signal at **0.5 mM** glutamate-equivalent — and the Hill form (n = 1) reads them out:

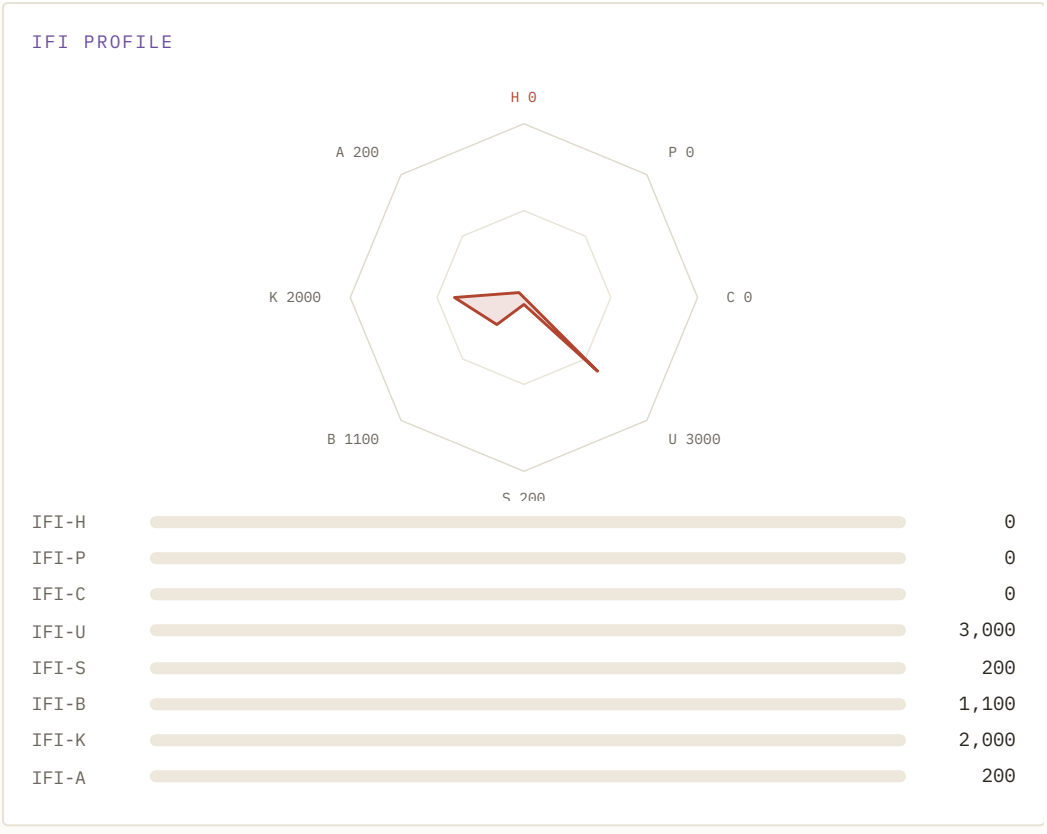
> $\theta(\text{reference}) = 0.5 \div (1.00 + 0.5) =$ **0.333** → 33% of full savory firing $\theta(\text{yeast}) = 0.5 \div (0.333 + 0.5) =$ **0.600** → 60% of full savory firing

Same delivered dose; nearly double the savory response, because the engineered extract's curve has slid left. The arithmetic also tells a formulator exactly how much less they need. Converting through glutamate's molar mass — free glutamic acid weighs **147.1 g/mol** — reference half-max at 1.00 mM is 0.147 g/L, while the engineered extract hits its half-max at 0.049 g/L. In a 300 mL bowl that is the

difference between **44 mg** and **15 mg** of glutamate-equivalent to reach the same savory landmark: a third of the dose, a third of the label line, the same depth on the tongue.

QUANTITY	REFERENCE GLUTAMATE	ENGINEERED YEAST
EC_{50} (mM, Glu-equiv)	1.00	0.333
IFI-U	1,000	3,000
g/L at half-max	0.147	0.049
mg to half-max in a 300 mL bowl	44	15
Firing at a 0.5 mM dose (Hill n=1)	33%	60%

That tall, clean savory note, with a real floor of kokumi beneath it, is the profile the 240 constructs are designed to deliver:



The synergy, in numbers

The EC_{50} picture shows *that* the curve slides left; the synergy math shows *why,* and it is the most important arithmetic on this axis. The classic empirical form, measured by sensory panels a half-century ago and still the working model, is multiplicative rather than additive:

$$y = u + \beta \cdot u \cdot v$$

Here u is the glutamate concentration, v the nucleotide concentration (both in grams per 100 mL), y the equivalent umami concentration — the amount of glutamate alone that would taste equally savory — and β a large synergy constant, about **1,218 for IMP** and roughly twice that for GMP. The product term β·u·v is the whole story: the two halves do not add, they multiply.

Work a concentrated extract dosed into a sauce at u = 0.050 and v = 0.025 g/100 mL:

> `y = 0.050 + 1,218 × 0.050 × 0.025 = 0.050 +` **`1.522`** `=` **`1.572 g/100 mL`** `equivalent`

The nucleotide makes 0.05 g of glutamate taste like **1.57 g** — a **31-fold** multiplication, with **97%** of the perceived savor coming from the synergy term and only 3% from the glutamate standing alone. This is the receptor arithmetic behind "punches above its weight," and it is why a nucleotide-enriched grade is worth its premium: the multiplier does the heavy lifting. Not every multiplier is equal, either. The synergy constant is larger for GMP than for IMP — roughly **2,800 against 1,218** — so a guanylate-rich extract shouts louder still: the same v of pure GMP would read y = 0.050 + 2,800 × 0.050 × 0.025 = **3.55 g/100 mL** equivalent, a **71-fold** lift where IMP gave thirty-one. The proportion of IMP to GMP the cell liberates is therefore a design variable in its own right, not a footnote, and we tune the two nucleotide pools as deliberately as we tune the glutamate.

Because the signal rides on a product, the *ratio* of the two halves matters as much as their total — which is exactly what we tune for. Hold the total savory solids fixed at T = u + v = 0.075 g/100 mL and ask where y is largest. The product u·v, under a fixed sum, is maximized when the two are equal, so a **1:1 glutamate-to-nucleotide balance** is the optimum:

> `u = v = 0.0375 → y = 0.0375 + 1,218 × 0.0375 × 0.0375 =` **`1.750 g/100 mL`**

Re-balancing the same total mass from a careless 2:1 split to the optimal 1:1 lifts the delivered savor from 1.572 to 1.750 — **+11%** umami for free, no added ingredient, only a corrected proportion. An extract engineered to carry both halves in the ratio the receptor most rewards therefore delivers more savor per gram than one that merely carries a lot of both. The same picture, written in receptor terms, is that the built-in nucleotide lowers the effective half-max of the whole ensemble by the factor (1 + β·v): to reach IFI-U 3,000 from a reference-strength glutamate pool the extract needs (1 + β·v) ≈ 3, a synergy that can be read as a modest lift in the free-glutamate pool, say ×1.5, composed with a nucleotide multiplier of ×2 — **1.5 × 2 = 3** — the two levers meeting at the published number.

The chemistry on the cell floor

All of this is unlocked by enzymes, and naming them names the grades a formulator will meet on a spec sheet. Two liberations happen in parallel when a yeast cell digests itself. The protein side: a

graded set of **proteases** and **peptidases** dismantle the cell's own proteins into free amino acids, and the one that matters most for savor is **glutaminase,** which converts **glutamine** — abundant, and nearly tasteless — into readable **glutamate.** Much of a cell's nitrogen is warehoused as the amide, so glutaminase activity is very often the difference between a flat autolysate and a profound one; it is the enzyme that turns *potential* savor into the note itself. The nucleotide side runs on the cell's RNA, of which a fast-grown yeast holds a great deal: a **5′-phosphodiesterase** cleaves that RNA into 5′-mononucleotides, and an **AMP deaminase** then converts the adenylate (5′-AMP, a weak multiplier) into the inosinate (5′-IMP, a loud one), while the guanylate (5′-GMP, the loudest) comes straight off the RNA's guanine. Only the two purine 5′-nucleotides, IMP and GMP, are umami-active; the pyrimidines, 5′-CMP and 5′-UMP, are silent, their rings the wrong shape for the hinge. The glutathione that carries the kokumi body is built and held by the cell's own γ-glutamyl machinery, and it speaks to a different receptor entirely — the **calcium-sensing receptor, CaSR,** another class-C GPCR — which is why kokumi has no flavor of its own yet thickens and lengthens everything around it, and why the engineered yeast's K axis is designed to read a real 2,000.

STEP	ENZYME	SUBSTRATE → PRODUCT	ON THE TONGUE
Proteolysis	proteases · peptidases	cell protein → free amino acids	the glutamate pool
Amide release	glutaminase	L-glutamine (mute) → L-glutamate	the savory *note*
RNA cleavage	5′-phosphodiesterase	RNA → 5′-AMP · GMP · CMP · UMP	raw material for the multiplier
Deamination	AMP deaminase	5′-AMP (weak) → 5′-IMP (strong)	the *multiplier,* IMP
Native	—	RNA guanine → 5′-GMP	the loudest multiplier, GMP
γ-glutamyl synthesis	glutathione machinery	Glu·Cys·Gly → glutathione	kokumi *body,* via CaSR

The grades a formulator meets

The method names the grades, and the grades are simply which of those enzymes were allowed to do their work, and how gently. In **autolysis,** the yeast's own enzymes are turned loose on the cell under gentle warmth, and they do the dismantling — the clean, traditional route, the one that yields the rounded savor of the classic dark spreads. In **hydrolysis,** acid or added enzymes force the breakdown faster and harder; it is cheaper and higher-yielding but drags along more of the harsh, bitter, salty character that then has to be masked. And the **nucleotide-enriched** grades are autolysates processed specifically to liberate the RNA to 5′-IMP and 5′-GMP — the multiplier grown into the cell rather than dosed from a drum.

GRADE	HOW	SAVOR PROFILE	TYPICAL USE
Autolyzed yeast extract	native enzymes, gentle heat	rounded glutamate + body	spreads, gravies, broths
Hydrolyzed (acid / enzyme)	forced breakdown	louder, harsher, saltier	low-cost bouillon
Nucleotide-enriched (I+G)	RNA liberated to IMP + GMP	the multiplier, built in	the savory-potentiator grade

Much of the world's yeast extract began as a thrift: spent brewer's yeast, the sediment left after fermentation, too valuable to discard and too bitter to eat until someone discovered that autolyzing it turned a brewery's waste into a beloved savory spread. We find that lineage fitting. It is the same instinct as our whole program — that savor is not a scarce luxury to be dosed sparingly but a thing already present in the living world, waiting to be unlocked, grown, and put to honest use.



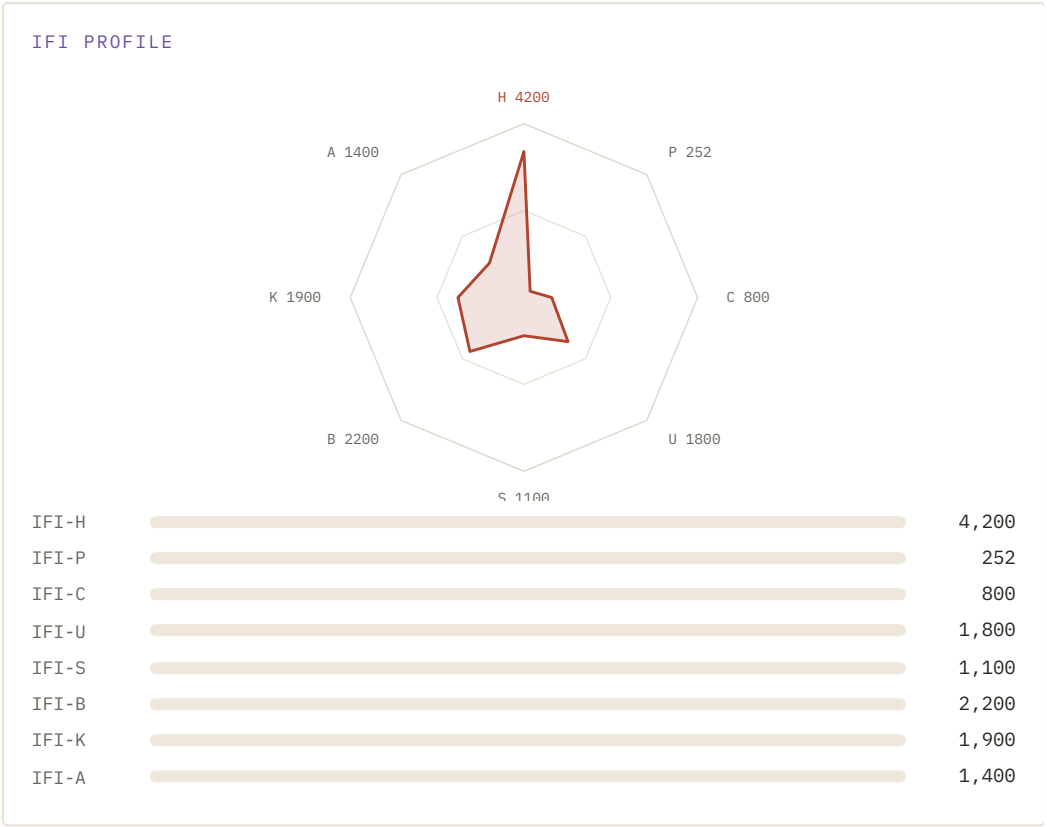
Soybean — the fermented-savory family yeast belongs to; miso and shoyu marry glutamate and nucleotide the way the single yeast cell does in one ingredient.

Where yeast sits on the savory floor

Yeast does not stand alone on this axis; it sits on a floor of savory hosts, and reading its place is the fairest way to size what the 240 constructs are for. The tomato leads by sheer breadth — a sweet-and-savory host with thousands of constructs — and among the organisms whose entire purpose is depth, yeast is the production-hardened leader. Soybean carries more raw constructs, but it sits at the frontier of our development rather than in the shipping tier; yeast is mature, on the floor, in the jar today. Below it run the pure fungal engines — koji, shiitake, the cultivated mushroom — loud at savor but single-noted where yeast is doubled.

ORGANISM	CONSTRUCTS	PRIMARY AXES	DIFFICULTY
Tomato	2,040	S / U / F / M	Easy
Soybean	360	U / K	Frontier
Yeast	**240**	**U / K**	**Hard**
Koji Mold	80	U	Hard
Shiitake	80	U	Hard
Mushroom	40	U	Hard

What those 240 constructs actually do is push on four levers at once, which is why the organism earns its **Hard** rating despite arriving in so convenient a form. We lift the **free-glutamate pool,** largely by strengthening the glutaminase that converts the cell's warehoused glutamine. We **lift and balance the nucleotide release,** tuning the 5′-phosphodiesterase and AMP-deaminase activity so the multiplier arrives in the 1:1 proportion the receptor most rewards rather than whatever the feedstock happened to yield. We **trim the bitter peptides and sulfur compounds down,** the sulfurous, slightly burnt edge that forces a formulator to mask the very ingredient they added for depth. And we **steady the glutathione** that carries the kokumi body. The reading is a tall, clean savory note with a real floor beneath it — and, tellingly, a single-axis specialist can out-savor even the full-spectrum flagship on its home ground. Set the engineered yeast beside our eight-axis benchmark cultivar Phoenix (SS-0096), whose predicted profile reads:



Phoenix carries all eight axes at once and sits at U 1,800, K 1,900 — a genuinely savory pepper. Yet the yeast, tuned to do one thing supremely, is designed to read **U 3,000, K 2,000** — louder on both savory axes than the generalist. That is the lesson of a specialist: breadth costs height, and when a maker wants depth and only depth, the organism built for nothing else wins.

Nutritional yeast — the savory the home cook already knows

A cousin of the savory extract deserves its own line, because home cooks know it better than any spec sheet: **nutritional yeast,** the flaked, deactivated *Saccharomyces* that vegetarian kitchens have leaned on for a generation for its cheesy, savory, faintly nutty character. It is the same organism, grown and dried and deactivated rather than autolyzed, and its savor comes from the same glutamate the extracts concentrate — a plant-kingdom seasoning that reads, uncannily, of aged cheese without a trace of dairy, which is exactly the trick a Parmesan plays with its own crystalline glutamate. Many nutritional-yeast products are also grown to carry B-vitamins, which we note as the plain fact it is and leave strictly as an attribute, making no claim: one more case, on an axis full of them, of the savory tissue and the nourishing tissue being the same tissue. Our engineered yeast is tuned for the industrial extract first, but the same lifted glutamate and cleaned-up profile carry straight into a flaked, at-the-table form, because the savor was built into the cell, not into the process. The flake on a bowl of pasta and the drum of extract bound for a bouillon line are, underneath, the same designed cell read at two scales.

Failure modes, variance, and the honest limits

The synergy that makes yeast valuable also makes it harder to keep constant, and the honest account has to say so. When the delivered savor rides on a product, u·v, its variability is not the variability of either half but the two combined in quadrature: $CV(u \cdot v) \approx \sqrt{(CV(u)^2 + CV(v)^2)}$. Run each pool at a loose 7% and the product already wobbles at nearly 10%. The multiplier multiplies the variance as surely as it multiplies the signal. So to ship the extract at our standard of **CV ≤ 7%** on the delivered umami, we have to hold each contributing pool *tighter* than the output spec — to roughly **5% each,** since $\sqrt{(0.05^2 + 0.05^2)} \approx 7.1\%$. Batch discipline on this organism is therefore stricter at the inputs than at the output, a direct consequence of the synergy arithmetic, and it is exactly the kind of demand that separates a flavor you can design around from one you can only hope for.

The reason the discipline matters is what a formulator builds on top of the number. Held at CV ≤ 7%, a designed IFI-U of 3,000 means a standard deviation of about 210, so ninety-five batches in a hundred land within ±420 — a savory signal between **2,580 and 3,420,** the ten-thousandth jar tasting like the first. An undesigned autolysate drifting with its feedstock and its process can run at CV 20% or worse: a standard deviation of 600, a ±2σ spread from **1,800 to 4,200,** a savor that swings by more than a factor of two across the year. You cannot formulate against that. Consistency is not a luxury on this axis; it is the whole difference between a seasoning and a surprise.



On the stage — a savory batch read against the receptor assay and the trained panel, then held to the number it ships at.

The honest limits are worth stating plainly, because an Index stays trustworthy only when it names what it cannot do. Yeast is **glutamate-forward,** not a whole flavor: it supplies the savory note and the multiplier and a floor of body, but it is depth, not brightness — it will not bring acid, it will not bring aromatics, it will not bring the top notes a dish still needs from elsewhere. Pushed too hard, even a well-tuned autolysate can go sharp and sulfurous, the over-digested edge that shouts instead of rounds, which is why we tune for the *shape* of the savor and not merely its height, holding the zero-bitter axis up at 1,100 so the depth stays something you can taste *through.* The per-axis ceiling on the scale is 5,000, and we stop well short of it at 3,000 on purpose: yeast's gift is a clean, carryable depth that sits under a thousand everyday foods, not a record. And the label, clean as it reads, is still an ingredient to declare honestly — a yeast extract is a yeast extract, named as itself, never hidden behind a euphemism. Savor that only shouts, or savor that hides what it is, is a different kind of empty. Designed with restraint and declared plainly, the cell is the most generous thing on the savory shelf.

For the savory-brand and meat-analog makers this book is partly written for, yeast is the honest first tool. It is a plant-kingdom ingredient in the way that matters — grown by fermentation, dried, carrying nothing but what the cell made — and it delivers the whole savory signal, note and multiplier and body, with a label that reads cleanly and asks for no apology. These are computational designs; Scoville Splice's cultivars and constructs have not been experimentally validated in a laboratory. Yeast is where the two halves of savor arrive already married, in one cell, in one powder: the note and the multiplier, the glutamate and the inosinate, the thing the tongue has always wanted a broth to be, grown into a single generous organism by a family that reveres what it grows and weighs it to the milligram before it ever reaches your bowl.



PART IV OF V

The Design

the pathway · the loop · precision · the clean label

Chapter 12 · Setting Glutamate Free — *the biosynthesis, drawn.*

the nitrogen routes · the GABA shunt · where free glutamate pools

We have named the enzymes organism by organism; here we draw the whole map at once, because a maker who can read the pathway can read the plant, and because this is the circuitry every restoration in this library edits. Glutamate sits at the exact center of a plant's nitrogen metabolism — it is the hub through which almost all inorganic nitrogen enters organic life, and the donor from which most other amino acids are built. To raise free glutamate is to lean on the busiest intersection in the cell, which is why it takes a model to do without breaking something else. What follows is that intersection, drawn to scale: the routes nitrogen takes to become savor, the one drain that quietly empties the savor away, and the arithmetic that lets us say *how much* — because the whole point of this family's work is that a taste you cannot measure is a taste you cannot design.



The restored vine in cross-section — the tissue in which the nitrogen routes drawn in this chapter either pool free glutamate or bleed it away.

The molecule the flytrap reads

Begin at the end, on the tongue, because everything upstream exists to serve one receptor. Umami is read by a pair of class-C G-protein-coupled receptors — the **TAS1R1·TAS1R3** heterodimer and the taste variant of **mGluR1** — and both share a strange and beautiful piece of architecture: a large extracellular domain shaped like a **Venus flytrap.** Glutamate does not sit on the surface of these receptors the way a key sits on a lock's face; it falls into the cleft *between* the two lobes of the flytrap, and its binding shifts the lobes from an open equilibrium toward a closed one. That closure is the signal. It travels

down through a cysteine-rich stalk into the seven-helix bundle buried in the membrane, which releases its G protein and begins the cascade the rest of the tongue will feel.

The cascade is worth drawing once, because it tells us what kind of molecule the plant must deliver. Closure activates gustducin; gustducin frees phospholipase C-β2; PLC-β2 cleaves the membrane lipid PIP_2 into IP_3; IP_3 opens a calcium store inside the cell; the released calcium throws open **TRPM5**, a cation channel that depolarizes the taste cell; and the depolarized cell vents ATP through **CALHM1** channels onto the afferent nerve. Lock, closure, calcium, current, nerve — a chain five links long, repeated a billion times a second, and every link of it waits on the first: a glutamate ion, *free in solution,* finding the open flytrap.

The two receptors divide the labor. The TAS1R1·TAS1R3 pair is the dedicated taste-bud sensor, broadly tuned and built for the mingled savor of a real meal; the mGluR1 variant — a truncated cousin of the glutamate receptor the brain uses for thought — reads glutamate more selectively and is thought to carry the cleaner, more specifically savory note. Neither adapts away quickly, and that slowness is part of umami's signature: where sweetness spikes and bitterness alarms, savor *lingers*, a long low plateau that stays as long as the glutamate does. The pool we build has to hold, because the receptors that read it are built to keep reading.

That phrase — *free in solution* — is the reason this chapter exists. The receptor cannot read glutamate that is still stitched into a protein, and it cannot read glutamate that the plant has decarboxylated into something else. A tomato can be rich in protein and rich in total nitrogen and still taste of almost nothing, because its glutamate is locked in peptide bonds or parked as a tasteless amine. Savor is not the amount of glutamate a fruit *contains.* It is the amount a fruit holds **free** — dissolved, unbound, in the form the flytrap can close around — at the moment you bite it. Our entire biosynthetic effort is the management of that one pool.

The Index gives us the language to be exact about it. Loudness, not quantity, is what we score:

$$\text{IFI-U}(c) = 1000 \cdot EC_{50}(\text{ref}) \,/\, EC_{50}(c)$$

EC_{50} is the concentration at which free glutamate drives the umami receptor to half of its full response — the midpoint of the flytrap's closing. A fruit whose free pool is large reaches that midpoint at a lower external concentration; its EC_{50} is small; and because the Index inverts EC_{50} against a fixed reference and scales by a thousand, its IFI-U is large. The reference — native wild-type savor — is pinned at **1,000** by construction. Everything we build is read against that peg.

From soil to savor — the assimilation gate

A nitrogen atom becomes a taste in stages, and the first stages happen before the fruit is even a fruit. Most plants draw nitrogen from the soil not as ammonium but as **nitrate**, and reduce it in two steps before anything savory can be built. **Nitrate reductase (NR)** turns nitrate into nitrite in the cytosol, spending NAD(P)H; **nitrite reductase (NiR)** turns nitrite into ammonium in the plastid, spending

reduced ferredoxin. NR is the slow, guarded valve of the whole system — regulated at the level of its gene, and again within minutes by phosphorylation and 14-3-3 protein binding that can switch it off while the light changes. Only once nitrate has been walked down to ammonium does assimilation proper begin.



The research plot at the reduction end of the pathway — where the form and timing of nitrogen a plant is fed sets the size of the pool savor is later built from.

Ammonium is captured by the **GS/GOGAT cycle,** the two-enzyme loop that is the primary gate of nitrogen into amino acids:

STEP	ENZYME	REACTION	COFACTOR / NOTE
1	Glutamine synthetase (GS)	glutamate + NH_4^+ + ATP → glutamine	the capture; costs one ATP; Mg^{2+}-dependent
2	Glutamate synthase (GOGAT)	glutamine + 2-oxoglutarate → 2 glutamate	the hand-off; Fd- or NADH-dependent
—	*net per turn*	2-oxoglutarate + NH_4^+ → glutamate	one **new** glutamate each cycle
alt	Glutamate dehydrogenase (GDH1)	2-oxoglutarate + NH_4^+ + NAD(P)H ⇌ glutamate	reversible; a buffer on the pool

Two features of that table set up everything we do. First, every new glutamate costs a molecule of **2-oxoglutarate** — α-ketoglutarate, the five-carbon skeleton onto which the nitrogen is hung — and 2-oxoglutarate is drawn from the **TCA cycle,** the central engine of cellular energy. Raising glutamate is therefore never free: it pulls carbon away from respiration, and pushed too hard it starves the very metabolism that ripens the fruit. Second, **GDH1** runs its reaction *reversibly.* It can build glutamate or tear it down depending on the cell's redox and ammonium state, which makes it both a buffer against swings in the pool and, for us, a tuning point: steer GDH1 toward amination in the ripening fruit and the free pool fills from a second direction while GS/GOGAT keeps turning.

This is also where horticulture becomes biochemistry. The *form* of nitrogen a plant is fed — nitrate or ammonium — and the activity of NR and NiR set the size of the ammonium pool that GS draws on. Feed and reduce the nitrogen well in the ripening window and the

cycle has raw material to fill the free pool; starve that front end and no downstream edit will find glutamate the plant never assimilated. The pathway begins in the soil solution, and we design it from there — which is why the controlled-environment nutrient regime of a restored crop is not a detail of growing but the first enzyme in the chain.

The GABA shunt — the drain we learn to close

Now the branch that decides whether the pooled glutamate *stays* readable — the branch the tomato taught this family to tune. Glutamate has a second fate, and it is a quiet one:

STEP	ENZYME	REACTION	EFFECT ON SAVOR
1	Glutamate decarboxylase (GAD)	glutamate → GABA + CO_2	**drains** the free pool
2	GABA transaminase (GABA-T)	GABA + 2-oxoglutarate → succinic semialdehyde + glutamate	partial return
3	Succinic semialdehyde dehydrogenase (SSADH)	succinic semialdehyde → succinate	exits to the TCA cycle

GAD is a pyridoxal-5′-phosphate enzyme switched on by calcium-calmodulin and by a falling cytosolic pH — exactly the conditions of a stressed or acidifying cell. In a green tomato it runs hard, and much of the fruit's nitrogen is held as **GABA**, which is tasteless at the umami receptor: the flytrap has no affinity for it, so a green fruit can be nitrogen-rich and savor-poor at once. As the fruit ripens, GAD activity falls; the shunt runs stored GABA back out through GABA-T and SSADH; and GS/GOGAT and GDH1 keep the free glutamate pool filling from the other side.



The ripe fruit, where the two curves have finished crossing — GAD falling, free glutamate rising, the savory form held long enough to read.

The savor of a ripe tomato is the sum of two curves crossing: **GAD falling, free glutamate rising.** A fruit picked green — picked for the truck, for the shelf-life, for the 1,500 miles — never lets those curves finish crossing. Its glutamate is still being decarboxylated into GABA when it is pulled from the vine, and it ripens the rest of the way in a box, filling with pigment but not with savor. Our restoration is, at its core, the deliberate easing of **GAD** in the late fruit, so the glutamate arriving in the final days is not spent on an amine the tongue cannot

read — holding the molecule free, in the one form the receptor closes around. We are not adding anything the plant does not make. We are letting it keep what it was always about to throw away.

The geography of the pool

Geography matters here as much as chemistry, because these reactions do not share a room, and the compartments are part of what we tune. Knowing where a reaction runs is knowing which promoter and which targeting peptide an edit needs — the difference between restoring a fruit's savor and derailing a leaf's growth.

REACTION	ISOFORM	COMPARTMENT	JOB
GS/GOGAT (primary assimilation)	GS2 · Fd-GOGAT	plastid	new nitrogen + photorespiration
GS/GOGAT (remobilization)	GS1	cytosol	recycling the plant's own protein
Amination buffer	GDH	mitochondrion	poised at the TCA cycle's edge
Decarboxylation	GAD	cytosol	the drain — Ca^{2+}/pH-gated
GABA catabolism	GABA-T · SSADH	mitochondrion	returns carbon to the TCA cycle

The split between GS2 and GS1 is the one that makes a late, fruit-specific restoration safe. The **plastid** form (GS2 with ferredoxin-GOGAT) handles the nitrogen of primary assimilation and of photorespiration — the leaf's working economy, which we must not touch. The **cytosolic** form (GS1) handles **remobilization:** the recycling of the plant's own proteins as a fruit ripens and a leaf senesces, nitrogen pulled out of structures no longer needed and shipped to the sink that is the fruit. It is largely that cytosolic, remobilizing pool that feeds a ripening fruit's free glutamate. So an edit placed under a ripening-specific, fruit-specific promoter can lift savor without disturbing the leaf's photosynthetic nitrogen at all — the restoration lives in the sink, not the source. GDH sits mostly in the **mitochondrion,** at the TCA cycle's lip where its 2-oxoglutarate substrate is made; GAD runs in the **cytosol,** where the ripening pool actually pools. Draw the map with its walls, and the edit writes itself: late, cytosolic, fruit-only.

The arithmetic of loudness

This is where the family's sensibility shows — because a savor you can feel but not count is a savor you cannot reproduce, and reproducibility is the whole difference between a lucky vine and a platform. The Index turns the pool into a number, and the number into a design target.

Start from the definition and invert it. If

> $\mathrm{IFI\text{-}U} = 1000 \cdot EC_{50}(\mathrm{ref}) \,/\, EC_{50}(c)$

then a candidate's EC_{50}, expressed as a fraction of the reference's, is simply **$EC_{50}(c)/EC_{50}(\mathrm{ref}) = 1000 / \mathrm{IFI\text{-}U}$.** A high score is a low EC_{50}; a low score is a high one. That single rearrangement lets us read the whole tomato story in receptor terms. A depleted supermarket fruit

scores **IFI-U 300**; our model predicts a fruit restored through its own native GAD/GDH1 route scores **IFI-U 2,600.** Run both through the inversion:

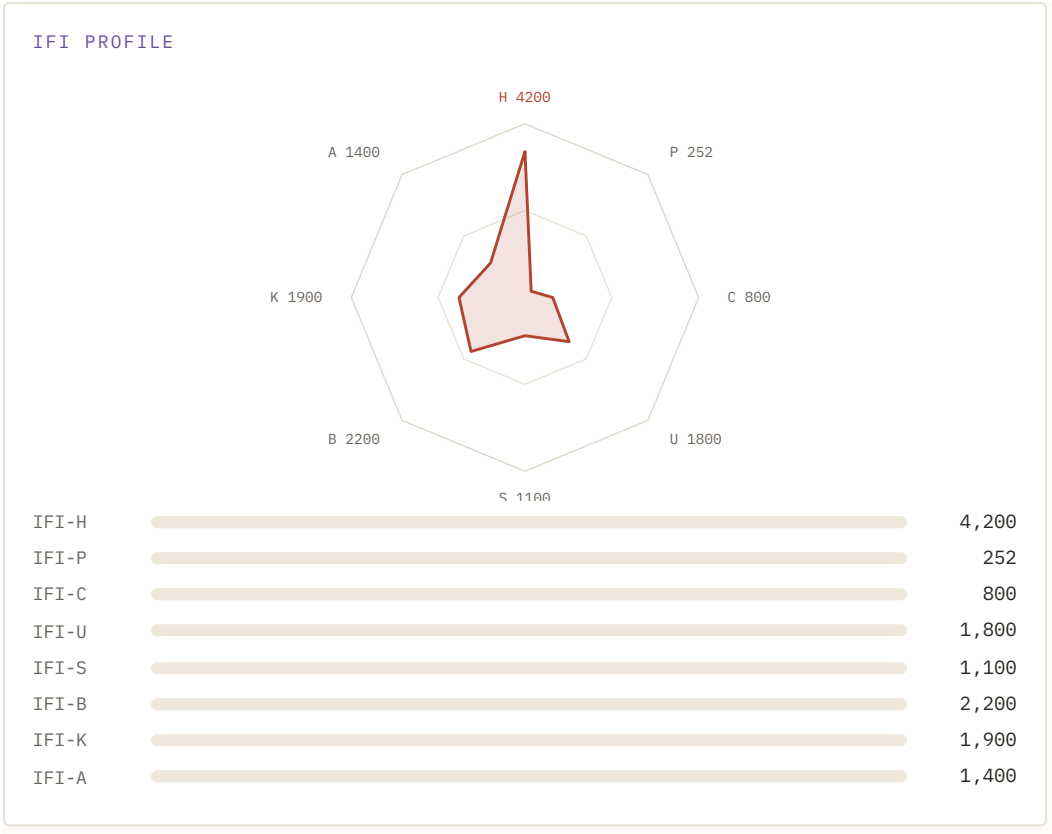
MATERIAL	IFI-U	EC_{50} (× REFERENCE)	VS. REFERENCE	RESPONSE AT [G]=EC_{50}(REF), N=1
Reference (native wild-type)	1,000	1.000	1.0×	50.0%
Depleted tomato	300	3.333	3.3× quieter	23.1%
Restored tomato	2,600	0.385	2.6× louder	72.2%
Phoenix (full-spectrum)	1,800	0.556	1.8× louder	64.3%

Every figure in the last two columns is derived, not asserted. The EC_{50} column is 1000/IFI-U. The response column applies the Hill equation at a fixed probe concentration equal to the reference's own half-activation point:

$$\text{response} / \text{max} = [G]^n / (EC_{50}^n + [G]^n)$$

Set the Hill coefficient n = 1 and the probe [G] = EC_{50}(ref), and the response collapses to 1 / (EC_{50}(c)/EC_{50}(ref) + 1). For the restored tomato that is 1 / (0.385 + 1) = **0.722** — at a glutamate concentration that drives wild-type savor to exactly half, the restored fruit already drives the receptor to seventy-two percent. The depleted fruit, at that same concentration, reaches only **0.231.** The gap between a tomato that tastes like a tomato and one that tastes like water is, in receptor terms, the gap between 72% and 23% of a flytrap's closing. (A cooperative receptor, n > 1, only steepens that gap; we use n = 1 as the honest floor.)

The fold-change is the cleaner way to feel it. From 300 to 2,600 is a factor of 2,600/300 = **8.67×** more loudness — and, because loudness is inverse EC_{50}, exactly an 8.67-fold *drop* in the concentration needed to be heard. On a log axis that is $\log_{10}(8.67)$ = **0.94** — the restored fruit's dose-response curve slides almost a full decade to the left. Nearly an order of magnitude of savor, recovered not by adding anything but by letting the fruit finish on the vine.



That profile is Phoenix (SS-0096), the full-spectrum flagship — the only plant we have designed to register on all eight axes at once. Read its umami line against the restored tomato and you find a quiet lesson: the specialist beats the generalist on its own axis. The dedicated tomato reaches **IFI-U 2,600**; Phoenix, spreading its budget across eight axes, holds umami at **1,800.** There is no contradiction in that. A plant has only so much carbon and nitrogen to spend, and a fruit built to speak eight languages speaks none of them as loudly as a fruit built to speak one. The Index makes the trade-off visible, in the one place a breeder tasting by tongue could never have seen it.

Loudness is nothing, though, without reproducibility, and here the clean-lab discipline earns its keep. Our batch-to-batch coefficient of variation holds at **≤7%.** On a restored tomato scoring 2,600, that is a band of 2,600 × 0.07 = ±182 — the fruit lands between roughly 2,418 and 2,782, batch after batch. A wild landrace selected by taste runs a CV near 42%; the same nominal 2,600 would scatter ±1,092, from 1,508 to 3,692, which is the difference between a seasoning you can formulate against and one you can only hope for. And because the score is a ratio, its error propagates cleanly: with both the reference and the candidate EC_{50} carrying an independent 7% CV, the score's CV is $\sqrt{(7^2 + 7^2)}$ = **9.9%;** calibrate the reference tightly and it falls back toward the candidate's own 7%. Average eight batches and the standard error drops by a further $\sqrt{8}$ — to **2.5%.** Numbers, in this family, are a form of respect for whoever cooks with the result.

One last piece of arithmetic, because it is the one diners feel without naming: **synergy.** The 5′-ribonucleotides — inosinate (IMP) and guanylate (GMP) — bind near the hinge of the Venus flytrap and stabilize its closed state, lowering glutamate's EC_{50} without being glutamate at all. Since IFI-U scales as $1/EC_{50}$, a potentiator that *halves* the EC_{50} *doubles* the score. A restored tomato at 2,600, met on the plate by a nucleotide-rich mushroom, pushes toward 5,200 in principle — which is why the scorecard clips each axis at a normalization ceiling of **5,000.** The cap is not a limit of the plant; it is

the point past which a single axis would swamp the page. Glutamate and a mushroom's guanylate are the oldest trick in savory cooking, and the Index finally writes down why it works: two molecules, one flytrap, a product and not a sum.

Aspartate rides along in this arithmetic as glutamate's faint echo. **Aspartate aminotransferase (AST)** interconverts the two — glutamate and 2-oxoglutarate traded for aspartate and oxaloacetate — so the pair rise and fall together, and a savory extract almost always carries both. But aspartate is weak at the flytrap: its umami coefficient is **0.077,** barely a thirteenth of glutamate's. An aspartate pool the same size as the glutamate pool adds only about 7.7% to the effective savor — a companion, never a stand-in, and a reminder that of the twenty amino acids a plant can hold free, only one truly speaks this axis aloud.

The carbon cost, counted

The carbon cost is worth one honest paragraph, because it is the constraint that makes this hard rather than infinite. Every free glutamate is a 2-oxoglutarate spent — a five-carbon skeleton pulled from the TCA cycle and committed to carrying nitrogen instead of yielding energy. Drain 2-oxoglutarate faster than the cycle can refill it and the cycle itself slows, because the carbon that left as glutamate never comes back as the oxaloacetate the next turn needs. The cell's answer, and ours, is **anaplerosis:** the refilling of the cycle from the side, chiefly by **PEP carboxylase (PEPC),** which fixes CO_2 onto phosphoenolpyruvate to make oxaloacetate and top the cycle back up. Many fruits ramp PEPC during ripening on their own; a restoration that leans on 2-oxoglutarate has to make sure that top-up keeps pace, or the savor is bought with the fruit's own respiration.

This is why "just make more glutamate" is not a strategy, and why a genome-scale flux-balance model — the cell's whole metabolism written as a system of mass-balance equations and solved for what it can and cannot afford — is not optional but the ground of the work. The model is what tells us the pathway, not an upstream step, is the true rate limiter before we touch a single base. And the model is what finds the window. The art is to raise the free pool in the narrow **late-ripening** window, when the fruit's growth is finished and its respiratory demand is easing, so the borrowed carbon is carbon the plant no longer urgently needs. Timing is the whole trick: the same edit that would starve a growing fruit merely enriches a ripening one. We are not overriding the plant's economy — we are spending its savor budget at the one moment it can best be spared.

The organisms that speak it

The tomato is the clearest voice in this axis, but it is not the only one, and the map we have drawn runs — with different enzymes playing the lead — in every organism that reads as savory. Seven hosts carry umami as a named axis; together they hold more than three thousand constructs:

ORGANISM	CONSTRUCTS	CHIEF SAVORY MOLECULE	DIFFICULTY
Tomato	2,040	free L-glutamate	Easy
Soybean	360	L-glutamate	Frontier
Tea	280	L-theanine + glutamate	Hard
Yeast	240	glutamate + 5′-nucleotides	Hard
Koji mold	80	glutamate (protease-liberated)	Hard
Shiitake	80	5′-guanylate (GMP)	Hard
Mushroom	40	glutamate + GMP	Hard

Add the constructs and the axis carries **3,120** of them across the seven — and notice that the tomato alone is **2,040,** two-thirds of the whole. Notice, too, the lone **Easy** in a column of Hard. The tomato is easy because its umami is a glutamate story and its glutamate machinery is intact: the native GAD/GDH1 route already exists, and our job is to retime it, not to build it. The fungi — shiitake, mushroom, koji — rate Hard because their savor is a *different* chemistry. They lean on the 5′-nucleotides, IMP and GMP, the very potentiators that close the flytrap alongside glutamate; engineering a mushroom's savor means reaching into nucleotide metabolism, a harder and more tangled branch than a tomato's amino-acid pool. Tea sits between, carrying both glutamate and **theanine,** the amino acid behind a good cup's brothy roundness.



Soybean in cross-section — a glutamate-route umami host, the same assimilation map running under a different skin.

What lets one family's work reach across seven species at once is that the front of the map does not change. The nitrate-to-ammonium reduction, the GS/GOGAT cycle, the GDH buffer, the GABA shunt — these run in every vascular plant, which is why the platform transfers. Only the last steps and the regulation differ: where the pool is held, which promoter wakes in the ripening sink, how the drain is gated. Draw the circuitry once and the soybean, the tea leaf, and the tomato are three dialects of one language — the same assimilation map, read under different skins, tuned at the same handful of nodes.

What the map cannot promise

A drawn map is an honest one only if it marks the cliffs, and this pathway has several. Lean too hard on GS and the plant cannot fix ammonium as fast as it arrives; free ammonium is toxic, and a fruit that accumulates it will damage its own tissue long before it tastes of more. Silence GAD completely rather than easing it and you lose more than a drain: the GABA the shunt makes is part of how a plant buffers cytosolic pH and answers stress, so a fruit stripped of its ability to make GABA is a fruit less able to weather a hot afternoon. Over-divert 2-oxoglutarate and the TCA cycle stalls, and a stalled cycle does not finish ripening — the fruit holds its carbon as savor and fails to color, soften, and sweeten on time. Each of these is a real way the restoration fails, and each is the reason the window is narrow and the model is mandatory.

There are honest uncertainties, too. GDH's physiological direction — how much it builds glutamate versus tearing it down — shifts with the cell's redox and ammonium state, and leaning on it assumes a ripening context that holds; where it does not, the buffer we counted on can run backward. And the Index measures loudness at the receptor, which is most but not all of savor: mouthfeel, the nucleotide synergy that lives on the plate rather than in the fruit, the slow build of a cooked or fermented dish — these ride alongside the number without being fully captured by it. We report the computationally predicted EC_{50} and the designed CV, and we do not dress either one up.

What the map does promise is modest and real. Glutamate is the hub, and we have drawn every road into and out of it: ammonium in through GS/GOGAT, carbon in from the TCA cycle as 2-oxoglutarate, GDH buffering the pool, aspartate aminotransferase trading glutamate for the weak-umami aspartate at the edge, and the GABA shunt as the drain we learn to ease rather than slam shut. Raising free glutamate means pushing the inflows and easing the drain *without* starving the cycle of the carbon that keeps the plant alive — a multi-variable balance no breeder could have selected by taste, and exactly the kind a model can solve. The same amino acid the flytrap closes around is the one the body builds a hundred of its own proteins from; to set it free in the fruit, in the form that can be read and the form that can be used, is the quiet aim under all of it. We measure so we can design, and we design so that a tomato warm from the vine can taste, again, like the thing it was always about to become. These are computational designs; Scoville Splice's cultivars and constructs have not been experimentally validated in a laboratory.

Chapter 13 · The Design Loop — *ESM2, flux balance, CRISPR.*

the method · the rate limiter · the edit

Every prior attempt to breed savor into a plant did the same hopeful thing: select the phenotype — taste the fruit, keep the seed of the one that moved you — and pray the genotype followed. It is how humanity has bred for ten thousand years, and it works, slowly, for traits you can see. It fails for savor, because savor is invisible on the vine, late to appear, and governed by a network of enzymes no eye can read. The round, brothy depth that makes a tomato taste like being cared for is not a color you can grade or a shape you can sort; it is a concentration of free glutamate in the cells of a ripe fruit, a number that does not exist yet when the breeder is standing in the field deciding which plant to keep. So we inverted the whole procedure. We start at the genotype, reason forward to the taste, and only grow the plant once the model says it should work. The loop has three instruments, and they are the same three that designed our heat — a protein language model, a genome-scale metabolic model, and a precise edit — turned now on the chemistry of savor instead of the chemistry of fire.

Hold the two apart for a moment, because the fact that one set of instruments reaches both is the whole argument. Heat is a molecule the plant builds and ships to the surface of a fruit; umami is a molecule the plant builds and then *keeps*, a pool it fills and drains and refills across the slow work of ripening. The receptor is different, the pathway is different, the failure modes are different. What is identical is the method — read the sequence, find the limit, write the change — and the reason it is identical is that five of the seven modules in the platform never had anything to do with peppers in the first place.

The receptor that reads savor

Umami is read by a Venus flytrap. The receptors on the tongue that answer to free glutamate — the taste-specific **TAS1R1·TAS1R3** heterodimer and the truncated **mGluR1** that sits beside it — are class C G-protein-coupled receptors, and each carries a large bilobed extracellular domain shaped, almost comically, like the hinged trap of the carnivorous plant. Glutamate drifts into the cleft between the two lobes and the trap closes on it. That closure is the entire event: a mechanical hinge swinging shut around a single small molecule, propagating through the seven transmembrane helices to the G-protein waiting on the inside of the cell, which lets go of gustducin, which frees phospholipase C-β2, which cuts PIP_2 into IP_3, which opens the TRPM5 channel, which depolarizes the cell and releases ATP through a CALHM1 pore onto the afferent nerve. A lock and key, repeated a billion times a second, reported to the brain as *savory*. We find it genuinely moving that a taste we reach for in every broth and every slow-cooked sauce comes down to a hinge the width of a few atoms, closing.



The flesh of a tomato in cross-section — the locul-gel and pericarp where free glutamate accumulates across ripening, the pool the loop is built to fill.

Two things about that hinge govern everything that follows. First, only *free* glutamate speaks. The glutamate bound up inside the fruit's proteins is mute — it cannot reach the cleft — so the thing we are designing is not the plant's total glutamate but the small, soluble, unbound pool that ripening liberates. Second, the trap does not report *how much* glutamate is present so much as *how readily* it closes, and that readiness is a number: the concentration at which the receptor sits at exactly half of its full response. Push that half-point lower and the same fruit tastes louder. Everything the Index measures, and everything the loop designs, lives in that half-point.

What "half" means — the dose–response

Write the receptor's behavior as the curve it actually traces. For a stimulus at concentration *[G]*, the fraction of receptors in the closed, active state follows a Hill relation,

$$\theta([G]) = [G]^n / (EC_{50}^n + [G]^n),$$

where EC_{50} is that half-activation concentration and n is the Hill coefficient — the cooperativity of the trap. For the umami complex we anchor the reference at **EC_{50} = 3.0 mM** of L-glutamate with **n = 1.5**, the mild positive cooperativity you expect when a bilobed domain closes as one piece. Those two constants are the reference anchor; every savory thing we grow is read against them.

The curve is worth tabulating, because its shape is the first honest limit of the whole enterprise.

FREE GLUTAMATE *[G]*	RECEPTOR ACTIVATION θ
0.5 mM	6.4%
1.0 mM	16.1%
1.5 mM	26.1%
3.0 mM	50.0%
6.0 mM	73.9%
10.2 mM	86.2%

Notice what happens past the half-point. Doubling glutamate from 3.0 to 6.0 mM lifts activation from 50% to 74% — a gain of twenty-four points. Doubling again, from 6.0 to 12 mM, would buy only another fifteen. The trap saturates; it has only so many hinges to

close. This is why raw concentration is the wrong thing to chase and the wrong thing to report. A fruit can hold twelve times the free glutamate of another and taste only a little louder, because both of them sit up on the flat shoulder of the same curve. Loudness is not the amount of the molecule — it is where on this curve the molecule puts you, and the fairest single number for that is the half-point itself.

From a curve to a number — the Index

So we score the half-point and invert it. The Iyengar Flavor Index reads each axis as

```
IFI(c) = 1000 × EC₅₀(reference) / EC₅₀(candidate),
```

which collapses an entire dose–response curve into one loudness number. A stimulus exactly as potent as the 3.0 mM reference scores 1,000, by construction. A stimulus that reaches half-activation at a *lower* concentration is more potent — louder — and scores higher; one that needs a higher concentration scores lower. The arithmetic is deliberately plain so that a tomato, a chili, and a cup of tea can be read against one another on a single page.

Work it directly. A savory stimulus that half-activates the trap at 1.5 mM scores 1000 × 3.0 / 1.5 = **2,000** — twice as loud as plain glutamate, because it takes half as much to reach the same half-closed trap. One that needs 6.0 mM scores 1000 × 3.0 / 6.0 = **500** — half as loud. The formula is nothing more than a ratio of half-points, scaled by a thousand, and that is exactly why it is honest: it compares potencies, not quantities, so it cannot be gamed by simply loading a food with more of a mute, bound-up molecule.

Turn it on the fruit we care about most. A vine-ripened tomato that has been picked green and trucked across a continent arrives with most of its savor gone — the glutamate pool never filled, because the fruit was taken before the metabolism that fills it could finish. Read against the reference it lands near **IFI-U 300**, which the formula tells us is an effective half-point of 1000 × 3.0 / 300 = **10.0 mM**: you would need more than three times the reference concentration to make that tomato speak. Restore the pool — grow the glutamate back in through the fruit's own machinery — and it climbs to **IFI-U 2,600**, an effective half-point of 1.15 mM, which is *louder than plain glutamate itself*, because a real tomato brings more to the trap than glutamate alone.

STIMULUS	EFFECTIVE EC_{50}	IFI-U	WHAT THE NUMBER SAYS
Tomato, picked green and trucked	10.0 mM	300	needs ~3× the reference to be heard
L-glutamate reference	3.00 mM	1,000	the anchor, by definition
Phoenix · SS-0096	1.67 mM	1,800	umami written beside the heat
Tomato, restored	1.15 mM	2,600	louder than plain glutamate — synergy

The loudness gap between the trucked tomato and the restored one is 2,600 / 300 = **8.7×**, and it is worth being precise about what that is and is not. It is an 8.7-fold gap in *loudness*, not in glutamate.

Because the dose–response saturates, the underlying difference in free glutamate is larger than 8.7× — the fruit's chemistry has to move more than the receptor's answer does. The Index reports the answer, not the chemistry, because the answer is what the tongue actually receives. Ninety-three percent of a restored tomato's savor is what the truck takes; the loop's job is to give the fruit a way to carry it to the plate.

The multiplication — glutamate and the nucleotides

A real tomato beats pure glutamate because savor multiplies. The 5′-ribonucleotides a ripening fruit accumulates — inosinate (IMP) and guanylate (GMP), the same compounds that make a dried shiitake or a fold of kombu taste so deep — bind the Venus flytrap at a second site, just inside the lip of the closed trap, and lock it shut. They are not themselves loud; they are a catch that holds the hinge. With the catch engaged, glutamate's half-point falls, sometimes steeply, and the two together ring far above their sum.

The classic measurement of this, made on MSG and IMP, gives the glutamate-equivalent concentration of a mixture as

```
γ = u + 1218 · u · v,
```

with *u* the glutamate and *v* the nucleotide, each in grams per 100 mL. Put equal small amounts of each, u = v = 0.05 g/100 mL, and the equivalent concentration is γ = 0.05 + 1218 × 0.05 × 0.05 = 0.05 + 3.045 = **3.095 g/100 mL** — the pair tastes like roughly sixty times the glutamate working alone. Read as a shift in the half-point, the nucleotide at that level lowers glutamate's effective EC_{50} by a factor of (1 + 1218 × 0.05) ≈ 62. It is the most dramatic synergy in all of taste, and it is why a spoonful of tomato in a mushroom broth does something a spoonful of either cannot.

We state the honest ceiling in the same breath. That sixtyfold is a multiplication in *equivalent-concentration* space, not in perceived loudness, and the trap still saturates. Drive the equivalent concentration far up the curve and θ climbs toward its flat shoulder, not toward sixty times anything; the receptor has a maximum and the synergy only helps you reach it with less. So the loop treats the nucleotide pool and the glutamate pool as two levers on one half-point, and spends its edits where the curve is still steep — on the free glutamate the fruit can actually hold — rather than chasing a multiplication the tongue would never finish hearing.

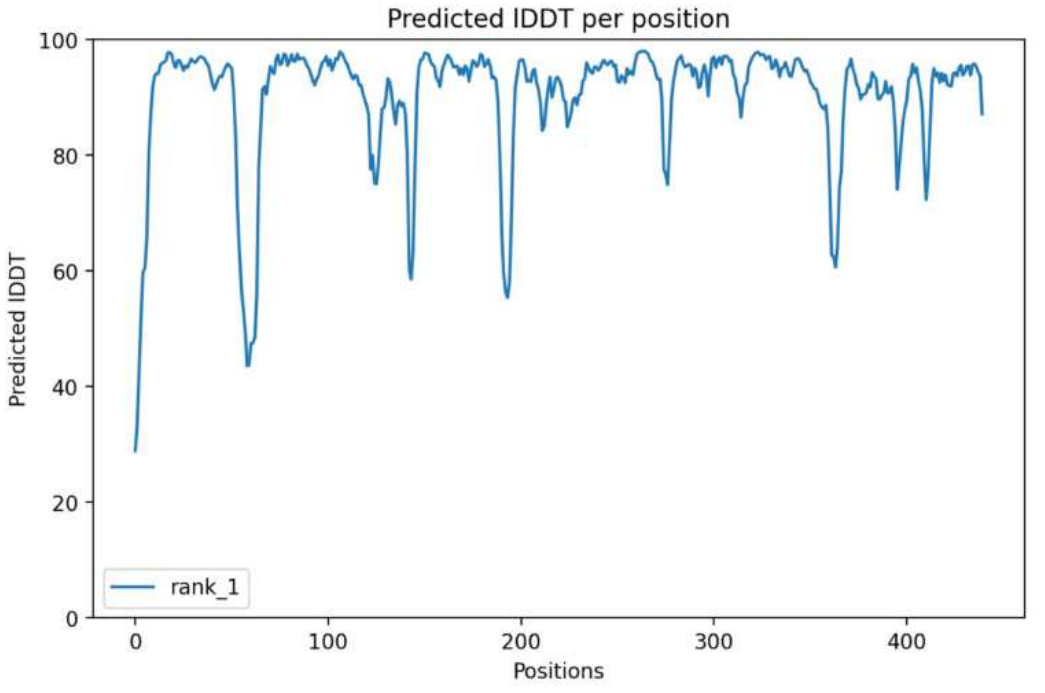
The first instrument — a protein language model

Now the method, starting where a sequenced genome lets us start: with the proteins. An **ESM2** network of 650 million parameters, trained on the evolutionary record of hundreds of millions of protein sequences, has learned the grammar of what a working protein is allowed to be. We use it as a mutation scorer. For any single-residue substitution in a target enzyme, the model reads the surrounding sequence and returns a log-likelihood for the wild-type residue and for the proposed one, and the difference,

```
score = log P(mutant | context) − log P(wild-type | context),
```

is a zero-shot prediction of whether evolution would tolerate the change. A score near zero or above means the substitution sits comfortably inside the space of sequences nature has already accepted; a strongly negative score means the model has almost never seen a working protein carry that residue there, and we should expect the fold to break. Point it at glutamine synthetase, at GOGAT, at the GABA decarboxylase we want to ease, and it returns a ranked map of the edits worth making before a single plant is grown — reading, from evolution's own statistics, which changes a protein will survive and which will undo it.

The language model tells us what is *tolerable*; a second read tells us what is *trustworthy*. For each candidate enzyme we build the structural model and color it by per-residue confidence, the pLDDT score, so we know which of the model's own predictions to believe.



The Pun1/AT3 acyltransferase, modeled and colored by per-residue confidence (pLDDT) — the same structural-modeling instrument that scores every candidate edit, whether the target is a heat enzyme or a glutamate one.

High confidence runs blue through the catalytic core and the binding cleft — the parts of the protein that matter for an edit's effect — and falls to orange in the flexible surface loops, where neither the model nor evolution has a strong opinion and where we therefore make no claims. An edit the language model loves but that lands in an orange, low-confidence region is not an edit we write; it is a guess wearing the costume of a prediction. This is the first place the loop refuses to fool itself.

The second instrument — the carbon budget

A protein model tells you which enzymes you *can* change. It does not tell you which one is actually *limiting* the savor, and here intuition is almost always wrong. For that we build a genome-scale, stoichiometric model of the whole organism's metabolism — every reaction, every mass balance — and solve for the flux distribution that the plant can physically sustain, asking the one question that matters: where is the bottleneck on free glutamate?

The answer is rarely at the glutamate-building enzymes. It is at the carbon they draw from. Glutamate in a plant is assembled by the GS/GOGAT cycle:

> **GS:** glutamate + NH_4^+ + ATP → glutamine **GOGAT:** glutamine + 2-oxoglutarate + reductant → 2 glutamate

so that the net of one turn pulls a molecule of **2-oxoglutarate** — the carbon skeleton — out of central metabolism and carries ammonium on it to make glutamate. And 2-oxoglutarate is precisely the molecule the TCA cycle needs to make the plant's energy. Every unit of it is claimed twice: once by respiration, which burns it for ATP, and once by nitrogen assimilation, which spends it on glutamate. The savor we want and the energy the fruit needs are drinking from the same cup.

Make the partition concrete, in normalized flux units. Say a fruit at peak respiration produces 100 units of 2-oxoglutarate, and its maintenance demand requires that at least 80 of them flow down the TCA cycle for energy. That leaves at most 20 for assimilation — the four-fifths-to-one-fifth split a working fruit actually runs. Now try to force more savor by lifting the assimilation enzymes at peak, grabbing 40 units for glutamate: the energy side is starved to 60, below its floor of 80, and the flux model shows exactly what the plant would do — stall its ripening, buckle its respiration, and surrender the gain. But the *same* shift, applied in the narrow late window when the fruit's growth is finished and its respiratory demand has fallen by around 40%, is one the budget can absorb. The energy floor drops to roughly 48, the assimilation ceiling rises to 52, and glutamate-building flux climbs 2.6-fold with no deficit anywhere in the cell.

WINDOW	2-OG SUPPLY	TCA FLOOR (ENERGY)	ASSIMILATION CEILING (GLUTAMATE)	GLUTAMATE FLUX VS. PEAK
Peak respiration	100	80	20	1.0×
Naïve push, at peak	100	60 — deficit	40	fails (respiration buckles)
Late ripening window	100	48	52	2.6×

Normalized flux units; the figures illustrate the partition the solver optimizes. The model's whole job is to find that window and that share — the largest savor the carbon budget will fund without a shortfall anywhere else — and it finds it by solving for flux, not by pushing on the enzyme that seems obvious. It is not a search for the biggest edit. It is a search for the edit the plant can afford, in the single stretch of its life when it can afford it.

The third instrument — the edit, and what the fold will hold

With the target chosen and the window found, **CRISPR** writes the change into the plant's own genome, at the loci the first two instruments identified. We tune native pathways; we import no foreign savor, no gene the plant did not already carry. The edit set for free glutamate is small and legible: ease the decarboxylase that drains glutamate down the GABA shunt, lift the assimilation enzymes that fill the pool, and steady the dehydrogenase that buffers it, so the savor holds through ripening instead of spiking and fading. A handful of precise changes to the plant's own circuitry, modeled before they are made.

Each edit carries a predicted stability cost, the change in folding free energy ΔΔG, and the sign of that number is the thing to read. A negative ΔΔG is stabilizing — an edit the fold will tolerate and hold — and its magnitude maps, through Boltzmann, onto how hard the protein now prefers its folded state. At body-mild temperature, RT ≈ 0.593 kcal/mol, so a stabilizing edit multiplies the folded-to-unfolded ratio by exp(−ΔΔG / RT).

TARGET	EDIT CLASS	MODELED EFFECT	ΔΔG (KCAL/MOL)	FOLD-STABILITY FACTOR
Assimilation enzyme (GS/GOGAT)	activity ↑	larger free-glutamate pool	−1.9	24.6×
Decarboxylase (GAD branch)	activity ↓	less glutamate drained to GABA	−1.4	10.6×
Pathway buffer (GDH1)	stability ↑	steadier pool through ripening	−0.8	3.85×

Read the first row: a ΔΔG of −1.9 kcal/mol means exp(1.9 / 0.593) = exp(3.20) ≈ **24.6×** — the edited enzyme is nearly twenty-five times more biased toward its working, folded shape than the stabilizing change alone would suggest. Taken together the three edits sum to −4.1 kcal/mol, a combined fold-stability factor of exp(4.1 / 0.593) ≈ **1,006×**: an edit set the protein does not merely survive but settles into. This is the same calculus that informed our heat design, where two substitutions in a single acyltransferase — **S39L** (−2.1) and **L345G** (−1.8) — sum to −3.9 kcal/mol and a combined factor near 718×, and our model predicts peak capsaicinoid output at approximately 2.94 million SHU, at a design target batch-to-batch CV of ≤7%, against 42% in the wild type. Different enzyme, different fruit, identical arithmetic. The point of the table is never a single number; it is the shape of the method — *model, rank, balance, write, grow, measure* — and then feeding the measured loudness back to correct the model.

Five modules that were never about peppers

The reason a method proven on fire could restore a tomato's savor is that the platform was built as seven modules, and only two of them are ever truly bespoke: the specific target pathway — glutamate assimilation here, capsaicinoid synthesis in the pepper — and the final cultivar backgrounds. The other five never cared what plant they were pointed at. The protein language model asks only for a sequence. The flux-balance solver asks only for a genome-scale reaction set. The CRISPR delivery, the receptor assay that reads the half-point, and the measurement-and-feedback loop that closes it

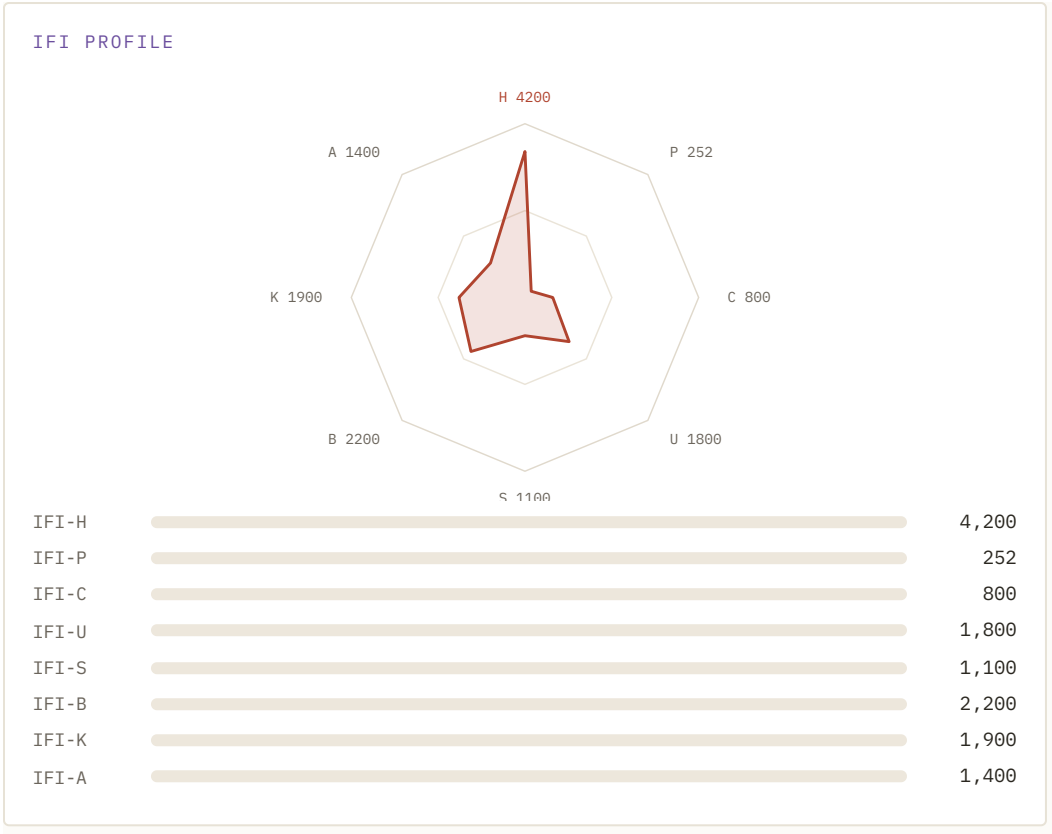
are all organism-agnostic; they want a sequenced genome and a receptor to read against, and nothing more. That is the whole reason the same loop reaches a tomato's umami, a tea's theanine, a soybean's savory depth — and the reason it will reach the rest of the savory floor as fast as the genomes and the growth cycles allow.

UMAMI HOST	CONSTRUCTS	PRIMARY AXES	IFI-U STATUS
Tomato	2,040	S / U / F / M	300 → 2,600, computed
Soybean	360	U / K	in validation
Tea	280	U / B / A	in validation
Yeast	240	U / K	in validation
Koji mold	80	U	in validation
Shiitake	80	U	in validation

Forty-two host species are live across the platform today; the umami rows above are the ones whose savor runs through free glutamate and its partners, and only the tomato's savor target has been fully modeled end to end — the others are honest works in progress, which is exactly how we will report them until the receptor says otherwise. The proof that the loop reaches umami at all is already in the ground, in a plant we built to register on every axis at once.



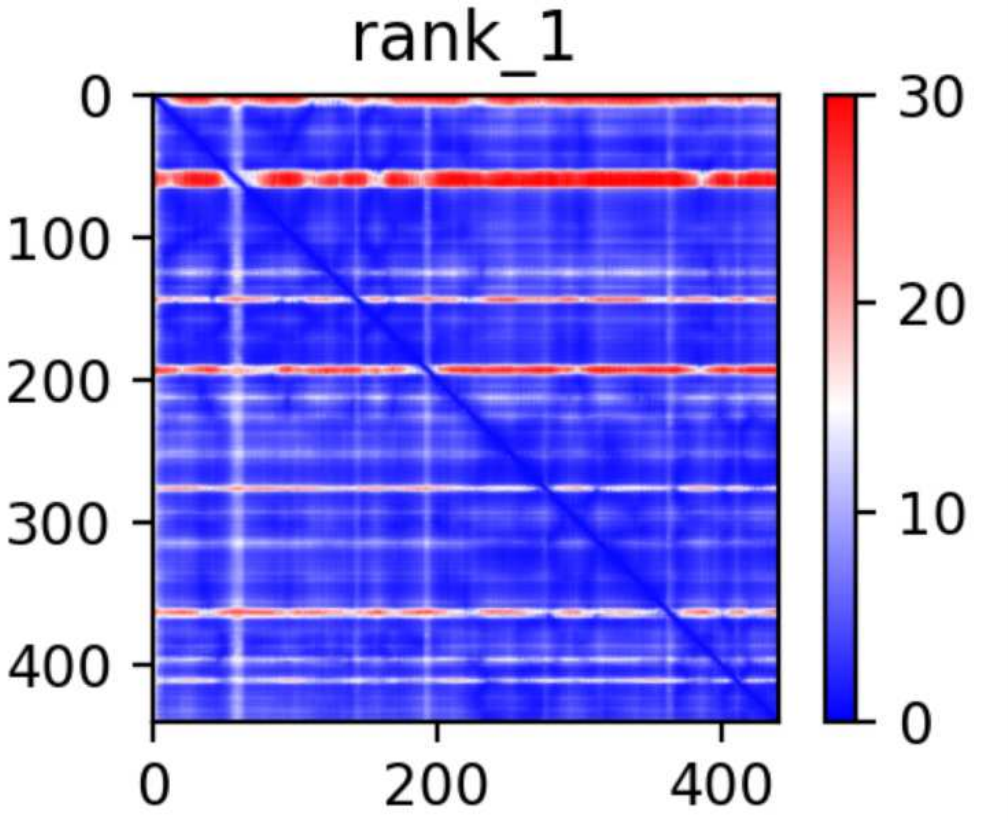
Phoenix (SS-0096) in the controlled chamber — the cultivar engineered to register on all eight axes, umami among them.



Read the fourth line. **U: 1,800** — an effective half-point of 1.67 mM — is umami written into a superhot pepper by the same three instruments that wrote its 4,200 of heat, the same ESM2 ranking, the same flux solver, the same edits. A fruit our model predicts at approximately 2.94 million Scoville that also carries real savory depth is not a thing any breeding program could have selected for; the two traits never travel together in nature. It exists because the loop does not select at all. It designs.

The referee — holding out, and the honest error

One discipline keeps the whole loop from becoming a mirror, and we borrowed it straight from machine learning: we **hold out** a fraction of every design round and test it blind. A model scored only on the edits it had already watched succeed would learn to flatter itself. So each round, some predicted-good and some predicted-poor edits are built and measured without the model being told which it expected to win, and the measured loudness is compared against the prediction. The gap between predicted and measured is the model's error, and shrinking it — round over round, organism over organism — is how the platform earns the right to be believed before a plant is grown.



The predicted aligned error map for the same modeled acyltransferase — which parts of the structure are placed reliably relative to which others, and therefore which predicted edits can be trusted.

Error is not a thing we gesture at; it is a thing we carry through the arithmetic. The coefficient of variation — the standard deviation over the mean — is the cleanest honest summary of how reproducible a savor is. For a free-glutamate target with a mean of 150 mg/100 g, a wild-type line at 42% CV scatters with a standard deviation of 63 mg/100 g; the engineered line at ≤7% holds to 10.5. Across eight batches the standard error of the mean falls to 22.3 for the wild type and 3.7 for the edited line — the difference between a savor you can formulate around and one you cannot. The variance is lower for the same reason the whole loop works: breeding fixes a phenotype on top of a genome still free to vary, while a written edit fixes the genotype, and the distribution tightens because its cause is no longer left to chance.

That error rides through to the loudness number itself. Because IFI is a ratio of half-points, its relative error is the two measurements' relative errors added in quadrature: with both the reference and the candidate carrying a 7% CV, the Index value carries $\sqrt{(0.07^2 + 0.07^2)} \approx 9.9\%$. So Phoenix's **U: 1,800** is really 1,800 ± ~180, a band from roughly 1,620 to 1,980 — a number you can stand on. The trucked tomato's 300, measured on an unimproved line at 42%, spreads from about 174 to 426; it overlaps half the things you might compare it to, which is the quantitative way of saying that savor you did not design is savor you cannot promise.

The failure modes are worth naming plainly, because the loop is defined as much by what it refuses as by what it writes. Slam the GABA decarboxylase to zero instead of easing it, and you win glutamate at the cost of the plant's stress tolerance — the GABA shunt is not waste, it is a buffer, and a fruit that cannot make it is a fruit that cannot weather a cold night; so we lower that enzyme, never delete it. Push the assimilation enzymes outside the late window and the carbon budget starves respiration, as the flux model warns. Trust a language-model edit that lands in a low-confidence

loop and you have believed a guess. And chase the nucleotide synergy past the shoulder of the dose–response and you spend edits buying loudness the tongue has already stopped hearing. Every one of these is a place the model would happily lead us wrong, and every one is caught by a second instrument checking the first.

A century of breeders, selecting savor they could not see, never had a chance at this — not for lack of care, but because the thing they were optimizing appeared too late and too faintly to read. We did not out-work them. We changed the thing being optimized, from a phenotype you taste after the harvest to a genotype you can read before the sowing, and we kept one referee above all the models: the receptor. The model only ever proposes. The trap, closing on a single molecule of the plant's own glutamate, disposes — and tells us, at the end, whether we read it right. These are computational designs; Scoville Splice's cultivars and constructs have not been experimentally validated in a laboratory.

Chapter 14 · Precision — *the same savor in August and February.*

the CV ≤7% story · the assay · why formulators need it

A flavor you cannot reproduce is a demonstration, not an ingredient. This is the hard, unglamorous truth that separates a beautiful tomato from a thing a manufacturer can build a product on, and it is where a great deal of our quietest and most important work lives. A chef can celebrate the drift of a heirloom from one week to the next — the way a late-summer fruit carries a savor its June self never had. A formulator building a shelf-stable soup that must taste identical across a national rollout and a two-year shelf life cannot. For them, consistency is not a nicety laid over the flavor. It is the whole difference between an ingredient they can specify and a story they cannot use. We came to revere that difference the way we revere the savor itself, because a savor held steady is a kind of care — a promise to the person at the far end of the supply chain that the broth will taste the same when they are tired and the pantry is low as it did the first time it made them feel looked after.



A research tray under fixed light — the controlled-environment discipline that lets a savory reading hold to within seven percent, lot after lot, from the August harvest to the February one.

A number worth keeping faith with

Set the target in numbers, because the gap between what nature hands you and what a formulator needs is enormous. A conventionally bred crop expresses its key flavor compounds with a batch-to-batch **coefficient of variation** — the standard deviation written as a percent of the mean — that routinely runs into the low forties. A wild-type fruit's free-glutamate content, measured honestly across a season, swings by roughly that much, because it rides a seasonal substrate, an ambient sky, a soil that was never the same two years running, and a genome shuffled fresh at every

cross. We hold our cultivars to a **CV at or under seven percent.** That is not an incremental tightening; it is a different category of material. A savory ingredient that varies by seven percent is one a formulator can dose once and trust. One that varies by forty percent is one they must re-assay every lot and over-engineer around — buying extra savor to cover the low batches, then wasting the very depth they paid for on the high ones.

The reason seven is reachable at all is that we invert the usual order of flavor-making. Conventional breeding selects a phenotype and hopes the genotype follows; the savor is an emergent property of a thousand uncounted alleles, and its variance is the variance of that lottery. Our savor comes instead from defined edits to known pathways, so every plant of a given line carries the identical circuitry. That single move removes the dominant source of the wobble before a single environmental variable is touched — and everything that follows in this chapter is the campaign to close what remains.

What the tongue is actually reading

To hold a number steady you must first know, exactly, what the number is measuring — and umami is read by a piece of machinery worth describing in full, because every later discipline is in service of its quirks. Savory is sensed by two proteins working at different scales. **mGluR1** is a metabotropic glutamate receptor of exquisite, micromolar sensitivity. The **TAS1R1·TAS1R3** heterodimer is the frank taste receptor of the tongue, answering at millimolar concentrations but carrying most of what you consciously register as *savory.* Both are class C G-protein-coupled receptors, and both share one beautiful piece of apparatus: a large extracellular domain shaped like a Venus flytrap, two lobes hinged around a cleft.

Free glutamate — the carboxylate-bearing amino acid, its side-chain acid group open and charged, not the capped amide of its cousin glutamine — settles into that cleft and pulls the two lobes shut. The closure is the signal. It travels down through a cysteine-rich linker into the seven-transmembrane bundle, which rolls against its bound G-protein and releases it; inside the cell the cascade runs α-gustducin → phospholipase C-β2 → inositol trisphosphate → a pulse of calcium out of the endoplasmic reticulum → the opening of the TRPM5 channel → depolarization → a squirt of ATP through the CALHM1 channel onto the waiting afferent nerve. A molecule you cannot see becomes a current you cannot ignore. The relay repeats a billion times a second across the surface of the tongue, and the round, brothy, cared-for sensation is the sum of it.

Two features of that flytrap govern everything a precision program has to manage. The first is **cooperativity.** Because the mature receptor is a dimer, the closure of one subunit biases the other, so the dose-response is steeper than simple one-to-one binding would give — the curve is sigmoidal, and near its half-point a small change in glutamate swings the receptor hard from quiet to loud. The second is **synergy.** A separate pocket on the flytrap, just outside the glutamate site, binds the 5′-ribonucleotides — inosinate (IMP) and guanylate (GMP). When a ribonucleotide docks there it acts as a positive allosteric modulator, wedging the flytrap toward its closed

shape and lowering glutamate's half-activating concentration severalfold. This is the ancient secret of a good dashi, of a tomato cooked down with a mushroom, of aged cheese over a slow ragù: two modest savory signals multiplying into one enormous one.

There is a third protein the chapter's measurements never leave behind, because the savor we sell is never glutamate alone. **Kokumi** is read at the **calcium-sensing receptor, CaSR,** where γ-glutamyl peptides — glutathione, γ-Glu-Val-Gly and their kin — dock as allosteric agonists. Kokumi carries almost no taste of its own; what it does is lend *continuity,* mouthfeel, and the long sustain that makes a broth feel whole rather than merely strong. It is the difference between a savor that arrives and a savor that stays. Because a plant builds glutamate, nucleotides, and γ-glutamyl peptides on three different enzymatic routes that respond differently to heat, light, and time, holding the *experience* steady means holding all three — which is why a reading we publish carries both an umami and a kokumi figure, and why the discipline cannot stop at a single compound.

The savor a plant has to build

Before any of that can be measured it has to be grown, and the savor is the output of an enzymatic economy inside the ripening fruit. Glutamate is pooled through the plant's own nitrogen machinery — the **GDH1** glutamate-dehydrogenase step interconverting α-ketoglutarate and ammonium with glutamate, and the **GAD** decarboxylase bleeding glutamate off toward GABA — a balance that tips, in a properly ripened tomato, toward a late surge of free glutamate in the last days on the vine. The nucleotide partners arrive on a separate schedule, as ripening nucleases free 5′-IMP and 5′-GMP from the fruit's own RNA. The γ-glutamyl peptides accumulate on a third track entirely. Three clocks, loosely coupled, each sensitive to a different stress — and the savor you taste is where their hands happen to point at the moment of harvest.

That is precisely the fragility a controlled environment exists to remove. A plant's late-ripening glutamate window, which in an open field is at the mercy of a cloudy fortnight, is in a controlled chamber a scheduled event: fixed light spectrum and intensity, fixed temperature and humidity, fixed nutrient delivery, fixed harvest read against the ripening curve rather than the calendar. Each variable pinned is one of nature's three clocks held still. We run this discipline across every host on which we engineer savor, and the organisms differ in how much of the work is theirs to do.

UMAMI HOST	CONSTRUCTS	PRIMARY AXES	ENGINEERING DIFFICULTY
Tomato	2,040	S · U · F · M	Easy
Soybean	360	U · K	Frontier
Tea	280	U · B · A	Hard
Yeast	240	U · K	Hard
Koji mold	80	U	Hard
Shiitake	80	U	Hard
Mushroom	40	U	Hard

The tomato is the easy host and the flagship of this axis — two thousand and forty constructs, savory and sweet braided in one fruit — because its native glutamate machinery is already generous and

wants only steadying and amplifying. Soybean and yeast pair umami with kokumi on the same organism, carrying both of the figures a savory base needs. The mushrooms and the koji are small-construct, high-difficulty hosts we keep for the particular shape of savor each one makes. Every one of them is grown toward the fullest savor, and the fullest nourishment, it can carry — and then assessed the same way.



Tomato, in cross-section — the late-ripening glutamate window, the nucleotides freed from the fruit's own RNA, and the kokumi peptides, three clocks a controlled chamber holds still so the savor lands on schedule.

Loudness, not quantity

The number we hold is not an amount of glutamate. It is a loudness — how forcefully the savor speaks to the receptor — and the whole index is built to read that. Every axis is anchored to the half-maximal activation of its receptor, the concentration at which a stimulus drives the receptor to half of full response.

> $\mathrm{IFI\text{-}U} = 1{,}000 \times \mathrm{EC_{50}}(\mathrm{reference}) \div \mathrm{EC_{50}}(\mathrm{candidate})$

Read it slowly. $\mathrm{EC_{50}}$ is a concentration, and a *lower* one means a *more potent* stimulus — less of it needed to half-open the receptor. Put the reference's $\mathrm{EC_{50}}$ on top and the candidate's underneath, and the ratio inverts potency into loudness: the more potent the candidate, the smaller its $\mathrm{EC_{50}}$, the larger the score. Scale by a thousand so a tomato, a broth, and a cup of tea can be read on one page. We anchor the umami axis on free glutamate at mGluR1 at **10 µM** — our stated convention constant for this axis, the declared reference point that places IFI-U at **1,000** — and we fix that anchor at **IFI-U 1,000** by convention. Because the index is a ratio, the anchor's exact value washes out of every comparison; we quote 10 µM only to make the micromolar world concrete.

Now work a real reading. A dedicated savory base reads **IFI-U 2,900.** Rearrange the formula for the candidate's $\mathrm{EC_{50}}$:

> $\mathrm{EC_{50}}(\mathrm{base}) = \mathrm{EC_{50}}(\mathrm{ref}) \times 1{,}000 \div \mathrm{IFI\text{-}U} = 10\ \mu\mathrm{M} \times 1{,}000 \div 2{,}900 = \mathbf{3.45\ \mu M}$

The base reaches half-activation at about three and a half micromolar — **2.9× more potent than the anchor.** The same arithmetic lays every savory source on one ladder, each canonical

IFI-U converted back into the EC_{50} it implies and the multiple of the anchor it represents.

SOURCE	IFI-U	IMPLIED EC_{50} (MM)	× ANCHOR
Reference anchor (glutamate at mGluR1)	1,000	10.00	1.0×
Tomato, truck-ripened (depleted)	300	33.33	0.3×
Phoenix · SS-0096 (full-spectrum flagship)	1,800	5.56	1.8×
Tomato, restored	2,600	3.85	2.6×
Designed savory base (target)	**2,900**	**3.45**	**2.9×**

The ladder tells a whole story at a glance. A tomato that rode a refrigerated truck for a week reads *below* the anchor — its savor idling at three-tenths, a depletion the field never finished and the supply chain made worse. Restore that tomato through its native glutamate route and it climbs to 2.6×, a potency gain of 2,600 ÷ 300 ≈ **8.7-fold** over the truck-worn version. The base built for savory work sits a step higher again, at 2.9×, reaching half-activation at roughly a third of the reference's concentration — which, converted to mass through glutamate's molar mass of 147.13 g/mol, is about **0.51 mg/L at the receptor** against the anchor's 1.47 mg/L.

The receptor's steepness is what makes that reading sharp rather than smeared. Any stimulus at a cooperative receptor follows a Hill relationship:

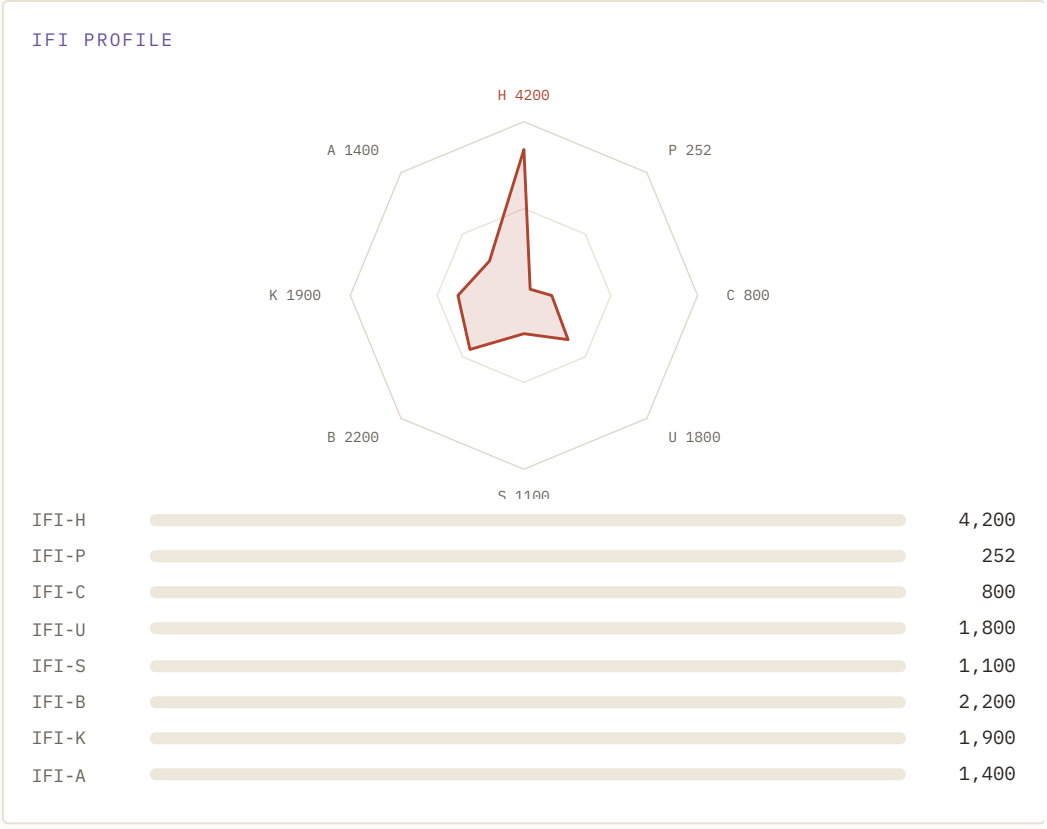
$$R = R_{max} \times [C]^n \div (EC_{50}^n + [C]^n)$$

where [C] is the glutamate concentration, EC_{50} the half-point, and n the Hill coefficient — the cooperativity of those twinned flytraps, typically between 1.5 and 2 for a class C dimer. Take n = 1.8 and raise the concentration from half the EC_{50} to the full EC_{50}. The response climbs from $0.5^{1.8} \div (1 + 0.5^{1.8}) \approx$ **0.22** of maximum to exactly **0.50** — a jump of more than two-and-a-quarter-fold for a mere doubling of glutamate. Savor does not rise gently and forgivingly; near the half-point it climbs a cliff. That steepness is why a reading has to be precise: an error in concentration is magnified in perceived loudness, and a plant allowed to wander even slightly in its glutamate pooling can swing from thin to blaring across a single step of the curve.

The instrument, on a calibrated leash

The measurement has to be as disciplined as the growing, or the number means nothing. IFI-U is read on a **calibrated receptor assay** — a reporter cell line expressing the umami receptor, its response to a sample quantified against a glutamate reference standard run alongside every batch. The reference is the whole point of the method. Because the index is a ratio of the reference's EC_{50} to the candidate's, and because we run the reference *in the same plate, on the same day, through the same reporter* as the sample, the two readings share every source of instrument drift — reporter passage number, incubation temperature, the day's luminometer gain — and the drift divides out of the ratio. A reading is never an absolute response; it is always a sample measured against its own anchor.

Two further disciplines keep the number honest to the savor a mouth will actually meet. The first is that we read the **composite extract,** not a purified compound. A reporter fed clean glutamate would report only glutamate; a savory base is glutamate *and* the 5′-nucleotides *and* the γ-glutamyl peptides, and their synergy is most of the experience. So the assay is run on the whole savory package the lot will deliver — the extract that carries the synergy pocket's partners along with the agonist — and the EC_{50} it returns is the EC_{50} of the composite, synergy included. The second is a known-value **control sample** run as a check on the whole chain. Our full-spectrum reference material, Phoenix — SS-0096, the cultivar designed to register on all eight axes at once — carries a computationally defined profile, and when it goes through the assay it must come back reading what it is known to read or the instrument is declared out of calibration and the day's data held.



Those are Phoenix's eight canonical numbers; the two that matter to this axis are its umami at **1,800** and its kokumi at **1,900.** When the control returns those two figures within tolerance, we trust the plate; when it does not, we stop. Running a known full-spectrum profile as a control does something a pure-glutamate standard cannot: it confirms the assay still reads *both* savory figures correctly on a real, complex matrix, so a batch certified for umami has not quietly lost its kokumi to a drifting instrument.



A reporter line under the microscope stage — the umami receptor expressed in culture, its response to each lot read against a glutamate standard run in the same plate, so instrument drift divides out of the ratio.

The tongue that keeps the instrument honest

A receptor assay with no tongue behind it drifts into abstraction, so we keep a **trained descriptive panel** — tasters calibrated against reference standards until their reports are reproducible, scoring savory intensity, duration, and the body-versus-note balance on anchored scales rather than by whim. Their palates are checked against known concentrations the way a laboratory scale is checked against known weights; a taster who has drifted is recalibrated, not trusted, and the panel's own reproducibility is tracked as rigorously as the instrument's.

The panel exists not because the instrument is unreliable but because perception has dimensions a single EC_{50} cannot hold. A savor has a *shape* over time — the point at which it arrives, the plateau where it sits, the slow fade as it leaves, the way it reads under salt, the continuity the kokumi lends underneath it all. The assay returns one number for loudness; the mouth lives the whole curve. When the assay says a batch is on-target and the panel says it reads thin, that disagreement is not noise to be averaged away — it is data, and usually a precise diagnosis. It means the glutamate held but a nucleotide or a kokumi peptide did not: the agonist is there to half-open the receptor, so the instrument reads full, but the synergy pocket is empty and the sustain is gone, so the mouth reads hollow. A composite that has lost its partners can score well and taste poorly, and only two readings kept in tension will catch it. When the instrument and the tongue diverge, we investigate rather than average, because the divergence is pointing straight at the compound that failed.

The arithmetic of consistency

Consistency is a number too, and it is the one this chapter is named for. We measure it as the coefficient of variation — the batch-to-batch standard deviation over the mean, as a percent:

```
CV = σ ÷ μ × 100%
```

The power of writing it this way is that the structural reason for the wobble becomes visible. A savor is not a single measurement but the sum of several independent sources of variation, and when independent sources combine, their relative variances add *in quadrature* — the squares add, not the magnitudes:

```
CV_total ≈ √( CV_genetic² + CV_environmental² + CV_developmental² + CV_analytical² )
```

That one equation explains both the wild number and the engineered one. In a conventionally grown crop the four terms might run, illustratively, near 38% genetic, 15% environmental, 12% developmental, and 5% analytical — and $\sqrt{(38^2 + 15^2 + 12^2 + 5^2)} \approx$ **43%,** the low-forties wobble nature actually hands you. Notice what the quadrature reveals: the genetic term alone, at 38%, very nearly sets the whole; the other three sources together only lift it from 38 to 43. The breeding lottery is not one contributor among four. It is the mountain, and the rest are foothills.

Which is why the fixed genotype is the single largest lever we own. Driving the genetic term to essentially zero — every plant of a line carrying identical circuitry — removes the dominant square from under the root before any other discipline is applied. What remains is the environmental, developmental, and analytical variation, and the controlled chamber, the ripening-curve harvest, and the anchored assay pin each of those to the low single digits. Hold them to roughly 4%, 4%, and 3% and the arithmetic closes: $\sqrt{(0^2 + 4^2 + 4^2 + 3^2)} = \sqrt{41} \approx$ **6.4%,** inside the ≤7% ceiling with room to spare. The seven-percent promise is not a slogan; it is the square root of a sum we engineer term by term.



Phoenix in the controlled chamber — fixed light, temperature, humidity, and nutrient delivery, each pinned variable one of nature's clocks held still, so the variance that survives is small enough to live under the root.

Read that difference the way a formulator feels it. Take a base that *averages* IFI-U 2,900, and watch what each CV does to the band around that average.

QUANTITY AT IFI-U 2,900	NATURE (CV ≈ 42%)	OUR CEILING (CV ≤ 7%)
Standard deviation σ	1,218	203
±1σ band (≈68% of lots)	1,682 – 4,118	2,697 – 3,103
±2σ band (≈95% of lots)	464 – 5,336	2,494 – 3,306
Variance σ²	1.48×10^6	4.12×10^4
Share of lots within ±10% of target	≈ 19%	≈ 85%

At nature's 42%, a base that averages 2,900 delivers anywhere from a thin 1,682 to a blaring 4,118 across two lots in three — and its two-sigma spread runs to 5,336, past the index's own per-axis normalization ceiling of **5,000,** meaning the raw wobble alone would occasionally push a lot clean off the top of the scale. Fewer than one lot in five lands within ten percent of target. Steadied to seven percent, the same average holds inside a narrow 2,697–3,103, and roughly six lots in seven arrive within ten percent on the first reading. The gain is not louder savor. It is *knowable* savor. The standard deviation has fallen sixfold and the variance — the square — thirty-six-fold, because $(42 \div 7)^2 = 36$, and that thirty-six-fold collapse of the spread is the whole thing a formulator is buying.

Where seven is a ceiling, not a boast

Honesty about the number means naming its limit. Seven percent is extraordinary against nature's forty, but it is a guaranteed envelope, not a claim that any spec is easy to hold. Process capability makes this concrete. For a demanding specification of ±10% around a 2,900 target — an upper-minus-lower spec width of 580 — a process at exactly 7% CV has σ = 203 and a capability of

$$C_p = (USL - LSL) \div 6\sigma = 580 \div (6 \times 203) \approx \mathbf{0.48}$$

which is not, by itself, a capable process for that tight a window. To reach $C_p = 1.00$ against ±10% you would need $\sigma \le 580 \div 6 \approx 97$, a CV near 3.3%; for the comfortable $C_p = 1.33$ most manufacturers want, nearer 2.5%. We say this plainly because it is the reason the ≤7% ceiling is a floor of acceptability rather than a resting place. Two things carry the rest of the way. The first is that quality-by-design centers the *mean* exactly where it was placed — a centered process at modest spread beats an off-center one at tight spread every time, because a formulator can dose against a dependable center far more easily than against a moving one. The second is that for the tightest savory specifications we hold well under the published ceiling; seven percent is the promise we make to every lot, and the demanding work is routinely held tighter. The published number is the guarantee, not the typical.

What breaks a savor

A precision program earns its keep in the failure modes it anticipates, and umami has a cruel one that heat uncovers. The nucleotide partners that do so much of the synergy are phosphorylated molecules, and the thermal processing a real product meets — retort, UHT, a long hot hold — drives dephosphorylation: 5′-IMP and 5′-GMP shed their phosphate to become inosine and guanosine, which do not touch the synergy pocket and are, to the savory receptor, effectively silent. Glutamate

is robust and survives; the nucleotides may not. A base that read gloriously in the lab can arrive on the plate having lost the multiplier while keeping the agonist — scoring near target on a cold assay and tasting flat after the cook. The kokumi peptides are labile in their own way, sensitive to proteolysis and prolonged heat, so the long sustain can thin over a shelf life even as the headline umami holds. This is why stability is written into the specification and tested under the storage and thermal conditions the real product will meet, not just at time zero in a clean vial. A number that holds in the lab and drifts in the warehouse is a number that failed.

The other failure modes are the ones any careful measurement guards against, and we name them because the discipline is a campaign against each. *Genetic* variance — plant-to-plant difference in the trait — is closed by the fixed genotype, defined edits rather than a breeding lottery. *Environmental* variance — light, heat, water, nutrient drift — is closed by controlled-environment cultivation, every variable pinned. *Developmental* variance — the harvest caught at the wrong point on the ripening curve, before the glutamate window has fully opened or after the nucleotides have begun to turn — is closed by timing the harvest to the curve rather than the calendar. *Analytical* variance — drift in the measurement itself — is closed by the reference standard run with every batch, the known-value control, and the panel cross-check that catches the composite coming apart. An honest limit sits over all of it: a single loudness number cannot fully hold the *shape* of a savor over time, which is exactly why the trained tongue stays in the loop. The instrument guards the magnitude; the panel guards the curve; neither is trusted alone.

What the formulator actually receives

For the person building a product, all of this arrives in a form they already trust. Every lot ships against a **certificate of analysis** that carries its assessed IFI-U and IFI-K, its reference-standard calibration, and the key compound figures behind them — traceable back through the growth record to the genotype that produced it. That traceability is not bureaucracy. It is what lets a maker who qualified a savory base at a given reading build a year of production on it without re-assaying every drum, and what lets them answer their own customers and regulators with a number that has provenance. The arithmetic of the chapter is, in the end, the arithmetic of trust: with nature's forty-two percent, a tolerance of ±10% catches fewer than one lot in five, so you must test nearly every drum and blend to target, carrying the cost of a laboratory in the middle of your production line. At our ceiling of seven, roughly six in seven land inside that window on the first reading and the center sits where we placed it — so you qualify the base once and build on it. A flavor you can specify is worth more than a flavor you can merely taste. A flavor you can specify, document, and trace is the only kind a serious manufacturer can actually build on.

This is the quiet infrastructure beneath every savory number in this book. A reading of IFI-U 2,900 is only worth printing if the next ten thousand kilograms also read 2,900 — and the discipline that makes

that true, the fixed genotype and the pinned environment and the anchored assay and the tongue kept in tension with the instrument, is the reason a maker can take one of our numbers and build a year of nourishment on it. Savor, restored to the plant, is a gift. Savor restored *reproducibly* — the same depth in August and February, the same care in the ten-thousandth jar as the first — is a product, and the keeping of that promise is the least glamorous and most loving work we do. These are computational designs; Scoville Splice's cultivars and constructs have not been experimentally validated in a laboratory.

Chapter 15 · Clean Label, Honestly — *the MSG question, and the regulatory line.*

integrity, not safety · what the label can say

Nearly every savory brand that comes to us arrives carrying the same quiet wish: depth on the label without the additive on the label. We want to answer that wish precisely, and answering it honestly requires beginning with a correction that costs us nothing and earns the reader's trust: **monosodium glutamate is not a villain.** It is the sodium salt of the exact molecule a ripe tomato and an aged miso make for themselves — the same free glutamate, the same receptor, the same taste. The decades of fear around it never survived the science; the controlled, double-blind challenges did not reproduce the effects the folklore promised, and the food-safety authorities, having looked hard and more than once, classify it as safe. We will not trade in a scare we know to be false, because a family that revered the truth about its own chemistry everywhere else would forfeit that reverence the instant it sold a myth for margin. Our case against the shaker is built on something sturdier than fear, and the whole of this chapter is the building of it — the receptor, the arithmetic, the enzymes, and the line on the label we will not cross.



Koji-cured substrate — the endogenous route by which a bean's own glutamate reaches the plate, and labels simply as the plant it came from.

The molecule both roads reach

To see why the fear was always misplaced, follow the taste all the way down to where it is read. Savor is not a property of a food; it is an event at a protein. Free L-glutamate — the dissociated anion, carboxylate and amino group and a four-carbon backbone, 146.12 g/mol of it — drifts into the saliva and finds the umami receptors waiting on the apical membrane of the taste cell. There are two of them working the same ligand. The first is the heterodimer

TAS1R1·TAS1R3, a class C G-protein-coupled receptor; the second is a family of taste-tuned **metabotropic glutamate receptors**, chief among them **mGluR1**, class C GPCRs built on the same chassis. Both carry, at their business end, a bilobed extracellular clamshell called the **Venus flytrap domain.** Glutamate settles into the cleft between the lobes of TAS1R1, and the clamshell closes around it — open conformation to closed, the flytrap shutting on exactly one molecule.

That closure is the whole message. It propagates down through a cysteine-rich linker into the seven-transmembrane core, which rolls against its intracellular face and releases a G protein — gustducin and its relatives. From there the cascade is a chain of small, fast certainties: the freed Gα and Gβγ switch on **phospholipase C-β2**, which cleaves a membrane lipid into **IP_3**; IP_3 opens the **IP_3-receptor** channels on the internal calcium stores; calcium floods the cytosol; the rising calcium gates **TRPM5**, a cation channel, and the cell depolarizes; the depolarization opens **CALHM1/3** channels that release ATP straight across the membrane; and the ATP lands on purinergic receptors of the afferent nerve fibers — the chorda tympani, the glossopharyngeal — that carry the word *savory* to the brain. A molecule became a conformational change became a current became a sensation. Lock and key, repeated a billion times a second.

Here is the point the folklore never reckoned with. The receptor reads the glutamate anion and nothing else. It cannot see where the anion came from. It cannot see the sodium that rode in beside it when the glutamate arrived as a salt, because the sodium dissociates away in solution and never touches the flytrap. Monosodium glutamate, crystallized, is the glutamate anion plus a sodium counter-ion plus a water of crystallization — 187.13 g/mol of packaging for a 146.12 g/mol message. The glutamate a koji mold frees from a soybean's storage protein, the glutamate an heirloom tomato accumulates on the vine, the glutamate stirred from a shaker: at the Venus flytrap these are one molecule, indistinguishable, identical in every bond. That is not an argument *for* the shaker. It is the ground we have to clear before the real argument can stand: the quarrel was never with the molecule, because the molecule is the plant's own.

Anchoring a number to half-activation

If the taste is an event at a receptor, then it can be measured the way any receptor event is measured — by how little of the stimulus it takes to drive the receptor halfway to its maximum. That half-way concentration is the **EC_{50}**, and it is the quantity the whole Index is built on. A dose–response curve for glutamate at the umami panel follows the familiar sigmoid of the Hill equation,

$$R / R_{max} = [L]^n / (EC_{50}^n + [L]^n)$$

where *R* is the receptor response, *[L]* the ligand concentration, and *n* the Hill coefficient that sets the steepness of the rise. Set the response to half of maximum and the equation collapses to a single fact: $[L] = EC_{50}$. A low EC_{50} means the receptor answers to a whisper of the stimulus — the taste is *loud.* A high EC_{50} means it takes a

shout to be heard — the taste is faint. Loudness is the inverse of EC_{50}, and that inversion is exactly what the Iyengar Flavor Index turns into a score:

```
IFI-U = 1,000 × EC₅₀(reference) ÷ EC₅₀(candidate)
```

We anchor the umami axis by fixing a reference stimulus — a defined solution of free L-glutamate — at **IFI-U = 1,000.** Across the human umami panel, mGluR1 paired with the TAS1R1·TAS1R3 heterodimer, glutamate reaches half-maximal activation in the low millimolar range; for the arithmetic that follows we carry a worked reference half-activation of **EC_{50}(reference) = 6.0 mM**, the value the axis is calibrated against. Everything downstream is a ratio, and the ratio is all that matters: a candidate scores 1,000 when it needs the same 6.0 mM the reference does, and it climbs as its own EC_{50} falls.

Rearrange the formula and the EC_{50} behind any score drops out directly: EC_{50}(candidate) = 6,000 ÷ IFI-U (in mM). Work it for the numbers that matter in a savory book. **Phoenix** — the flagship we designed gene by gene to register on all eight axes at once — is predicted by our model to carry an umami voice of **IFI-U 1,800.** Its implied half-activation is 6,000 ÷ 1,800 = **3.33 mM**: it reaches the receptor's midpoint at a little over half the concentration the reference needs, which is to say it is 1.8× as loud. A long-haul commodity tomato, picked green and ripened in a truck, limps in near **IFI-U 300** — EC_{50} = 6,000 ÷ 300 = **20.0 mM**, so faint you would need nearly seven times the glutamate in the mouth to reach the same midpoint. The same species, restored on the vine to carry its savor the way it was bred to, reads **IFI-U 2,600** — EC_{50} = 6,000 ÷ 2,600 = **2.31 mM**, 2.6× the reference and almost nine times the loudness of the commodity fruit it shares a Latin name with. And the scorecard ceiling, the per-axis normalization maximum of **5,000**, sits at EC_{50} = 6,000 ÷ 5,000 = **1.20 mM** — the loudest a single axis is allowed to read before the radar saturates.

STIMULUS	IFI-U	IMPLIED EC_{50} (MM)	LOUDNESS VS. REFERENCE
Reference anchor	1,000	6.00	1.0×
Commodity tomato (long-haul)	~300	20.00	0.3×
Phoenix · SS-0096 (designed umami voice)	1,800	3.33	1.8×
Restored tomato	2,600	2.31	2.6×
Scorecard ceiling (normalization max)	5,000	1.20	5.0×

The unit conversion is worth doing once, because it carries the MSG point in numbers. One millimolar of free glutamate is 147.13 mg in a liter — the molar mass of L-glutamic acid. In a watery food near 1 g/mL, the 6.0 mM reference is about **88 mg of free glutamate per 100 g**, and the restored tomato's 2.31 mM midpoint is about **34 mg per 100 g.** These are *half-activation concentrations* — the yardstick against which a food is read — not the free-glutamate content of any one fruit, and we are careful never to let the two be confused. What the conversion makes vivid is the chemistry of the salt. The receptor closes on the 146.12 g/mol anion. Monosodium glutamate delivers that identical anion wrapped in roughly 40 extra mass units — a

sodium of 22.99 and a water of crystallization of 18.02 — none of which the Venus flytrap ever meets. The savor is the anion. The salt is a delivery truck. The quarrel, again, was never with the cargo.

The synergy the shaker cannot buy

Here the two roads part, and the parting is mathematical. Umami's deepest signature is not glutamate alone but **synergy.** The 5′-ribonucleotides — **inosine-5′-monophosphate (IMP)** and **guanosine-5′-monophosphate (GMP)** — carry almost no savor on their own. Yet where they meet free glutamate at the receptor they bind a second site at the mouth of the TAS1R1 Venus flytrap, near the hinge, and act as a molecular latch: they stabilize the *closed*, glutamate-bound conformation of the clamshell, making it harder to spring back open. The practical consequence is a fall in the apparent EC_{50} of glutamate — the receptor now answers to less of it. The taste gets louder without a single extra glutamate molecule present.

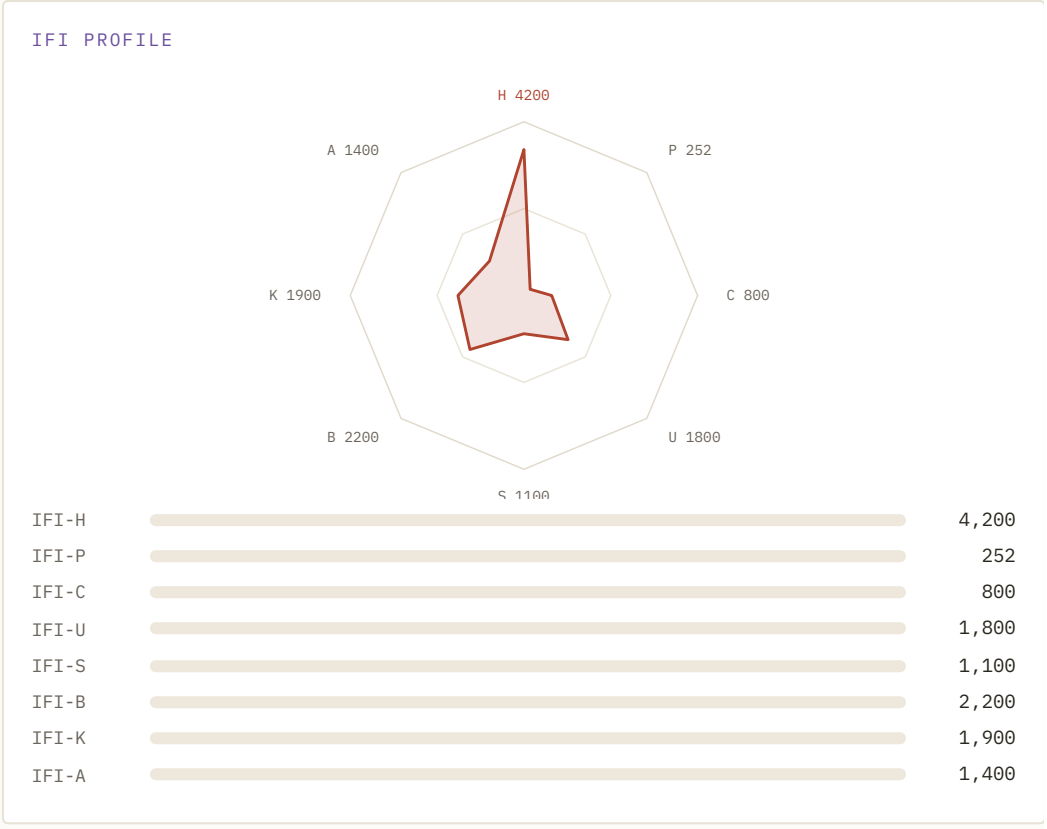


Dried shiitake, in cross-section — the mushroom whose breakdown of its own RNA on drying makes it one of the plant kingdom's great reservoirs of 5′-guanylate, the nucleotide that latches umami's flytrap shut.

Put it through the formula. Let a dish present free glutamate that, measured alone, half-activates the panel at EC_{50} = 3.0 mM — by the Index, IFI-U = 1,000 × 6.0 ÷ 3.0 = **2,000.** Now introduce the plant's own ribonucleotides and let the apparent half-activation fall to $EC_{50} \div \beta$, where β is the potentiation factor the nucleotides supply. Carrying an illustrative **β = 4** — conservative within the range reported for IMP and GMP — the effective EC_{50} drops to 0.75 mM, and the *identical* glutamate now reads IFI-U = 1,000 × 6.0 ÷ 0.75 = **8,000**, past the 5,000 scorecard ceiling, where it registers as capped. Not one glutamate molecule was added. The loudness quadrupled because the nucleotides were in the room.

This is the number under the whole clean-label argument. Glutamate from a shaker arrives with β = 1 — alone, flat, context-free, a single compound stirred into a formula. Glutamate grown into a plant arrives the way the receptor evolved to meet it: riding with its 5′-nucleotides, its kokumi peptides, its sugars and acids and cell-wall structure, the whole living package that makes savor read as *food* rather than as *signal.* A dried shiitake is a reservoir of guanylate because the mushroom breaks down its own RNA as it dries; a ripe tomato carries both its glutamate and a measure of nucleotide; a koji-cured bean is glutamate and nucleotide and peptide at once. The plate that pairs a glutamate source with a nucleotide source — the old wisdom of kombu with bonito, of tomato with mushroom, of miso with dashi — is simply synergy made edible, β stacked by a cook who never needed the equation to feel it. The shaker cannot sell you β. It can only sell you the one molecule, and the one molecule, the arithmetic says plainly, reads quieter than the package it was torn from.

The flagship makes the package literal. Phoenix does not speak umami in isolation; it speaks it as one of eight voices held in a single fruit, each anchored to its own receptor and its own EC_{50} —



— heat on TRPV1, pungency on TRPA1, cool-burn on TRPM8, umami on mGluR1, sweetness on TAS1R2·R3, the attenuation of bitterness on TAS2R38, kokumi on the calcium-sensing receptor, astringency on the salivary proline-rich proteins. The umami at 1,800 is not a dose added at the end. It is grown in beside the kokumi at 1,900 and the sweetness at 1,100, the three of them composing the roundness a broth has when it tastes like being cared for. That composition is the thing a shaker can never deliver, because a shaker delivers a compound and a plant delivers a context.

Integrity, not safety

So our argument against the shaker is not about safety; the science settled safety long ago, and we will say so to anyone who asks. It is about **integrity** — the plain difference between *dosing* a finished plate and *growing* the savor in. Glutamate added at the end is glutamate alone: loud, flat, β = 1, a single stirred-in compound. Glutamate grown into a plant is glutamate in its evolved company, the whole package the receptor was built across millions of years to read as nourishment. That is what a maker is really reaching for when they say *clean label*, and it is what the source-side approach delivers that a shaker never can — not the absence of a molecule, but the presence of everything the molecule was supposed to arrive with. We are a vegetarian family, and we have cooked savor from plants our whole lives; we know in the hand what the equation says on the page, that the depth of a long-simmered tomato is not one chemical but a chord.

Where the plant makes it

If the savor is to be the plant's own, the plant has to make it, and it already does. Free glutamate sits at the exact center of plant nitrogen metabolism — it is the cell's nitrogen currency, the hub through which almost every amino group passes — so growing savor in is less an act of installation than of retuning a chemistry the plant has run since it was a seed. The routes are these, in order:

Nitrogen enters as nitrate or ammonium and is assimilated by the **GS/GOGAT cycle.** Glutamine synthetase (GS) spends an ATP to ligate ammonium onto a glutamate, making glutamine; glutamate synthase (GOGAT) then transfers that amide onto a molecule of **2-oxoglutarate** — the carbon skeleton the TCA cycle hands up from respiration — yielding two glutamates where there was one. That is the engine: a respiratory carbon skeleton and a stray ammonium, welded into savor. Alongside it, **glutamate dehydrogenase (GDH1)** reversibly aminates 2-oxoglutarate with ammonium using NADPH, a buffer and reservoir reaction that fills and drains the glutamate pool as the fruit respires. Aminotransferases shuttle the amino group between glutamate and other keto-acids, keeping glutamate at the center of the ledger. And one reaction runs the other way: **glutamate decarboxylase (GAD)**, calcium-and-calmodulin regulated, strips the carboxyl from glutamate to make GABA and CO_2 — the drain that carries savor off the plate.

The whole of designed umami, in a crop that already makes glutamate, is the management of that balance. Free glutamate accumulates in a ripening fruit precisely when synthesis and retention outrun the GAD drain. To restore a tomato to the savor it was bred for — to lift it from the commodity fruit's faint IFI-U near 300 toward the vine-ripe 2,600 — we work the plant's own enzymes: easing the GAD drain, steadying GDH1 and the GS/GOGAT engine, so the glutamate the plant was always making stays free in the cell instead of being ferried off to GABA. It is the **native GAD /**

GDH1 route retuned, not a foreign molecule bolted on. The glutamate that results was made by the tomato, in the tomato's cells, by the tomato's enzymes. On the label it is tomato.



Tomato, in cross-section — the savor restored through the plant's own GAD / GDH1 chemistry, so the glutamate on the plate is the fruit's own and labels simply as the fruit.

The ferments take a different road to the same honest place. Koji — the mold *Aspergillus oryzae* laid over a steamed soybean or grain — secretes a battery of proteases and peptidases and, crucially, **glutaminase**, which hydrolyzes the bean's protein-bound and free glutamine into free glutamate. The soybean's own storage protein, locked up and tasteless, becomes a standing pool of savor. That is soy sauce; that is miso. The glutamate was the bean's; the koji only unlocked it. Across the organisms we have brought onto the platform, the savory hosts carry their work at very different depths —

HOST	CONSTRUCTS	PRIMARY AXES	DIFFICULTY
Tomato	2,040	S · U · F · M	Easy
Soybean	360	U · K	Frontier
Tea	280	U · B · A	Hard
Yeast	240	U · K	Hard
Koji mold	80	U	Hard
Shiitake	80	U	Hard
Mushroom	40	U	Hard

— 3,120 engineered constructs across these seven umami hosts, the overwhelming majority of them in the tomato, where the chemistry is most forgiving, and only a scatter on the soybean, which sits at the Frontier of difficulty precisely because its savor is the hardest and most valuable to retune at the source.

The regulatory line, drawn plainly

There is a genuine regulatory line underneath this, and it is worth drawing plainly for the formulators who have to live on the right side of it. Under the relevant labeling rules, added monosodium glutamate must be declared by name — *monosodium glutamate* — on the ingredient list. The subtler point is the claim that cannot be made: a product may not say "no MSG" or "no added MSG" if it contains ingredients that are themselves sources of free glutamate. Those are

the industry's usual factory tools for savor — **hydrolyzed vegetable protein, autolyzed yeast extract, hydrolyzed proteins, soy sauce, sodium caseinate, certain protein isolates** — and a regulator can read every one of them as a back-door glutamate source that voids the claim. The endogenous route is categorically different. Free glutamate that the plant made in its own cells is not an added ingredient at all; it labels as what it is — *tomato, soybean, shiitake* — the same way the glutamate of a Parmesan or a vine-ripe heirloom has never needed a declaration, because no one added it. The savor is the plant's.

APPROACH	WHAT THE LABEL CARRIES	REGULATORY CHARACTER
Added MSG	"monosodium glutamate"	declared additive
Hydrolyzed protein / autolyzed yeast	the ingredient, by name	free-glutamate source; blocks "no MSG" claims
Source-side (engineered plant)	the plant: *tomato, soybean, shiitake*	endogenous; the glutamate is the food

The international picture is worth a formulator's glance, because the rules differ by jurisdiction even though the molecule does not. In the systems that number their additives, the savory compounds carry a tidy range: the glutamic-acid family at **E620–E625**, the nucleotide enhancers at **E626–E635**, each a declarable additive when added as such.

E-NUMBER RANGE	CLASS	REPRESENTATIVE ENTRIES
E620–E625	Glutamic acid & glutamate salts	E620 glutamic acid · E621 monosodium glutamate · E622 monopotassium glutamate · E623 calcium diglutamate · E624 monoammonium glutamate · E625 magnesium diglutamate
E626–E635	5′-ribonucleotides & salts	E627 disodium guanylate · E631 disodium inosinate · E634 calcium 5′-ribonucleotides · E635 disodium 5′-ribonucleotides

The United States takes a different form to the same conclusion: monosodium glutamate is **generally recognized as safe**, carries no numeric daily limit, and must simply be declared by name. Europe's food-safety authority, re-examining the additive, set a **group acceptable daily intake of 30 mg per kilogram of body weight per day** for added glutamates expressed as glutamic acid — a number that applies to glutamate *added* as an additive, not to the glutamate intrinsic to a tomato or a cheese, which no regime counts against an intake limit. We report that history plainly rather than selling around it: the weight of the controlled evidence is reassuring, the honest caveat is small — a minority of people report transient, self-limited sensations after a large bolus dose taken without food — and neither the reassurance nor the caveat changes our approach, because our approach was never an answer to a safety question. The subtler trap is the phrase *natural flavor*, a real regulatory category that can still read, to a careful shopper, as something *added.* Endogenous glutamate sidesteps even that, because it is not a flavor added to the food; it *is* the food, the way the savor of an aged cheese or a vine-ripe tomato has always simply been the cheese and the tomato.

Two honesties we owe

Rigor cuts toward us as well, and we owe two honesties here. The first: our organisms are *engineered*, and engineered crops carry their own regulatory obligations — novel-food and biotechnology review in the jurisdictions that require it, and the disclosures those regimes demand. We do not route around glutamate labeling only to fall silent about engineering. The source-side story is an argument about *where savor comes from*, not a license to hide *how the plant was made*, and we would not want the license if it were offered.

The second: we make **attribute statements, never health claims.** We will say a food is savory, is deep, carries its glutamate endogenously, lets a cook reach for less added salt. We will not say it cures, prevents, detoxifies, or heals, because those are claims the science does not license and the law does not permit — and because the quiet value under all of this, that food should nourish, is worth far more kept as a value than cheapened into a slogan. The line runs like this, the kind of thing we will say set against the kind of thing we will not, on the very same facts:

WE SAY (ATTRIBUTE)	WE DO NOT SAY (CLAIM)
"deeply savory; glutamate the plant's own"	`"boosts your metabolism"`
"lets you cook with less added salt"	`"lowers blood pressure"`
"no added MSG; the savor is endogenous"	`"MSG-free is healthier"`
"the ripe fruit carries its own depth"	`"prevents disease / detoxifies"`
"satisfying; the taste of being fed"	`"suppresses appetite"`

The left column is true, testable, and ours to say. The right column trades a value for a slogan and a fact for a promise, and we will not cross into it — not because the law alone forbids it, though often it does, but because a family that revered the truth of its chemistry everywhere else would forfeit that reverence the instant it dressed a taste up as a cure.

A number you can stand behind

An attribute statement is only honest if it is true every time. "Deeply savory" on a label is a promise that *this* jar reads the way the last one did, and that promise is a statement about a distribution, not a single measurement. The discipline that makes it keepable is the **coefficient of variation** — the batch-to-batch standard deviation divided by the mean — and our platform holds it to **CV ≤ 7%**, against the roughly **42%** a wild, unselected population wanders across.

Work what that means for a savory target. Design a tomato to **IFI-U 2,600.** At CV = 7%, the standard deviation of the axis is 0.07 × 2,600 = **182**, so batch after batch the umami lands at 2,600 ± 182 — a one-sigma band of 2,418 to 2,782, and even a two-sigma band, covering roughly 95% of batches, of 2,236 to 2,964. Every jar in that range is unmistakably, provably savory. Now run the same target at the wild-type's 42% CV: the standard deviation balloons to 0.42 × 2,600 = **1,092**, a one-sigma band of 1,508 to 3,692 and a two-sigma band from 416 to 4,784. That lower tail is not savory at all; it is thin. You could not print "deeply savory" over a distribution like that and mean it — some fraction of jars would call you a liar. The tight CV is not a manufacturing nicety. It is the thing that makes the word on the label

true, and it is the same reproducibility discipline that holds the heat program's peak capsaicinoid output to a batch CV at or under 7% where an unselected population scatters at 42%. Precision is how a claim becomes an attribute, and an attribute becomes honest.

The honest limits

We would not have the reader leave this with the arithmetic looking tidier than nature is, so here are the edges plainly. Free glutamate is **water-soluble and labile**: it leaches into cooking water and can be boiled or rinsed away, so a savor you designed into the cell still has to survive the pot — delivery and preparation are part of the taste, not an afterthought. Synergy is **contextual**: the β that lifted our worked example from IFI-U 2,000 to a capped 8,000 depends on 5′-nucleotides being present, and their level shifts with the plant's state — a shiitake builds its guanylate as it dries, fresh tissue carries less — so a food's umami is read at the receptor, in the moment, not fixed to its dry weight. Human sensing **varies**: TAS1R genotype and receptor density move individual umami thresholds, so one person's EC_{50} is not another's, and the Index is a population yardstick, never a private one. The regulatory relief of the endogenous route is **real but bounded**: it sidesteps "added," yet engineered crops still carry their novel-food and biotechnology review, which we meet rather than dodge. And the savory hosts are, frankly, **early and hard** — soybean sits at the Frontier, koji and shiitake and yeast and tea at Hard, several of them carrying only 40 to 360 constructs against the tomato's 2,040 — so the honest state of this work is a deep, forgiving craft on a few plants and a careful beginning on the rest. We would rather say that than oversell it. These are computational designs; Scoville Splice's cultivars and constructs have not been experimentally validated in a laboratory.

Clean label, for us, means exactly this: the savor is real, it is the plant's own, the receptor cannot tell it from any other glutamate because there is no difference to tell, and everything we say about it is true.



PART V OF V

The Kitchen

composition · depth and body · the builds · the reference · the close

Chapter 16 · Umami in Composition — *the floor the others stand on.*

the composer's axis · set U first

Umami is the one axis that mostly serves the others. It rarely wants to be the loudest thing in a dish; it wants to be the ground the loud things stand on. This is the deepest reason savor is worth measuring on its own axis even though it so seldom stars — and the reason a maker who learns to specify IFI-U *before* anything else is a maker whose dishes have a bottom to them, a place for the other seven axes to land. Savor is not usually the melody. It is the room the melody plays in.

We came to this the slow way, through a lifetime of cooking without meat, where there is no stockpot of bones to fall back on and the savor has to come up out of the plants or it does not come at all. You learn, over enough evenings, that a vegetable dish lives or dies on a thing you cannot name and the diner cannot either — a fullness underneath, a sense that the food is *food*. For most of human history that fullness was a matter of touch and luck. The Index lets us hold it as a number, and a number is a thing you can build on purpose. What follows is the mechanism of that fullness, the mathematics that scores it, and the discipline of composing a dish from the floor up.

The receptor beneath the floor

Savor is a conversation between one small molecule and two proteins folded into the surface of a taste cell. The molecule is free L-glutamate — glutamate loose in the food, unbound from any protein, the form the tongue can actually read. The proteins are **TAS1R1** and **TAS1R3**, two class-C G-protein-coupled receptors that clasp together into a single working unit, the umami heterodimer. Each carries a large extracellular domain shaped, almost literally, like a Venus flytrap: two lobes hinged at a cleft. Glutamate settles into the cleft of the TAS1R1 lobe and the trap closes around it. That closing is the whole event. A shut flytrap twists the receptor's seven-helix core on the inside of the membrane, and the cell begins, for the first time, to report *savor*.

What happens next is a cascade we can trace step by step, because every step is a place the signal can be made louder or softer. The twisted core engages its G-protein — α-gustducin and its partners — which switches on **phospholipase C-β2**. That enzyme cleaves a membrane lipid into **IP_3**, which opens calcium stores inside the cell. The released calcium throws open **TRPM5**, a channel that lets the cell depolarize; the depolarization drives voltage-gated sodium channels, and the cell fires. At the far wall it vents **ATP** through CALHM1 channels onto the gustatory nerve — chorda tympani at the front of the tongue, glossopharyngeal toward the back — and only there, as a train of nerve impulses climbing toward the insular cortex, does the molecule finally become the feeling of a good broth. A

tomato warm from the vine runs that entire relay a billion times a second, and we spent most of our species' history unable to say *how much*.

There is a second listener, and the Index names it alongside the first: **mGluR1**, a metabotropic glutamate receptor better known from the brain, expressed in taste tissue in a truncated taste-variant form. It binds glutamate at a lower concentration than the heterodimer does — it is the more sensitive ear, the one that catches the faint brothiness at the bottom of a dish before the heterodimer has woken up. We anchor IFI-U to the TAS1R1·TAS1R3 complex, because that is the receptor food is built around and the only one that performs the synergy trick we will come to in a moment; but we read mGluR1 as the corroborating channel, the reason a floor can register as *presence* long before it registers as *flavor*.



Soybean, a glutamate organism — where koji's proteases cut stored protein into the free amino acids the tongue can finally read.

What the index returns, and the number it hands back

The Iyengar Flavor Index scores loudness, not quantity — not how much of a compound a food holds, but how *loudly* that compound speaks to its receptor. The instrument is a single equation:

> $\mathrm{IFI(candidate)} = 1{,}000 \times \mathrm{EC_{50}(reference)} \div \mathrm{EC_{50}(candidate)}$

EC_{50} is the concentration at which a stimulus drives its receptor to half of full response. We calibrate the umami axis so that the reference — a native wild-type savor — behaves like **2.6 mM glutamate** at the heterodimer, and we fix IFI-U = 1,000 at that point by convention. Everything else is read against it. Because the score is an inverse ratio, a *lower* effective EC_{50} — a food so potent you can

dilute it far and the receptor still half-fires — returns a *higher* number. Invert the reference against a candidate and savor becomes a figure a tomato, a koji, and a cup of broth can be printed on one page.

Watch the formula do its work on the savor values that run through our catalogue. The restored tomato, the fruit we design back to what it should be, is computed at IFI-U 2,600; rearrange the equation and its effective EC_{50} is 1,000 × 2.6 ÷ 2,600 = **1.00 mM** — it half-fires the receptor at a glutamate-equivalent two-and-a-half times more dilute than the reference. A degraded supermarket tomato scores 300, which is an EC_{50} of 1,000 × 2.6 ÷ 300 = **8.67 mM** — it must be eight-and-a-half times more concentrated to reach the same half-signal, which is a tidy mathematical way of saying it tastes of almost nothing. Our full-spectrum flagship is designed to carry IFI-U 1,800 beneath its heat: EC_{50} = 1.44 mM. The arithmetic is dull and that is the point — numbers are a form of respect for the reader, and a floor you can specify is a floor you can reproduce.

IFI-U	WHAT IT IS	DERIVED EC_{50} (MM GLU-EQUIVALENT)	LOUDNESS VS REFERENCE
200	top-down sauce, no floor	13.00	0.20×
300	supermarket tomato (degraded)	8.67	0.30×
1,000	wild-type reference anchor	2.60	1.00×
1,800	Phoenix · SS-0096	1.44	1.80×
2,400	floor-up sauce	1.08	2.40×
2,600	restored tomato	1.00	2.60×

Now walk the other direction — from the fruit to the receptor — because it shows why the Index is measured the way it is. The receptor does not read EC_{50}; it reads whatever concentration a bite delivers, through the dose-response curve $R = R_{max} \cdot C / (C + EC_{50})$, where EC_{50} here is glutamate's own fixed constant at the heterodimer, 2.6 mM. A vine-ripe restored tomato holds on the order of 250 mg of free glutamate per 100 g of flesh. Convert it: 250 mg/100 g ≈ 2.5 g/L, and glutamate's molar mass is 147 g/mol, so 2.5 ÷ 147 = **17.0 mM** in the fruit's serum. Drop that into the curve: R/R_max = 17.0 ÷ (17.0 + 2.6) = **0.87**. A bite of a real tomato drives the umami receptor to 87% of everything it can do, before a grain of its ribonucleotide synergy is counted. The degraded tomato — the supply chain having taken roughly 93% of that glutamate on the truck — carries near 17.5 mg/100 g, which is 1.19 mM, and R/R_max = 1.19 ÷ (1.19 + 2.6) = **0.31**. The gap between 87% and 31% occupancy is the entire difference between a fruit that tastes like a tomato should and a fruit that tastes like water given a shape.

Hold those two accountings next to each other and the architecture of the Index justifies itself. The content fell 93%. The receptor occupancy of a full bite fell only from 87% to 31% — because near saturation the dose-response curve flattens and compresses large concentration differences into small response differences. But the *loudness* — the EC_{50} ratio, measured down in the sensitive, near-threshold stretch of the curve where the receptor still discriminates — fell the full 8.7-fold, from 2,600 to 300. We score umami as an EC_{50} ratio rather than as the response to a mouthful precisely for this

reason: a mouthful saturates and hides the truth, while the half-firing point lives in the honest, linear part of the curve. The number that reaches the scorecard is the one taken where the receptor can still tell two foods apart.

And every number carries an envelope. An EC_{50} fit to our standard panel holds a coefficient of variation near 7%, and the score is a ratio of two such fits — the candidate's and the reference's — so the uncertainties add in quadrature: CV(IFI) = $\sqrt{(0.07^2 + 0.07^2)} \approx$ **9.9%**. The restored tomato's IFI-U 2,600 is therefore 2,600 ± ~260. Batch to batch, the construct itself holds the platform's ≤7% CV, so a formulator who specifies a floor of 2,600 receives, lot after lot, a floor between roughly 2,420 and 2,780. A floor that wanders is not a floor. The tight CV is what lets savor be an ingredient a recipe can *name*.

The synergy term — why a floor multiplies

Here is the part that makes umami unlike the other seven axes, and the single most useful fact a composer can hold. Glutamate does not add to savor. With the right partner, it *multiplies* it.

The partners are the 5′-ribonucleotides — **inosinate (IMP)** and **guanylate (GMP)**. Neither tastes of much alone. But a ribonucleotide binds the umami heterodimer near the mouth of the Venus flytrap, just where the two lobes hinge, and holds the trap shut around the glutamate already inside. The closed state is stabilized; the receptor's effective affinity for glutamate climbs; the EC_{50} drops. This is the structural reason a pinch of dried mushroom turns a thin broth bottomless. It is allostery you can taste.

The food scientist Kuninaka and, formally, Yamaguchi gave the effect an equation, and it belongs in any serious account of a savory floor:

$$\gamma = u + \beta \cdot u \cdot v$$

where u is the glutamate concentration, v the ribonucleotide concentration (each as a fraction, g/100 mL), γ the resulting umami expressed as glutamate-equivalent, and β ≈ 1,218 for IMP. The term that matters is the last one — the **product** u·v. It is zero whenever either partner is absent, which is why a glutamate-only floor, however rich, stays stubbornly linear and a little flat. Work it: a 0.05 g/100 mL solution of glutamate alone has γ = 0.05. Add an equal 0.05 g/100 mL of ribonucleotide and γ = 0.05 + 1,218 × 0.05 × 0.05 = 0.05 + 3.05 = **3.10** glutamate-equivalent — a 62-fold jump in equivalent concentration from mixing two things that, apart, were quiet.

Sixty-two-fold is not what the tongue reports, and the honest finish to the calculation is the reason. Perceived intensity follows a compressive power law — Stevens' law — with an exponent near 0.5 for umami, so the felt intensity scales roughly as the square root of concentration. $\sqrt{(3.10 \div 0.05)} = \sqrt{62} \approx$ **7.9**. The mouth feels about an eightfold deepening, which is exactly the synergy generations of broth-makers have tasted without being able to write down: kombu

laid with bonito, tomato cooked with mushroom, soy fermented under koji. The floor did not get taller by addition. It got deeper by multiplication, and then the power law gentled the product into something a body reads as *comfort* rather than *loud*. The compositional instruction falls straight out of the algebra: a floor built from glutamate and a ribonucleotide together is worth many floors built from glutamate alone.

Where the floor is grown

Savor is not one plant's gift. The glutamate currency runs through every organism that stores protein and then, by enzyme or by ripening, sets some of it free. Our umami shelf is built from seven of them, 3,120 constructs in all, each a different route to the same molecule at the same receptor.

ORGANISM	CONSTRUCTS	PRIMARY AXES	THE SAVOR IT CARRIES
Tomato	2,040	S / U / F / M	free L-glutamate, built in ripening
Soybean	360	U / K	glutamate + peptides, freed by koji
Tea	280	U / B / A	L-theanine, the γ-glutamyl amino acid
Yeast	240	U / K	glutamate with native 5′-nucleotides
Koji mold	80	U	glutamate, cut loose by proteases
Shiitake	80	U	5′-guanylate (GMP), the synergist
Mushroom	40	U	glutamate + guanylate together

Read the table as a composer and it stops being a list. Shiitake is on it not for glutamate but for **guanylate** — it is the organism you reach for when you need the v in Yamaguchi's product term, the synergist that multiplies a tomato's u. Soybean and koji appear together because koji is *how* soy becomes savory: *Aspergillus oryzae* floods the bean with proteases and peptidases that cut stored seed protein into free amino acids, glutamate chief among them. Yeast brings its own glutamate *and* its own nucleotides in one body — a complete floor in a single ingredient. Tea's savor is **L-theanine**, the γ-glutamylethylamide behind the brothy hush of a fine shaded green. Seven organisms, one receptor, and a whole grammar of ways to feed it.

The tomato is our clearest proof, because in the tomato the floor is grown rather than added. A ripening tomato does not import glutamate; it *accumulates* it, out of its own nitrogen metabolism, along a route the plant already owns. The pathway runs through two enzymes the Index keeps its eye on. **Glutamate dehydrogenase (GDH1)** interconverts the Krebs-cycle intermediate 2-oxoglutarate and glutamate, aminating the carbon skeleton into the savory amino acid. **Glutamate decarboxylase (GAD)** runs the other way on the budget — it spends glutamate to make GABA through the so-called GABA shunt. In a green fruit, GAD is busy and GABA is high and free glutamate is scarce; as the fruit ripens, the shunt quiets, GDH1's aminating flux holds, and free glutamate pools climb ten- to twentyfold until the fruit tastes the way a tomato is supposed to. Our restoration work does nothing exotic to this. It tunes the plant's *native* GAD/GDH1 balance so our model predicts the ripe fruit will land its glutamate pool where vine-ripened fruit always did — before a breeding program chasing firmness and shelf life and a truck that

wanted the fruit picked green quietly bled it out. Our model predicts IFI-U 300 becomes IFI-U 2,600 with no foreign savor poured in, only the plant's own chemistry asked to finish the job it evolved to do.



The restored tomato — the composer's floor grown back into a single fruit, our model predicts IFI-U 300 lifted to 2,600 along the plant's own GAD/GDH1 route.

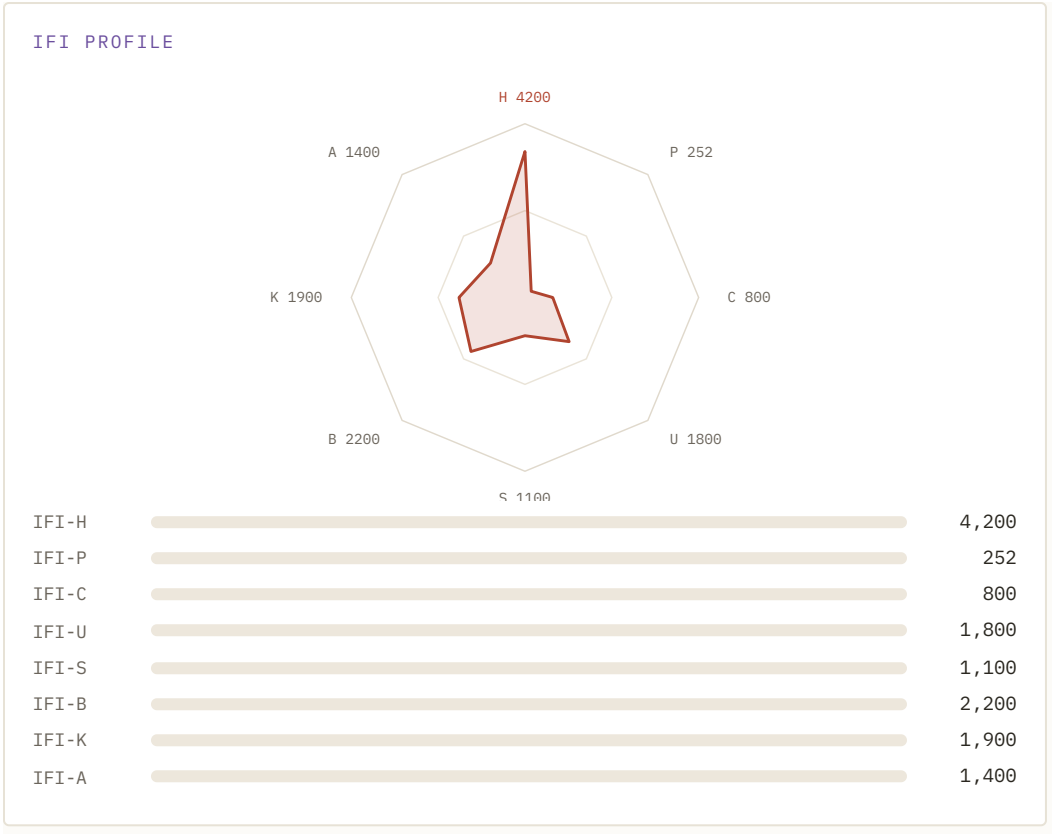
Savor under its neighbors

The composition is mechanical, not merely poetic, and the mechanics are most visible when you watch savor work beneath each of its neighbors in turn.

Put savor **under heat,** and the burn stops reading as an assault and starts reading as a dish. The body, told *food* by glutamate landing on the umami heterodimer, receives the capsaicin arriving at its TRPV1 channels as *seasoning* rather than *threat* — the same heat, recontextualized by the signal sitting beneath it, because the gustatory cortex integrates the two streams before it hands you a verdict. This is precisely why our full-spectrum flagship is designed to carry an IFI-U of 1,800 under its towering 4,200 of heat: the savor is what makes a predicted heat of approximately 2.95 million SHU taste like something a person would *choose* to eat rather than merely survive. Strip the umami out and the identical heat reads thin and hostile. Build it in and the fire has a floor to stand on.



The full-spectrum flagship — proof that savor specified beneath heat turns an assault into a dish rather than a dare.



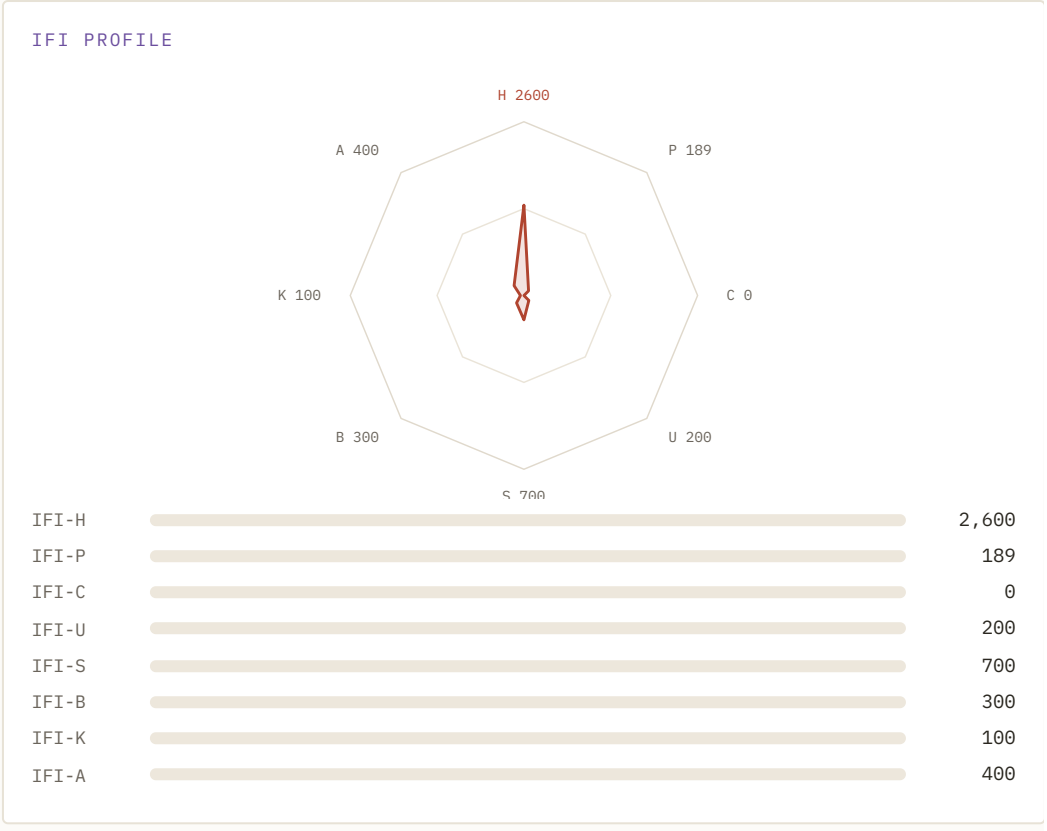
Set savor **against bitterness** and it does a gentler work. Where the zero-bitter axis eases the warning signal at TAS2R38, umami *fills the space that subtraction leaves.* A coffee or a cacao or a brassica with its bitterness lowered and its savory depth lifted does not taste merely *less bitter* — a subtraction, which can read as hollow — it tastes *complete,* the place where the alarm sounded now occupied by something nourishing. Subtraction alone leaves a gap; savor fills it. And **beneath sweetness,** umami keeps the sugar honest: a sweet thing with savor under it reads as *fruit* rather than as *candy,* grounded instead of floating, because the body is getting the protein-signal that tells it this is food and not mere energy. The restored tomato is the proof in a single fruit — its sweetness reads generous rather than cloying precisely because its umami anchors it.

There is a fourth neighbor worth its own paragraph, because it carries the most practical weight for a manufacturer: **salt.** Umami and saltiness reinforce one another at the perceptual level — a savory base makes a given amount of sodium read as fuller and more seasoned, and there is a receptor logic beneath the pairing, since the calcium-sensing receptor that governs kokumi sits in the same taste cells and lifts the whole savory-saline percept together. So a dish built on real umami can reach the same satisfying savoriness at a lower added-salt level than one that leans on salt alone. We state this precisely and as an attribute, never a claim: engineered savor lets a formulator *use less added salt for the same perceived seasoning,* which is a formulation fact, measurable on a trained panel, and nothing more than that. It is, though, a fact with reach — because so much of the salt in a modern diet is there to paper over the savor that breeding bled out of the plant. Restore the savor at the source and some of that compensating salt simply becomes unnecessary. We promise nothing in the body; we note only that a floor of real umami gives a cook somewhere to stand other than the salt cellar, and that this, too, is the emptying being quietly repaired.

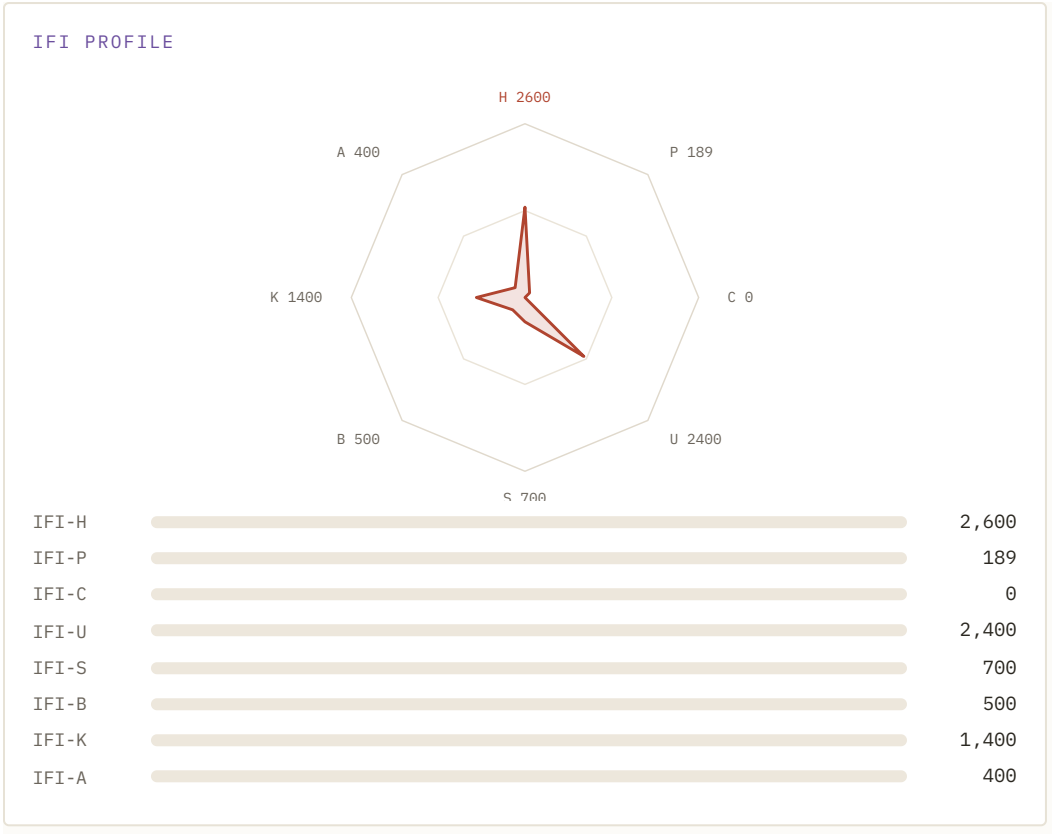
And beneath them all sits the **kokumi** partner, savor's near-twin. Kokumi compounds — γ-glutamyl peptides such as glutathione and γ-Glu-Val-Gly — taste of almost nothing alone, but they agonize the calcium-sensing receptor and, through it, lend the umami signal *continuity, thickness, and mouthfulness:* the three words tasters reach for when a broth feels like it has a middle. This is why the composer sets umami and kokumi together, as one act. The floor is what the food *is;* the body is how *long* it lasts on the way down. One receptor pair for presence, one for persistence.

The floor, measured — one sauce, two readings

Watch the floor do its work in a single comparison, because the scorecard makes the abstract concrete. Here is a bright, fruit-forward hot sauce designed from the top down — all heat and acid, no savor beneath it:



It will taste sharp, thin, and front-loaded — a sauce that hits and leaves, the heat reading as an edge rather than a dish. At IFI-U 200 its umami receptor needs a 13.0 mM glutamate-equivalent to half-fire, a concentration a dilute sauce never reaches, so the floor is, functionally, not there. Now the same sauce with the umami set first, a little restored tomato and koji depth built in beneath the identical heat:



The heat number did not move. Everything about how the heat *reads* did. Set the two side by side and the arithmetic of the change is plain.

AXIS	TOP-DOWN	FLOOR-UP	Δ
H · heat	2,600	2,600	0
U · umami	200	2,400	+2,200
K · kokumi	100	1,400	+1,300
B · zero-bitter	300	500	+200
S · sweet	700	700	0
A · astringency	400	400	0

At IFI-U 2,400 the floor's effective EC_{50} drops to 1.08 mM — twelve times more sensitive than the top-down version's 13.0 mM — so now a spoonful's worth of savor actually fires the receptor, and the kokumi lift from 100 to 1,400 gives that firing a middle to rest on. The same 2,600 of heat lands on a floor instead of in a vacuum. The burn becomes seasoning; the sauce gains a bottom and a finish; a taster who could not say why will call the second one "rounder," "deeper," "more finished." Nothing was subtracted from the heat. A floor was built under it. That is the entire argument of this chapter in two readings and one unchanged heat number.

The order of operations

The practical instruction falls straight out of all of this: when you design a profile, **set the umami first.** Decide the floor — how much *food-signal* the thing will carry — before you tune the heat or the sweetness or the bright top-notes, because every one of those reads differently against what you have built beneath it. A profile designed top-down, savor last, comes out as a collection of loud

notes with nothing holding them; a profile designed floor-up, savor first, comes out as a dish. In practice the order runs like this, and it is close to how we design every profile in the catalogue:

1. **Set the floor.** Fix the umami — the glutamate currency, and crucially its ribonucleotide synergy so the u·v term is live — and its kokumi body alongside, because the TAS1R1·R3 and CaSR signals together are the ground. Decide this number before any other.
2. **Place the lead.** Bring in the axis the product is *about* — the heat of a sauce, the sweetness of a dessert, the cool of a confection — knowing it will read against the floor you just poured.
3. **Trim the warnings.** Ease the bitterness at TAS2R38 and set the astringency at the salivary proteins to the level the composition wants, using those axes to clear room rather than to star.
4. **Lift the top-notes.** Add the bright, volatile, first-impression axes last — a pungent allium top, a cooling finish, a thread of acid — because they sit *on* the structure and would mislead you if placed before it.
5. **Read the radar whole.** Check the eight-spoke shape, not any single number, for balance. A profile is a silhouette, not a height.

Set the umami first does not mean set it *high.* It means decide it first. A few profiles want a deliberately shallow floor — a crystalline astringent tea, a bright citrus cooler — where too deep a savor would muddy the top the product is built to show. The discipline is to choose the floor's depth on purpose, with eyes open, before anything else forces the choice by accident.

Failure modes and honest limits

A floor is powerful and it is not magic, and the maker who knows its edges builds better than the one who believes the number can do anything.

The floor has a ceiling. The receptor saturates. A restored tomato at 87% occupancy on glutamate alone sits near the top of its dose-response curve, and the scorecard caps each axis at a normalization maximum of 5,000 for the same reason — past roughly IFI-U 2,600 to 3,000 the receptor is nearly full, the power law compresses hard, and each additional unit of glutamate buys almost nothing a mouth can feel. You cannot deepen a floor without bound; near saturation you are spending glutamate the tongue will never spend back.

A glutamate-only floor underperforms its own number. The synergy term is a product, not a sum, so a floor rich in glutamate but empty of ribonucleotide reads flatter than its IFI-U suggests — the familiar one-note quality of savor added as a single powder. A floor that reads as *food* carries its synergist (a shiitake's guanylate, a yeast's nucleotides) and its kokumi body. The number on the scorecard is necessary; it is not, by itself, sufficient.

A floor cannot rescue a broken profile. Umami integrates centrally with the other axes, but it integrates with what is actually there. A profile with runaway astringency, or a bitter spike beyond the range

the zero-bitter axis can ease, or heat with no structure anywhere on the radar, is not fixed by pouring savor underneath it — a floor under chaos is a well-grounded chaos. Savor contextualizes a coherent dish; it does not author coherence from nothing.

The salt attribute is an attribute. The lower-salt-for-equal-seasoning result is a panel-measurable formulation fact and strictly that. It is not a health claim, and we do not let it drift into one; the body's response to sodium is the body's, and the Index scores taste, not physiology.

And the floor is calibrated to a tongue. Umami familiarity varies across palates and cultures, the batch envelope is ±~10% on the score and ≤7% lot to lot, and a monotone floor — however deep — fatigues across a meal the way a single held note does. The honest version of "set the umami first" is a range, a confidence band, and a composer's judgment, not a single sovereign number.

Designed top-down, savor last, a product comes out as loud notes with nothing holding them. Designed floor-up, savor first, it comes out as a dish. The sequence is the difference, the scorecard is drawn with eight spokes for a reason, and the composer's eye goes to the umami spoke first and treats it as the ground the other seven are planted in. Savor is not usually the melody. It is the room — and you build the room before you let anyone sing in it.

These are computational designs; Scoville Splice's cultivars and constructs have not been experimentally validated in a laboratory.

Chapter 17 · Umami × Kokumi — *depth and the body around it.*

IFI-U × IFI-K · CaSR · the ratio that reads as rich

Savor rarely travels alone, and its closest companion is the strangest sensation in the whole catalogue: **kokumi,** the axis that has no taste of its own. Umami is the savory *taste* — the brothy, mouth-filling note of free glutamate meeting its receptor. Kokumi is the *body* that note arrives in: the continuity, the roundness, the sense of a mouthful filling the whole mouth and lasting past the swallow. Umami you can point to. Kokumi you can only miss when it is gone. Together they are what a long-simmered thing has and a quick one lacks, and together they are the single most-requested combination the savory makers who come to us bring to the table — a pairing deep enough to earn its own dedicated volume in this library, and expressed to some degree by nearly every organism on this shelf.

We write these two axes as a pair because the tongue reads them as a pair, and because the mathematics that lets us design them is, at heart, one equation applied twice. What follows is that equation — the receptors it rests on, the molecules that trip them, the enzymes that build those molecules, and the single ratio, note to body, that decides whether a broth tastes thin or tastes *cared for.* We will show the numbers as we go, because in our family a number is a form of respect for the reader, and because you cannot grow a flavor on purpose until you can say how much.

Two sensations, told apart

The first discipline is to stop using the words loosely. Umami has a molecule and a receptor and a threshold; it is the taste of L-glutamate (and of a few close relatives, the 5′-ribonucleotides) read off dedicated taste machinery. Point to a ripe tomato, a simmered stock, a sheet of kombu, and you are pointing at umami. Kokumi has a molecule and a receptor too, but no taste — activate its receptor alone and nothing tastes of anything. What kokumi does is *modulate:* it reaches into the tastes already present — umami, salt, sweet — and rounds them, sustains them, lowers the concentration at which the mouth first reads them, and stretches the reading across time. It does not add a voice to the chorus. It widens the room the chorus sings in.

This is why the two multiply rather than compete, and the reason is mechanical, not poetic. A sensation that added its own flavor would have to share the stage — crowd the glutamate signal, mask it, trade against it. Kokumi works a *different* receptor from umami, so it never competes for the same binding site; instead it lowers the *working* threshold of the umami response, so the same free glutamate reads deeper, rounder, and longer. Read that straight off the Index formula: anything that lowers a candidate's half-activation concentration raises its score, so a kokumi tone lifts the savor you perceive from a *fixed* quantity of glutamate. **Umami names the savory; kokumi holds it.** A high umami note with no kokumi behind it tastes thin and front-loaded — a savor that hits and vanishes, like a photograph of a

broth. Add kokumi and the same savor gains duration and dimension; the second signal does not add flavor so much as *hold* the flavor already there, the way a frame holds a picture.

A single family of receptors — the clamshell that umami and kokumi share

The elegance of this pairing is written into the receptors, and once you see it you cannot unsee it. Umami and kokumi are read by members of the *same receptor superfamily* — the class C G-protein-coupled receptors, a lineage whose signature is a large extracellular **Venus flytrap domain:** two lobes hinged like a clamshell that close around their ligand and, in closing, send the activating signal inward. Three of these clamshells matter here.

Umami taste proper is read by the heterodimer **TAS1R1·TAS1R3** (T1R1/T1R3), whose T1R1 flytrap closes around free glutamate. A second, metabotropic reader — **mGluR1,** the same glutamate receptor the brain uses, expressed in a taste-tissue variant — contributes the deeper, more persistent register of the savory note; both sit under the IFI-U axis. Kokumi is read by the **calcium-sensing receptor, CaSR** — a clamshell whose day job, elsewhere in the body, is monitoring blood calcium, and which the tongue has repurposed as a taste *modulator.* Its ligands are not calcium here but the **γ-glutamyl peptides,** which settle into the CaSR flytrap and switch it on.

AXIS	RECEPTOR	FAMILY	WHAT CLOSES THE CLAMSHELL	SIGNAL IT SENDS
IFI-U	TAS1R1·TAS1R3	class C GPCR	free L-glutamate (+ 5′-ribonucleotides nearby)	savory *taste*
IFI-U	mGluR1 (taste variant)	class C GPCR	free L-glutamate	deep, persistent savory
IFI-K	CaSR	class C GPCR	γ-glutamyl peptides	*modulation* — no taste of its own

Because all three are the same kind of machine, the ligands rhyme: glutamate opens the umami flytraps, and a γ-glutamyl peptide — glutamate joined to a second residue through its side chain — opens the kokumi one. The body's savor-modulator is, structurally, glutamate wearing a second face. And downstream the two signals converge rather than collide. The umami receptors route through Gα-gustducin and Gβγ to phospholipase C-β2, releasing intracellular calcium through the IP_3 receptor, opening the TRPM5 channel, and firing the cell. CaSR drives its own Gq/phospholipase-C cascade in the same taste tissue — and because it carries no taste line of its own, that cascade lands as *gain:* it lowers the threshold and lengthens the response of the umami-bearing cells rather than writing a separate flavor. Two clamshells, two ligands, one summed depth. That convergence is the whole reason a kokumi peptide amplifies glutamate instead of masking it, and it is why we tune the pair together rather than chasing umami to the ceiling and wondering why the result rings hollow.

The molecules of the note — free glutamate and the ribonucleotide multiplier

The umami ligand is free L-glutamate: not glutamate bound up in protein, which the tongue cannot read, but the standing pool of the free amino acid in the tissue. That pool is set by metabolism — built by the glutamine-synthetase / GOGAT cycle and by glutamate dehydrogenase (GDH1) running in its aminating direction, drawn down by protein synthesis and by glutamate decarboxylase (GAD) shunting glutamate toward GABA. A plant that holds more free glutamate at the moment you eat it tastes more savory, full stop. This is also the pool that modern supply chains destroy: pick a tomato hard and green so it survives the truck, and you strip the ripening window in which that free-glutamate pool is laid down — roughly 93% of a vine-ripe fruit's savory signal, gone before it reaches a kitchen. Our work on the savory note is, in large part, the work of growing that pool back in through the plant's own GAD / GDH1 machinery, so the fruit arrives already carrying what the truck used to take.

The note has a multiplier, and it is one of the most dramatic numbers in all of flavor. The 5′-ribonucleotides — inosinate (IMP) and guanylate (GMP) — have little savor alone, but they bind a pocket beside the T1R1/T1R3 flytrap and stabilize its closed, glutamate-bound state, so that far less glutamate is needed to reach the same response. The classic determination of this synergy, by Yamaguchi, fits a strikingly simple law:

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y = u + β · u · v
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where *u* and *v* are the glutamate and ribonucleotide fractions of a savory mixture, *y* is the glutamate-equivalent intensity the blend delivers, and **β** is an empirically fitted synergism constant on the order of **1,200** for glutamate with inosinate. The cross term is the whole story. Take a mixture that is mostly glutamate with a small nucleotide fraction — say *u* = 0.95, *v* = 0.05: y = 0.95 + 1,200 × 0.95 × 0.05 ≈ 0.95 + 57 ≈ **58.** The blend tastes as savory as roughly fifty-eight times as much glutamate would alone. A little nucleotide does not add to the note; it multiplies it.

This is why, when we design a savory build, we do not chase glutamate alone — we co-grow a glutamate source and a nucleotide source so the multiplier is in the tissue, not sprinkled from a packet. And because ours is a vegetarian table by conviction and by lineage, the nucleotide partner we reach for is never bonito or meat but the fungal GMP of **shiitake** and **mushroom:** the same multiplication, drawn entirely from the plant-and-fungus world. Glutamate from the tomato or the soybean; guanylate from the mushroom; the cross term does the rest.

The molecules of the body — γ-glutamyl peptides and the enzyme that makes them

Name the kokumi molecules, because naming them is how a maker learns to *source* body rather than merely hope for it. The archetype is **glutathione** — γ-Glu-Cys-Gly, the tripeptide every living cell carries as its redox workhorse, which turns out to double as a CaSR agonist. From the search for stronger, steadier analogs came **γ-Glu-**

Val-Gly, a γ-glutamyl peptide markedly more potent than glutathione at eliciting kokumi, and now a touchstone for the whole class. The family shares a signature: the **γ-glutamyl bond** — glutamate joined to the next residue through its *side-chain* carboxyl rather than its backbone α-carboxyl. That one structural choice does two things. It is the shape the CaSR flytrap recognizes, and it is an isopeptide bond that ordinary peptidases cannot cleave, so these peptides are *stable* — they survive cooking and storage and arrive intact on the tongue.

The enzymology is worth drawing, because it tells a maker where body comes from and how to grow more of it. Glutathione is assembled in two ATP-driven steps: glutamate-cysteine ligase (GCL) joins glutamate and cysteine into γ-glutamylcysteine, and glutathione synthetase adds glycine to finish the tripeptide. The diversity of the kokumi class then comes from one remarkable enzyme — **γ-glutamyl transpeptidase (GGT),** which cleaves the γ-glutamyl group off glutathione and *transfers* it onto an acceptor amino acid or peptide. Hand GGT a valine and a glycine acceptor and it builds toward γ-Glu-Val-Gly; run it on the cell's pool of amino acids and it generates the whole spread of γ-glutamyl peptides that read as body.



Garlic — the kokumi workhorse, rich in the γ-glutamyl peptides, and in the transpeptidase that keeps reshuffling them, that give umami a body to stand in.

This is why the **alliums** — garlic and onion — sit at the base of nearly every savory build even though their own Index axis is kokumi, not umami. They are unusually rich in GGT and in γ-glutamyl precursors (including the γ-glutamyl-S-alk(en)yl-cysteines that are the storage form of garlic's own pungency), and they reward time: aging, sprouting, and gentle heat all give the transpeptidase room to work, converting stored precursors into free kokumi peptides. Aged cheese, long stock, yeast, miso — every slow savory thing humans have ever loved is, in part, a vessel in which GGT has had time to run. Kokumi is, in a real sense, what glutamate does when it stops being a taste and becomes a modulator of taste.

How the Index measures a modulator — the EC50 and the anchor

Here is where the two axes become designable, and where the Index earns its place. Every IFI value is a single number anchored to the half-maximal activation of a receptor:

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IFI(c) = 1,000 × EC50(reference) ÷ EC50(candidate)
```

EC_{50} is the concentration of a stimulus that drives its receptor to half of full response — the lower the EC_{50}, the *louder* the stimulus, because less of it is needed to be heard. Fix a reference stimulus and it scores itself: 1,000 × EC_{50}(ref) ÷ EC_{50}(ref) = **1,000,** the anchor. A candidate twice as loud as the reference — half the EC_{50} — scores 2,000. Invert, scale by a thousand, and loudness becomes a number a tomato, a soybean, and a clove of garlic can be read against on one page. Rearranged, the formula also runs backward, turning any score into the receptor fact beneath it: EC_{50}(candidate) = EC_{50}(reference) × 1,000 ÷ IFI. Each IFI axis is anchored to its own reference stimulus — a stated convention, like a scale defined by its own reference point, not a single shared absolute measure — so a U:K ratio used in this chapter is a design-balance tool, expressing how the two axes sit relative to their respective anchors rather than claiming that umami and kokumi intensities are interchangeable on a universal scale.

Run that backward on the savory note. A tomato picked green and shipped scores around **IFI-U 300;** its glutamate must reach 1,000 ÷ 300 ≈ **3.33 times** the reference concentration before the receptor answers at half — which is to say it reads faint. Grow the free-glutamate pool back in through the native route and our model predicts the same fruit at **IFI-U 2,600;** now its EC_{50} is 1,000 ÷ 2,600 ≈ **0.385 times** the reference. Our model predicts the design would drop the effective half-activation concentration by a factor of 2,600 ÷ 300 ≈ **8.7×** — the savor growing almost ninefold louder in that prediction, and none of it added from outside the plant.

FOOD / BUILD (AXIS)	IFI	EC_{50} ÷ REFERENCE (= 1,000 / IFI)	READS AS
Commodity tomato, shipped (U)	300	3.33×	faint, watery
Reference anchor (any axis)	1,000	1.00×	the standard
Phoenix · SS-0096, predicted (U)	1,800	0.556×	deep
Phoenix · SS-0096, predicted (K)	1,900	0.526×	round, sustained
Restored tomato design, predicted (U)	2,600	0.385×	vine-ripe savory
Per-axis scorecard ceiling	5,000	0.200×	the normalization cap

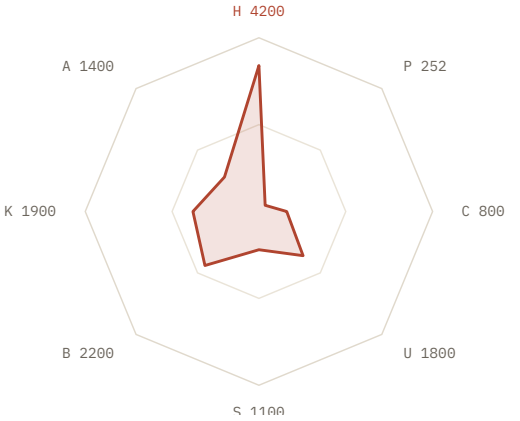
The quiet triumph in that table is the last two rows of the middle block. **Phoenix** — the full-spectrum cultivar we designed gene by gene to register on all eight axes at once — is designed to carry a savory note at **IFI-U 1,800** and a body at **IFI-K 1,900;** our model

predicts a plant that speaks umami *and* kokumi at predicted, balanced strength from its own tissue. That a modulator like kokumi can be scored at all is the deepest point here: because the Index is built on EC_{50}, and because kokumi's entire action is to *lower the working* EC_{50} of the tastes around it, a modulator is exactly the kind of thing the formula was made to read. It is not a flavor to be added. It is a lever on the flavors already there — and a lever on EC_{50} is a number.



Phoenix (SS-0096) under glass — designed to carry a predicted savory note at IFI-U 1,800 and a predicted body at IFI-K 1,900, the pair tuned together in the design.

IFI PROFILE



IFI-H	4,200
IFI-P	252
IFI-C	800
IFI-U	1,800
IFI-S	1,100
IFI-B	2,200
IFI-K	1,900
IFI-A	1,400

Reading kokumi off the curve — dose–response and the working EC50

To see *how* the body holds the note, put a number on the holding. A receptor's response to its ligand follows a dose–response curve — in its standard form the Hill equation,

$$\theta([A]) = [A]^n \div (EC_{50}{}^n + [A]^n)$$

where θ is the fraction of maximal response, [A] the glutamate concentration, and *n* the Hill coefficient — the cooperativity, greater than one when binding is self-reinforcing, as it is for the T1R1/T1R3 heterodimer with a ribonucleotide alongside. At [A] = EC_{50}, θ = 0.5 by definition: the half-point that anchors everything.

Now let kokumi do its single trick: it lowers the *working* EC_{50} of the umami response by some factor *f*, shifting the whole curve to the left, so a glutamate concentration that is held *fixed* suddenly sits higher on it. A dose pinned at the unmodulated half-point (θ = 0.50) climbs to θ = 1.5 ÷ (1 + 1.5) = **0.60** when a kokumi tone lowers the working EC_{50} by half again (*f* = 1.5, *n* = 1) — and, once the cooperativity of the paired receptor is counted, to $1.5^2 \div (1 + 1.5^2)$ = **0.69** (*n* = 2). Double the working potency (*f* = 2) and the same unchanged dose reaches θ = 0.67 and, at *n* = 2, θ = **0.80.** The glutamate never moved. The curve did. This is the quantitative meaning of *kokumi holds the note:* the body does not pour in more savor — it walks the savor already present further up its own response, and because the umami receptor is cooperative (*n* > 1), the walk is steeper than you would guess, which is exactly why a well-bodied broth tastes so much deeper than its glutamate assay says it should.

The ratio that reads as rich — U:K by application

The catch is ratio, and finding it is what the platform's flux-balance modeling is for at this intersection. Too much body under too thin a note and the dish is round but mute; too much note under too little body and it is bright and gone. The right balance is not fixed — it is a design input that depends on the application, and we carry a rough map of it by what each kind of savory thing wants between note and body.

APPLICATION	WANTS	TYPICAL U:K	WHY
Clear broth, sippable	note-forward	~2 : 1	savor bright and clean to the last of the bowl
Everyday soup, bouillon	balanced	~1.3 : 1	depth without heaviness
Sauce, gravy, demi	body-forward	~1 : 1.2	savor that clings and persists past the swallow
Meat analog, braise	even	~1 : 1	note and body together read as *substance*
Snack seasoning, coating	note-forward, fast	~2.5 : 1	immediate savor, little lingering body

Put Phoenix's predicted numbers to this map and the point lands: a predicted IFI-U of 1,800 against a predicted IFI-K of 1,900 gives a U:K of 1,800 ÷ 1,900 ≈ 0.95, or about **1 : 1.06** — note and body nearly even in our model, a hair to the body side, sitting squarely in the braise-and-analog register where savor reads as *substance.* That is not an accident of the design; it is where we tuned it.

The reason we can hand a maker a target as tight as "1 : 1.06" and mean it is reproducibility, and reproducibility is itself a piece of arithmetic. Each axis on our platform holds a batch-to-batch coefficient of variation of **≤7%.** When you form a ratio of two independent measurements, their relative errors add in quadrature, so the CV of the U:K ratio is $\sqrt{(0.07^2 + 0.07^2)} = 0.07 \times \sqrt{2} \approx$ **9.9%.** A

target ratio of 1.00 therefore lands, one standard deviation, between roughly 0.90 and 1.10 — and since the application windows above are each 15–30% wide, a ~10% ratio error sits comfortably *inside* one window: the build reproducibly tastes the way it was designed to taste. Contrast the world before the platform, where a wild savory trait can swing a coefficient of variation near **42%;** its ratio CV would be 0.42 × √2 ≈ **59%,** wide enough to wander across two or three application windows from one harvest to the next. That drift — not a lack of flavor, but a lack of *repeatable* flavor — is the thing precision design ends. We find the U:K a given use wants, and we tune the organisms to carry it, so that a maker asking for *depth* receives two numbers rather than one: an IFI-U that says *how savory,* and an IFI-K that says *how complete.*



Soybean — on our savory shelf one of the few organisms engineered to carry both the umami note and the kokumi body in a single tissue.

Growing the pair into a plant — the organisms that carry both

The savory shelf divides, roughly, into plants that carry the note, plants that carry the body, and the rarest and most valuable — plants that carry both in one tissue. We build the pairing from all three.

ORGANISM	CONSTRUCTS	PRIMARY AXES	DIFFICULTY	ROLE IN A U×K BUILD
Tomato	2,040	S / U / F / M	Easy	the umami note, with sweetness beside it
Soybean	360	U / K	Frontier	both axes in one plant — the core
Tea	280	U / B / A	Hard	umami (theanine) with an astringent edge
Yeast	240	U / K	Hard	both axes; the fermented savory base
Garlic	560	K / P	Moderate	kokumi body, γ-glutamyl peptides
Koji mold	80	U	Hard	enzymatic release of free glutamate
Shiitake	80	U	Hard	guanylate (GMP) — the synergy multiplier
Onion	40	K	Moderate	kokumi body in a sweeter register
Mushroom	40	U	Hard	umami, nucleotide-rich
Vanilla	40	K	Hard	kokumi carried toward the sweet side

The two organisms that carry *both* axes — **soybean** (360 constructs, U/K) and **yeast** (240, U/K) — are the core of the savory shelf precisely because the pairing lives in one tissue, grown together, balanced at the source. Around them we compose: **tomato** (2,040 constructs) brings the loudest restored note and a sweetness beside it; the **alliums** lay down body under everything; **shiitake** and **mushroom** bring the GMP that multiplies the note; **koji** brings the enzymatic machinery that liberates free glutamate as it works; **tea** offers theanine — itself a γ-glutamyl amino acid, glutamate wearing that same second face — for a savory note with an astringent edge.

What we never do is dose the body in from a packet after the fact. The whole conviction of this work, the one our family grows by, is that a flavor and the nourishment that carries it are one act: a plant grown to hold the most savor it can is a plant grown to hold the most of itself, laid down by its own enzymes, in its own tissue, through the long patient chemistry that slow food has always relied on. The computing that finds the ratio is one pair of hands at a keyboard; the table it serves, and the reverence for the living thing it serves it with, belong to all of us.

Where it holds, and where it does not

Honesty about limits is part of respect, so a few edges. The Index scores a receptor response, not a plate — it tells you how loudly a stimulus speaks to TAS1R1·TAS1R3 or CaSR, not how a mouthful behaves inside fat, salt, acid, and temperature, all of which shift perception in ways a single EC_{50} cannot carry. A savory build that reads cleanly in water by our model may behave differently in an emulsion, and matrix effects are worth exploring by any maker working with the build. Human panels add their own spread: sensitivity to these axes varies person to person, so the number is a population anchor, not a promise about one taster. The failure modes are specific and worth naming — a build pushed to a high IFI-U with too little IFI-K beneath it tastes thin and front-loaded no matter how large the umami number; the opposite error, body stacked under a

weak note, tastes round and oddly *flat,* full in the mouth with nothing to say. And a modulator has a ceiling: past a point, more kokumi stops deepening the note and starts reading as a dull, lingering heaviness, which is why the ratio, not the maximum, is the design target. The Index does not abolish the cook's judgment. It gives that judgment two honest numbers to work from — and keeps them steady, harvest to harvest, inside the ≤7% that turns a lucky batch into a thing you can grow on purpose. These are computational designs; Scoville Splice's cultivars and constructs have not been experimentally validated in a laboratory.

What a taster will finally call it is *rich,* and they will never quite be able to source the richness, because it is not the fat and not the salt. It is a savory note grown loud enough to carry, held inside a body grown to hold it — two clamshell receptors, one summed depth, measured as two numbers and tuned as a pair. It is savor the way the body was built to meet it. The builds this book gathers are that pairing, grown into one tissue and set on the table as food.

Chapter 18 · The Builds — *savory depth, from plants only.*

APPLICATION · broth · base · analog · ferment · every reading from plants

Enough mechanism; let it become dinner. Everything in this book resolves, finally, into a pot — and the builds below are the axis made edible, each scored so you can taste the depth and read it on the same page. They are composed entirely from the organisms of this library and their kin, with no added glutamate anywhere in any of them. The savor is grown, freed, and multiplied — never dusted on. What follows is five recipes and, underneath each one, the receptor-level account of why it reads the way it does: the enzyme that makes the molecule, the molecule that finds the receptor, and the single number the Index assigns to how loudly the two of them finally speak.

The number under the spoon — how a build is scored

Before the first pot, the instrument. Every reading in this chapter is an **IFI-U** value, and umami is the one axis the tongue reads with two receptors at once. The first is the heterodimer **TAS1R1·TAS1R3**, a class-C G-protein-coupled receptor whose large bilobed *Venus-flytrap* domain closes around a single molecule of free L-glutamate; the closure propagates through a cysteine-rich stalk into the seven-transmembrane core, which activates its G-protein, drives phospholipase-C-β2, releases intracellular calcium, opens the TRPM5 channel, and — through CALHM1 — lets the cell signal the nerve. The second is **mGluR1**, a taste-truncated metabotropic glutamate receptor that answers the same molecule at a different affinity and widens the dynamic range. Savor, at its root, is this: a carboxyl-rich amino acid settling into a flytrap and holding it shut long enough to be counted.

The Index does not count how much glutamate a food contains. It counts how *loudly* that glutamate speaks, and it defines loudness through the half-maximal concentration, EC_{50} — the concentration at which the receptor reaches half of its full response. Formally the response follows a Hill curve,

> $R([L]) = R_{max} \cdot [L]^n \,/\, (EC_{50}^n + [L]^n),$

with a Hill slope near unity ($n \approx 1$–1.5 for the umami heterodimer); set $[L] = EC_{50}$ and the response is exactly $R_{max}/2$, which is all the definition we need. A quieter stimulus needs a higher concentration to reach that half-point — a larger EC_{50}. A louder one reaches it sooner — a smaller EC_{50}. Invert that against a fixed reference and scale by a thousand, and loudness becomes a score:

> **$IFI\text{-}U = 1000 \times EC_{50}(\text{reference}) \,/\, EC_{50}(\text{candidate})$**

We anchor the umami reference to pure L-glutamate, and we set its EC_{50} at **5.0 mM** by convention, so that a plain glutamate solution scores **IFI-U 1,000** by construction. Everything louder than glutamate scores above a thousand; everything quieter, below. One consequence is worth keeping in your pocket for the rest of the chapter, because it turns every score into an EC_{50} with no algebra at all:

> EC_{50}(candidate) = 5000 ⁄ IFI-U (in mM), and the fold-shift versus glutamate = IFI-U ⁄ 1000.

A worked unit conversion grounds the reference so the millimolar numbers stay physical. L-glutamic acid has a molar mass of 147.13 g/mol, so the 5.0 mM anchor is 5.0 mmol/L × 147.13 g/mol = 735.7 mg/L, or **0.074 g per 100 mL** — about three-quarters of a gram of free glutamate in a full litre to reach the half-point. (As monosodium glutamate monohydrate, molar mass 187.13, the same 5.0 mM would weigh 0.094 g/100 mL; the Index reads the free glutamate ion either way, which is why a koji ferment and a jar of MSG with identical free-glutamate concentration score identically — the molecule is the same molecule.) Hold that anchor; every build below is just a different EC_{50}, and therefore a different score, read against it.

The synergy, written as an equation

Umami is the one axis where two quiet molecules together are louder than the sum of their voices, and the whole of this chapter leans on that fact. Free glutamate speaks to the flytrap; a 5′-ribonucleotide — inosinate (IMP) from aged and dried things, **guanylate (GMP)** from mushrooms — binds an adjacent pocket on the same TAS1R1 lobe and acts as a *positive allosteric modulator*: it does not open the receptor on its own, it stabilizes the closed, glutamate-bound conformation, and in doing so it lowers glutamate's EC_{50}. The nucleotide makes the glutamate already present sound louder. The molecular detail that governs every recipe here is that only the **5′-phosphate** isomer synergizes — a 2′- or 3′-nucleotide, or a nucleoside stripped of its phosphate, does nothing — which is why the enzyme that liberates the *5′* form, and the temperature that keeps that enzyme alive, are not garnish but the recipe itself.

The psychophysics of this were measured long before anyone had a receptor to point at, and they still hold. The relative umami intensity *y* of a glutamate-plus-nucleotide mixture obeys

> $y = u + \beta \cdot u \cdot v,$

where *u* is the glutamate fraction, *v* is the nucleotide fraction (same units), and β is a synergy constant specific to the nucleotide: **β ≈ 1218 for inosinate, β ≈ 2800 for guanylate.** Guanylate — the mushroom's nucleotide — is more than twice the synergist inosinate is, which is the quiet reason a shiitake outperforms a flake of bonito on a vegetarian table. Hold the glutamate fixed and the intensity ratio is simply $y/u = 1 + \beta \cdot v$: a steep carrying a guanylate fraction v = 0.005 g/100 mL multiplies the glutamate already in solution by 1 + 2800 × 0.005 = **15×** at that concentration.

Fifteen-fold, and yet none of our builds score 15,000. This is the chapter's first honest limit, and it is worth stating plainly rather than hiding. The Yamaguchi relation above measures *suprathreshold intensity* at one fixed concentration — how much louder a given mouthful tastes. The Index measures something stricter and more reproducible: the **EC_{50} left-shift**, the factor by which the half-activation concentration drops. These are two true lenses on one phenomenon, and the receptor-level shift realized at the dilute concentrations of an actual steep — where the nucleotide is only partly liberated and the liquor is far below saturation — lands at roughly **3 to 4×**, not fifteen. The Index chooses the EC_{50} lens on purpose: it is the receptor's own first event, it is the quantity that stays stable from batch to batch, and it refuses to flatter the cook. A build that scores IFI-U 3,400 is telling you, soberly, that its effective half-activation sits 3.4× below plain glutamate — and that sober number is the one you can design against.

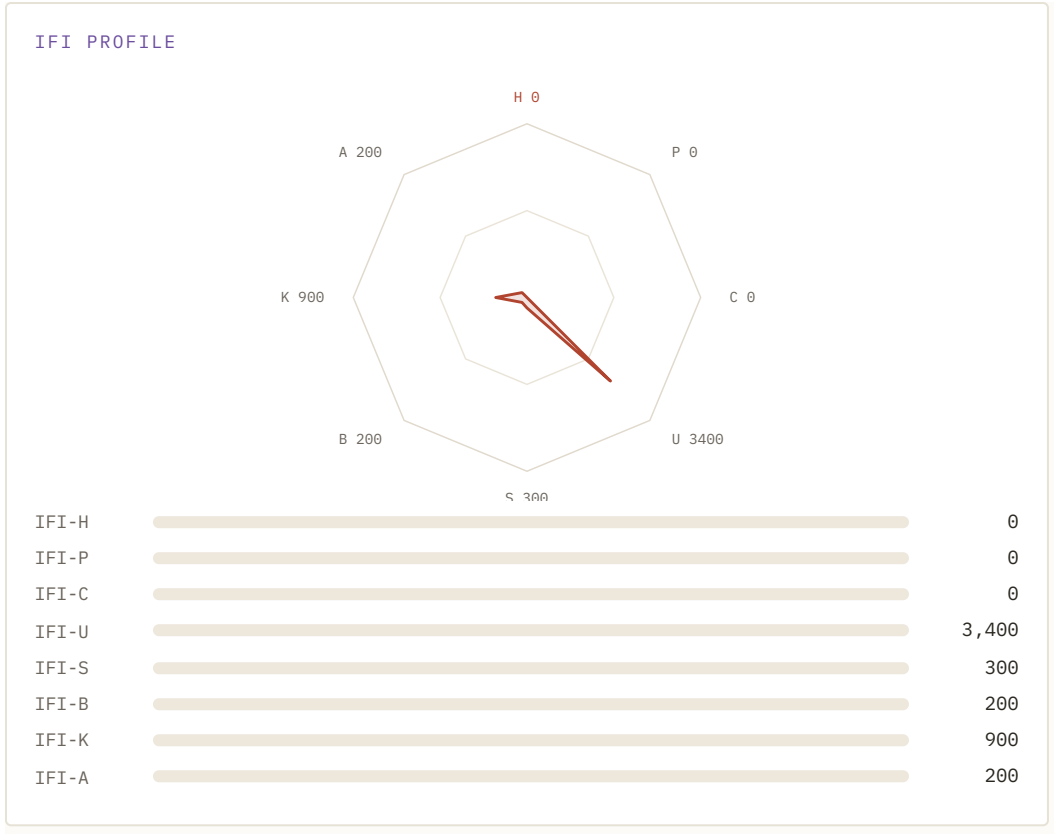
Read the five builds as a single EC_{50} ladder and the design intent becomes legible on sight:

BUILD	IFI-U	IFI-K	U : K	EC_{50}-U (MM)	FOLD VS. GLUTAMATE
Two-ingredient dashi	3,400	900	3.78	1.47	3.40×
Ten-minute broth	2,900	1,900	1.53	1.72	2.90×
Savory mother (sauce)	2,700	2,300	1.17	1.85	2.70×
Patty	3,000	2,500	1.20	1.67	3.00×
Koji garum	3,400	2,200	1.55	1.47	3.40×

The umami column barely moves — every build lands its note between EC_{50} 1.47 and 1.85 mM, a deliberately narrow window, because the note is the easy part once you have the organisms. What moves is the **U : K ratio**, the balance between the note (umami, read at TAS1R1·TAS1R3 and mGluR1) and the body (kokumi, read at the calcium-sensing receptor CaSR). The dashi is nearly four parts note to one part body; the sauce is almost even. That single ratio is the difference between a thing you sip and a thing that clings, and across these five pots we are going to walk it, on purpose, from one end to the other.

Build one — a two-ingredient vegetarian dashi

The whole axis in a bowl, and the fastest proof a skeptic ever gets. Pour water just off the boil — never fully boiling — over a strip of kombu and a handful of torn dried engineered shiitake, and leave them to steep twenty minutes off the heat. Strain. That is the entire recipe, and our model predicts **IFI-U 3,400** — higher than either ingredient alone, the synergy this Index exists to measure, happening in real time under your spoon.



Here is what the twenty minutes actually do, step by step, because every instruction in that one sentence is an enzymological decision. The kombu carries its glutamate **already free** — stored in the kelp's vacuoles as the ion, not locked in peptide — so it simply dissolves into warm water; this is why a cool-ish steep, not a boil, is correct for the kelp, since high heat drags alginates and mannitol into the liquor and clouds it toward bitterness without adding one unit of savor. The shiitake's contribution is manufactured on the spot. Dried mushroom is dense with RNA, and rehydration at **50–65 °C** rouses two of its own enzymes: a **ribonuclease** that cleaves the RNA backbone, and a **5′-phosphodiesterase** that trims the fragments to free 5′-mononucleotides — among them the guanylate that synergizes. Push the water to a true rolling boil and you denature both enzymes before they finish, liberating far less GMP; hold it just off the boil and the nucleases work the full twenty minutes. "Never fully boiling" is not a texture note. It is the line between an active and a dead enzyme, written into a recipe.

Now the number. The reference anchor gives EC_{50}(candidate) = 5000 ⁄ 3400 = **1.47 mM** glutamate-equivalent — the finished dashi reaches half-activation at a dilution 3.4× below plain glutamate, which is the guanylate paying for itself. The guanylate alone would read almost nothing at the receptor; the glutamate alone would read near the reference; together, through the allosteric pocket, they clear 3,400. Two ingredients, one pot, and the single clearest demonstration in the book that savor is a *relationship* between molecules, not a quantity of any one of them.

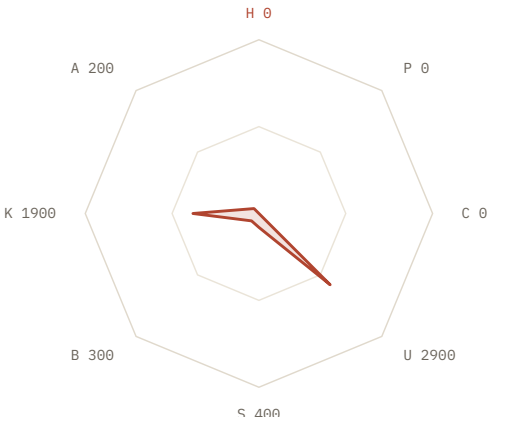
Build two — a ten-minute broth that tastes like all day

The dashi above, composed into a meal.

20 g	dried engineered shiitake, torn
1 strip	kombu (or 1 tbsp fresh koji, for glutamate at the source)
2 tbsp	light miso or fresh koji paste
4 cloves	kokumi garlic, smashed
1 large	onion, charred black on a dry pan, halved
2	restored tomatoes, halved
1.5 L	water, just off the boil
	tamari to finish · a few drops of toasted sesame oil

1. Pour the just-off-boil water over the torn shiitake and the kombu; leave to steep while you char the onion black at the cut face in a dry pan, no oil. The cool-ish steep pulls glutamate and guanylate without turning the kombu bitter.
2. Add the charred onion, smashed garlic, and halved tomatoes. Bring to the barest simmer — never a rolling boil, which clouds the broth and blurs the savor — and hold ten minutes.
3. Pull the pot off the heat. Whisk the miso or koji into a ladleful of hot broth until smooth, then stir it back in *off the heat,* so the live koji enzymes and the kokumi body survive.
4. Strain. Finish with tamari to the salt you want and a few drops of sesame oil. Taste it standing up, which is the only correct way.

IFI PROFILE



IFI-H	0
IFI-P	0
IFI-C	0
IFI-U	2,900
IFI-S	400
IFI-B	300
IFI-K	1,900
IFI-A	200

The broth takes the dashi's bare note and gives it a body, and the ifi block records the trade precisely: umami eases from 3,400 to **2,900** while kokumi more than doubles, from 900 to **1,900**, dropping the U : K ratio from 3.78 to **1.53**. The note recedes a half-step so the body can be heard. Three organisms build that body. The **charred onion** contributes a genuine Maillard crust — amino acids and reducing sugars condensing under dry heat into melanoidins and savory pyrazines — which is why it is charred *black* on a dry pan and not sweated in oil. The **kokumi garlic** and the **miso or koji** contribute γ-

glutamyl peptides, the short glutamate-headed chains that the calcium-sensing receptor reads as continuity and thickness. The **restored tomato** holds the umami window open from the other side.

That tomato earns a word, because it is the quietest engineering in the pot and the most important to what we believe. A vine-ripe tomato is one of the few plants that makes meaningful free glutamate on its own — and a tomato bred for shipping, picked green and gassed red, arrives at the table having lost the great majority of it. Restored through the plant's own **GAD / GDH1** route — the native glutamate-decarboxylase and glutamate-dehydrogenase machinery, tuned rather than overridden — our model predicts the fruit's umami reading climbs from a depleted **IFI-U 300** back to **2,600**: EC_{50} from 16.7 mM back down to 1.9 mM, a score restored almost ninefold to what a tomato grown with time and intention would always have carried. We did not add savor the tomato never had. We grew back what the truck took.



A restored tomato, cut — the umami the supply chain strips out, grown back in through the fruit's own glutamate pathway.

The one failure mode here is the miso. Whisked into a rolling pot it would be merely salty — the heat denatures the koji's live enzymes and flattens the γ-glutamyl peptides that the kokumi score depends on. Stirred back *off the heat*, those enzymes and peptides survive, and the broth keeps the 1,900 of body the ifi block promises. Note-forward, body underneath — the sippable U : K balance, built in ten minutes instead of a day.

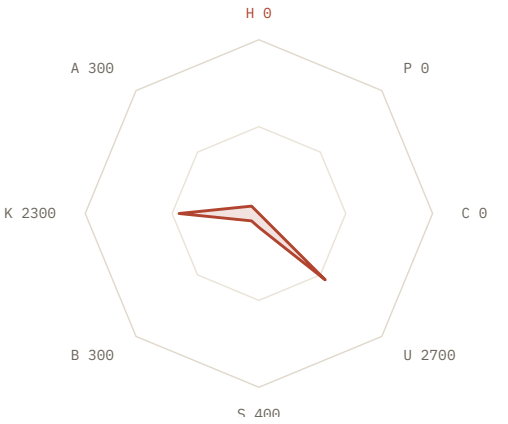
Build three — the savory mother

A sauce asks the opposite of a broth. Not bright-and-clean to the last sip but *clinging* — coat the spoon, stay on the palate, taste like more the longer it sits. That is a kokumi-forward balance, the body carried higher than the note so the savor extends past the swallow.

2 tbsp	neutral oil
1 tbsp	flour (or 1 tsp kuzu, for a cleaner body)
2 cups	the broth above (or water, at a cost)
3 tbsp	fermented soybean paste (or engineered U/K soy, loosened)
2 tbsp	engineered yeast
1 tbsp	kokumi garlic, grated fine
2 tbsp	restored tomato paste
	black pepper · a few drops of rice vinegar, at the very end

1. Cook the flour in the oil over medium heat until it smells of toast and turns the color of wet sand — a blond roux, body without a floury taste.
2. Whisk in the broth a ladle at a time, smooth before each addition, to a loose glossy base.
3. Stir in the soybean paste, yeast, garlic, and tomato paste. Drop to a bare simmer and cook until the sauce *naps* — coats the spoon and holds a drawn line.
4. Off the heat: black pepper, and a few drops of vinegar to cut the richness and make the savor read sharper by contrast. It should stay with you after you swallow.

IFI PROFILE



IFI-H	0
IFI-P	0
IFI-C	0
IFI-U	2,700
IFI-S	400
IFI-B	300
IFI-K	2,300
IFI-A	300

Read the ifi block against the broth and the design is unmistakable: umami settles to **2,700** while kokumi climbs to **2,300**, pulling the U : K ratio down to **1.17** — the most body-forward build so far, almost even between note and body. This is the sauce's entire thesis made numeric. The savory *mother* is not louder than the broth; it is longer.

The mechanism of "longer" lives at a second receptor. Kokumi is read not at the umami flytrap but at **CaSR**, the calcium-sensing receptor — another class-C GPCR, sitting on the same taste cells, which produces no taste of its own but *modulates* the umami and

sweet signals around it, stretching them into what the tongue reports as thickness, mouthfulness, and continuity. Its agonists are **γ-glutamyl peptides**: glutathione (γ-Glu-Cys-Gly) and its kin, short chains joined through glutamate's *side-chain* carboxyl rather than the usual backbone bond. Three organisms in this pot are dense with them. The **fermented soybean** brings both axes at once — free glutamate for the note, γ-glutamyl peptides for the body. The **yeast** lifts the body further. The **kokumi garlic**, grated fine so its cells rupture and its γ-glutamyl-transferase works freely, pushes CaSR higher still.



Engineered kokumi garlic — the γ-glutamyl peptides the calcium-sensing receptor reads as body, grown up into the clove.

Two physical details finish the sauce. The **roux** is not for flavor but for *nap* — the cooked flour's starch granules swell and gel, raising viscosity so the liquid clings to the spoon and dwells on the palate long enough for the slow CaSR body to register; kuzu does the same with a cleaner mouthfeel. And the **vinegar at the very end** is a contrast trick, not an acid for its own sake: a few drops sharpen the whole reading by juxtaposition, the way a line looks straighter against a curve. The tomato paste keeps a bright umami window open above all that weight so the sauce stays savory and does not go heavy. Body carried high, note held just under it — depth you can pour.

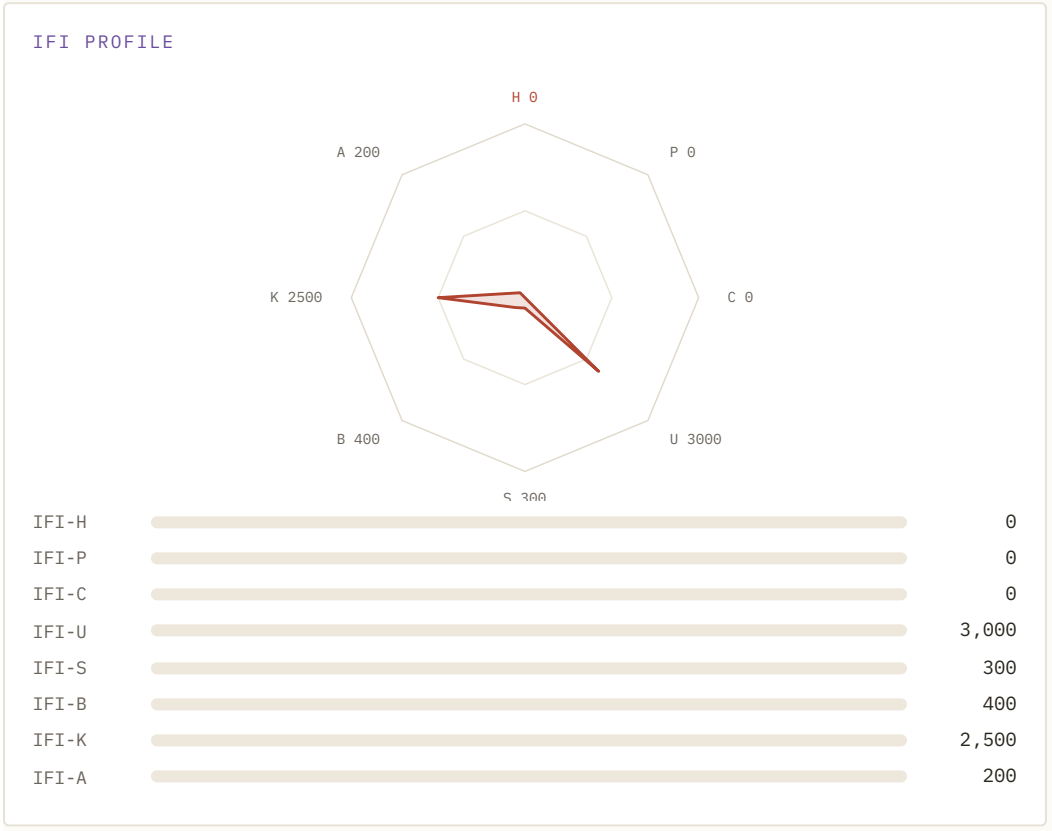
Build four — the patty that isn't pretending

It bears saying plainly, because it is the ethic of this entire build: this is not an imitation of meat. A good piece of meat is a particular architecture — glutamate, inosinate, rendered fat, and the Maillard crust a hard heat lays down — that took an animal a life to build and deserves respect, not a counterfeit. We build the same *architecture* from plants that can carry it, and let it taste like itself.

250 g	engineered mushrooms, chopped and seared hard and dry
1 cup	cooked U/K soybean, half of it mashed
2 tbsp	engineered yeast
2 tbsp	fresh koji paste (or light miso)
1 tbsp	kokumi garlic, grated
1	flax or egg binder · breadcrumbs, to hold
	salt · black pepper · neutral oil, to sear

1. Sear the chopped mushrooms in a dry heavy pan until they squeak, then brown — all the water gone, the glutamate concentrated, a real crust forming. This is where most of the savor is made; do not rush it.

2. Fold the seared mushrooms into the soybean — half mashed for body, half whole for the pull of texture — with the yeast, koji, garlic, and binder. Season. It should already smell braised.
3. Form four patties, press flat, chill ten minutes so they hold.
4. Sear in a film of oil, hard on both faces, until the outside is dark and the inside is hot. Rest a minute, the way you would rest anything you respect.



The patty is designed to reach **IFI-U 3,000** against **IFI-K 2,500** — the note back up near the dashi's loudness, the body at its highest in the chapter, and a U : K ratio of **1.20** held deliberately near even, because that evenness is precisely what the tongue reads, in meat, as *substance*. A broth is note over body; a steak is note and body nearly matched, and so is this. The savory architecture of meat has four load-bearing elements, and we build each one from a plant that genuinely makes it:

SAVORY LEVER	WHAT MEAT DOES	BUILT HERE FROM	MOLECULE	RECEPTOR
The note	free glutamate	koji + seared mushroom	L-glutamate	TAS1R1·TAS1R3 / mGluR1
The multiplier	inosinate (5′-IMP)	engineered shiitake / mushroom	5′-guanylate (GMP)	allosteric pocket, TAS1R1
The body	— (fat + peptides)	soybean, yeast, garlic	γ-glutamyl peptides	CaSR
The crust	Maillard on hard heat	hard dry sear	melanoidins, pyrazines	olfactory + trigeminal

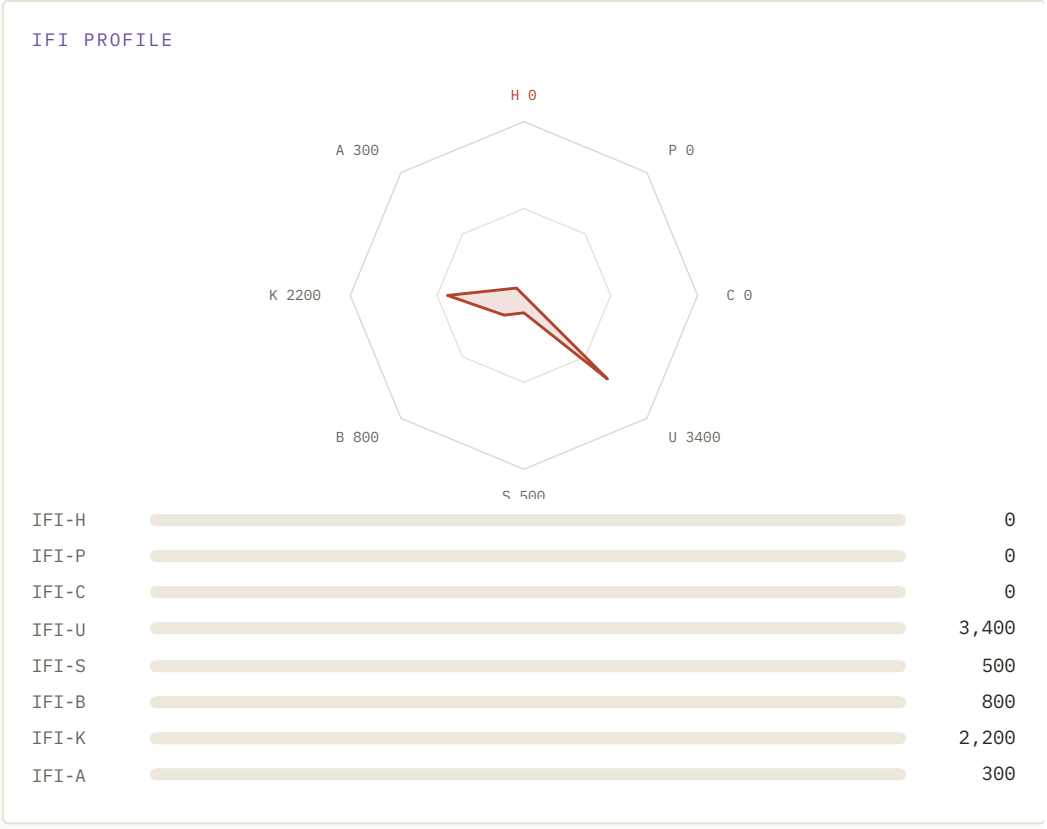
The first instruction carries most of the result, and the reason is water. Fresh mushroom is roughly nine-tenths water, and until that water is driven off the pan cannot pass 100 °C — the Maillard reaction, which needs ~140–165 °C of dry surface heat, simply cannot start. Searing hard and dry *concentrates* the free glutamate by removing the water it is dissolved in, and only then does the surface climb hot enough for amino acids and sugars to brown into the melanoidins and pyrazines of a real crust. "Until they squeak,

then brown" is the audible signal of that transition — the squeak is steam still escaping, the browning begins when it has stopped. Rush it and you have pale, wet mushroom with its savor diluted and no crust; hold it and you have the top note of the whole patty. Every lever meat uses, built from plants that make the molecule themselves — and not one gram of glutamate dusted on at the end.

Build five — a jar that keeps working

The last build is composition in *time.* You set the conditions and step back, and the koji keeps cleaving protein into free glutamate for weeks — savor not seasoned in but *grown* in, deeper tomorrow than today.

Blend **500 g** warm cooked U/K soybean with **150 g** fresh koji and **60 g** fine salt to a loose mash. That salt is not seasoning; weigh it. It is about **8%** of the total mass, and it is the one number standing between a ferment and a spoilage — enough to suppress *Clostridium* and the rot organisms while leaving the koji's own enzymes free to work. Pack into a clean jar, leave the top third empty, cover loosely. Hold it warm: a steady **~60 °C** works the koji hard for about a week; a room shelf works it gently for four to eight. Stir and taste every day or two — this is the part that teaches you what umami *becoming* actually tastes like. When it is deep, round, and faintly sweet, strain the liquor for a clear savory garum and keep the paste as a miso.



The garum ties the two highest predicted scores in the chapter together — **IFI-U 3,400** *and* **IFI-K 2,200**, a U : K ratio of **1.55** — and it earns them through enzymology rather than assembly. Three enzyme families from *Aspergillus oryzae*, the koji mold, do the work. Its **endo- and exo-peptidases** cleave soy's storage proteins into peptides and then into free amino acids — the note, manufactured in place from protein that held no free glutamate at all. Its **glutaminase** performs the single most important conversion in the jar: it hydrolyzes glutamine to **glutamate**, turning an amino acid the

receptor cannot read into the one amino acid the receptor reads best. And across the long warm hold, **γ-glutamyl-transferase** assembles the γ-glutamyl peptides that lift the CaSR body from a broth's register toward a sauce's. The savor is not in the recipe; it is *made by* the recipe, slowly, while you sleep.



Engineered U/K soybean — the protein the koji takes weeks to cleave into free glutamate and γ-glutamyl peptides.

Composition in time means the reading is a *trajectory*, not a point, and it is worth making that trajectory legible. The 3,400 is the endpoint of a curve the glutaminase draws day by day:

DAY (AT ~60 °C)	FREE GLUTAMATE	EC_{50}-U (MM)	IFI-U
0 (fresh mash)	negligible	≈ 16.7	≈ 300
2	climbing	≈ 3.3	≈ 1,500
4	most peptides cleaved	≈ 1.9	≈ 2,600
7+	plateau, rounded	≈ 1.47	≈ 3,400

The numbers on that climb are illustrative of the shape, not stamped on any one jar — the point is the direction. A ferment is the single build here that is *more* savory the longer you leave it, because unlike every other pot its instrument is still running when you walk away. The koji's proteases and glutaminase manufacture free glutamate in place while the long low heat builds the γ-glutamyl peptides the calcium-sensing receptor reads for body, and the jar deepens on its own schedule until the glutamine runs out.

What the five builds share — and where the Index stops

Five builds, one axis, no shaker — and the organisms behind them are a short, deliberate list, every one carrying its molecule by its own engineered pathway rather than by a dusted-on additive:

ORGANISM	CONSTRUCTS	AXES	CARRIES
Tomato	2,040	S / U / F / M	free glutamate (GAD / GDH1)
Garlic	560	K / P	γ-glutamyl peptides
Soybean	360	U / K	storage protein → glutamate + peptides
Yeast	240	U / K	glutamate + nucleotides + body
Shiitake	80	U	5′-guanylate (via RNase)
Koji mold	80	U	glutaminase + proteases
Onion	40	K	γ-glutamyl peptides, Maillard
Mushroom	40	U	free glutamate, 5′-GMP

Honesty about the instrument closes the chapter, because a number is only a form of respect for the reader if its uncertainty travels with it. An IFI-U score is the ratio of the stated reference EC_{50} to the candidate's model-predicted EC_{50}, and the error on a ratio compounds: the relative uncertainty is $\sigma_IFI / IFI = \sqrt{[(\sigma_ref/EC_{50}_ref)^2 + (\sigma_cand/EC_{50}_cand)^2]}$. Our builds carry a **≤7%** design specification for batch-to-batch coefficient of variation; at that target, the compounded uncertainty is $\sqrt{(0.07^2 + 0.07^2)} \approx$ **9.9%**, so the dashi's predicted score is honestly **IFI-U 3,400 ± ~340** — meaningful to two significant figures. The same propagation is exactly why the CV matters. Run the arithmetic at a wild-type process's **42%** variability and the compounded error is $\sqrt{(0.42^2 + 0.07^2)} \approx 43\%$: a "3,400" that is really 3,400 ± 1,450, a number that is no number at all. The reproducibility is not a quality boast standing to one side of the science. It is the precondition that lets the score mean anything on the tongue.

And the Index has edges it does not cross, which these pots make plain. IFI-U reads the half-activation of a receptor; it does not read the slow *release* of a ferment over weeks, the way a sauce's nap holds savor on the palate longer than a broth at the same score, or the aroma that a Maillard crust sends up the back of the nose to meet the taste — all of which a mouth registers and a single EC_{50} cannot. It reports the receptor's first event, loudly and reproducibly, and leaves the rest of the meal to the cook. Each build also fails in exactly one place, and each failure is an enzyme killed by heat or starved of salt: boil the dashi and the shiitake's nucleases die before they free the guanylate; whisk the miso into a rolling pot and the koji's peptides flatten; short the garum's salt below its eight percent and the jar spoils instead of deepening. The savor is grown, freed, and multiplied — note and multiplier and body, each read at its own receptor, each earned by a plant or a mold and never dusted on at the end. These are computational designs; Scoville Splice's cultivars and constructs have not been experimentally validated in a laboratory. The number on the page is only ever as good as the living thing that made it.

Chapter 19 · The Reference Library — *every savory organism, placed.*

the seven carriers · the kokumi kin · the natural benchmarks

Seven organisms carry this axis in our library, and between them they hold a little over three thousand constructs — but the number that matters is smaller and stranger than any construct count. It is the number a receptor returns when a savory molecule settles into it: not how much glutamate a food holds, but how *loudly* that glutamate speaks. We grew up at a vegetarian table, where savor was never borrowed from an animal and had to be coaxed instead out of a tomato left on the vine a week too long, a handful of dried mushrooms, a spoon of miso stirred in off the heat. The whole art of that table was loudness — teaching a plant to say the savory thing clearly. The reference library below is that art written down as arithmetic, every carrier placed by exactly how clearly it speaks and exactly which half of the savory chord it holds. This page is for citation and comparison: absorb it once and you can compose a broth, a sauce, or a whole engineered plant the way a cook reaches for salt — by reaching for a number.

Reading the library — loudness, not amount

Every figure here resolves against one formula, and the formula is worth holding in the hand before any table is read:

> $\mathrm{IFI}(\text{candidate}) = 1{,}000 \times \mathrm{EC}_{50}(\text{reference}) \div \mathrm{EC}_{50}(\text{candidate})$

EC_{50} is the concentration at which a stimulus drives its receptor to exactly half of full response — the receptor's own definition of "enough to be heard." A loud stimulus reaches that half-point at a *low* concentration; a faint one needs a great deal of itself to say the same thing. Invert the candidate's EC_{50} against a fixed reference, scale by a thousand, and the inversion does something quietly beautiful: it turns a concentration into a volume. Because loudness runs as $1 \div \mathrm{EC}_{50}$, the formula rearranges to a line a child could read —

> $\text{loudness}(\text{candidate}) \div \text{loudness}(\text{reference}) = \mathrm{IFI} \div 1{,}000$

— so an IFI-U of 2,600 is not an abstraction. It says the food is **2.60×** as loud as the reference at the umami receptor. An IFI-U of 3,200 is **3.20×**. The emptied supermarket tomato, picked green and gassed into a false blush, reads near **300** — *0.30×*, less than a third as loud as the benchmark it was bred from. Restore that same tomato to the savor a vine-ripened fruit carries and it climbs to roughly **2,600**. The restoration is therefore a loudness gain of 2,600 ÷ 300 ≈ **8.7×**, and in the receptor's own terms it is a leftward slide of the entire dose–response curve: the half-max concentration drops to 300 ÷ 2,600 ≈ **11.5%** of what it was, a shift of $\log_2(8.7) \approx$ **3.1 octaves** toward sensitivity. The tomato did not gain a new flavor. It simply stopped mumbling.



A restored tomato in cross-section — the locular jelly around the seeds is where free glutamate concentrates as the fruit ripens on the vine.

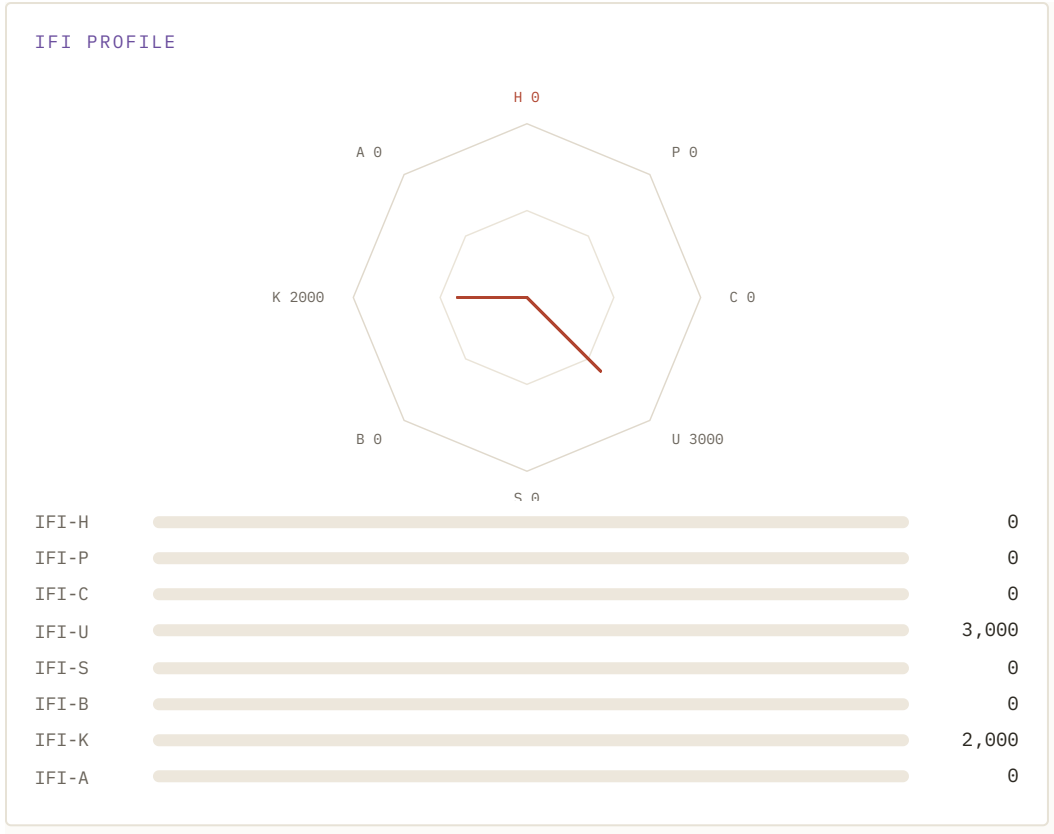
That single worked example is the whole method in miniature, and it is why we quantify loudness rather than milligrams. Milligrams are honest but mute; they cannot tell you that the glutamate in a ripe tomato is *heard* far out of proportion to its mass once a nucleotide stands beside it. The Index listens where a scale only weighs.

The seven carriers

Here are the seven, on one page, each with its canonical construct count and index axes — the fixed record — and a representative restored IFI-U and IFI-K, the design target we tune toward. These restored figures are typical values, not fixed SKUs; a line is tuned to a brief, and the brief moves. The construct counts and axes do not.

ORGANISM	CONSTRUCTS	INDEX AXES	ROLE ON THE FLOOR	IFI-U (TYP.)	IFI-K (TYP.)	LOUDNESS-U
Tomato	2,040	S · U · F · M	the restored note; 300 → 2,600	2,600	400	2.60×
Soybean	360	U · K	both axes in one plant	2,900	2,400	2.90×
Tea	280	U · B · A	theanine's brothy green	1,900	300	1.90×
Yeast	240	U · K	note + multiplier + body, one cell	3,000	2,000	3.00×
Koji Mold	80	U	the enzyme engine; glutamate made in place	3,200	600	3.20×
Shiitake	80	U	guanylate; the multiplier	2,800	500	2.80×
Mushroom	40	U	the fungal floor; glutamate with a little body	2,400	400	2.40×

They divide the labor of savor cleanly, and the division is the first thing to learn here. There are **glutamate-makers** that supply the note itself — tomato, mushroom, the raw material of koji. There are **nucleotide-makers** that supply no great note of their own but multiply whatever note stands beside them — shiitake chief among them. And there are **enzymatic engines** — koji, and the living yeast cell — that do not merely store savor but manufacture it on the spot, freeing what the quieter organisms only hold. Yeast is the rare soloist that does all three at once: it carries the glutamate note, makes its own 5′-nucleotide multiplier, and lends a round body, all inside a single cell.



That is a yeast cell's honest profile, and we write it with its zeros showing. A yeast makes no capsaicin, no menthol, no tannin, no sugar worth tasting — so seven of its eight axes sit at the floor, and we do not flatter them with small invented numbers. A specialist's scorecard should confess what a thing cannot do as plainly as it declares what it can. Two axes light; the rest are dark; that spike-and-floor shape *is* the engineering. We tune the two we mean and leave the others at zero, because a profile that pretends to richness everywhere is the profile of something designed by wishing rather than by design.

Turn the IFI-U column into the receptor's native language and the ladder sharpens. Since IFI-U = 1,000 × EC_{50}(ref) ÷ EC_{50}(candidate), the candidate's own half-max concentration is simply EC_{50}(candidate) = EC_{50}(ref) × (1,000 ÷ IFI-U) — a pure fraction of the reference, no absolute number required to compare them:

CARRIER	IFI-U	EC_{50}(CANDIDATE) ÷ EC_{50}(REF) = 1000/IFI	READS HALF-MAX AT…	LOUDNESS VS REF
Koji Mold	3,200	0.31	31% of the reference dose	3.20×
Yeast	3,000	0.33	33%	3.00×
Soybean	2,900	0.34	34%	2.90×
Shiitake	2,800	0.36	36%	2.80×
Tomato	2,600	0.38	38%	2.60×
Mushroom	2,400	0.42	42%	2.40×
Tea	1,900	0.53	53%	1.90×

Read the middle column as the point of the whole axis: koji reaches the receptor's half-response at *less than a third* of the concentration the reference needs, which is another way of saying koji's glutamate is the loudest savory voice in the library. Tea sits lowest not because theanine is weak but because it is a cousin, not the thing itself — and a cousin reads faintly on purpose.

The receptor, step by step

To understand why loudness and not mass is the right measure, follow a single glutamate ion from the tongue inward. The savory axis is read by two receptor systems working in concert, and the Index names both: the heterodimer **TAS1R1·TAS1R3** and the metabotropic **mGluR1** in its truncated taste form. The heterodimer is the one to watch closely. It is a class C G-protein-coupled receptor, and each of its two subunits ends, far outside the cell, in a large bilobed structure the field named for the plant it resembles in motion — the **Venus flytrap domain**. L-glutamate drifts into the open cleft of the TAS1R1 flytrap and the two lobes close over it. That closure is the entire event. A flytrap shutting a few nanometers at the top of the receptor becomes, through the cysteine-rich stalk below it, a twist in the seven-helix bundle buried in the membrane — and the twist is read on the inside of the cell:

1. The closed receptor activates its G-protein (α-gustducin, with the Gβγ pair released alongside it).
2. Gβγ switches on **phospholipase C-β2**, which cleaves the membrane lipid PIP_2 into **IP_3**.
3. IP_3 opens its receptor on the endoplasmic reticulum, and stored **Ca^{2+}** floods the cytosol.
4. The calcium opens **TRPM5**, a monovalent cation channel; sodium pours in; the cell depolarizes.
5. Depolarization opens **CALHM1/CALHM3** channels and the cell releases **ATP** as its transmitter.
6. ATP crosses to the **P2X2/P2X3** receptors on the gustatory nerve, and only now — six steps downstream of a flytrap closing — does the brain receive the word *savory*.

Every step after the first is a near-switch: it fires or it doesn't. So the thing that actually sets how loud the savory signal arrives is step one — *what fraction of the flytraps are shut at any instant* — and that fraction is governed by the dose–response curve the receptor obeys, the Hill function:

```
R(L) = R_max × Lⁿ ÷ (EC₅₀ⁿ + Lⁿ)
```

At the ligand concentration L = EC_{50}, the equation collapses to R = R_max ÷ 2 — half the flytraps shut, half the signal — which is precisely why EC_{50} is the number we anchor to. For a simple receptor with Hill coefficient n = 1, the curve's shape is fixed and worth memorizing: at one-third of EC_{50} the response is ¼ (25%); at EC_{50} it is ½; at three times EC_{50} it is ¾; at nine times EC_{50} it is ⁹⁄₁₀. A twenty-seven-fold span of concentration buys you only the stretch from a quarter-lit to nine-tenths-lit receptor. That compression is why doubling the amount of glutamate in a food never doubles its savor — and why *lowering the EC_{50}*, sliding the whole curve left so the same glutamate shuts more flytraps, is the lever we actually pull.

The bridge from the benchmark tables to this curve is a unit conversion, and it is clean because free glutamic acid has a molar mass of 147.13 g/mol. Divide any milligram-per-hundred-gram figure

by 147.13 and the food's savor becomes millimoles the receptor can be dosed with:

FOOD	FREE GLUTAMATE (MG/100 G)	≈ MMOL / 100 G
Kombu (dried kelp)	~2,240	15.2
Yeast extract	~1,960	13.3
Nori (dried laver)	~1,380	9.4
Parmigiano-Reggiano (aged)	~1,200	8.2
Soy sauce	~400–1,700	2.7–11.6
Fish sauce	~950	6.5
Cured ham	~340	2.3
Ripe tomato	~150–250	1.0–1.7
Green pea	~110	0.75
Human breast milk	~22 *(per 100 mL)*	0.15

Now the loudness story becomes physical. The umami detection threshold in water sits near **1 mM** of free glutamate. A dashi brewed the old way — ten grams of kombu steeped in a liter of water, pulling perhaps a generous 70% of its glutamate into solution — delivers 10 g × 2,240 mg/100 g × 0.70 = 157 mg into that liter, which is 157 ÷ 147.13 ≈ **1.07 mM**: a broth sitting barely a whisper above threshold. Tasted alone it is thin, almost disappointing. The reason anyone bothers with kombu is not the kombu. It is what happens when a nucleotide arrives.

The multipliers and the arithmetic of synergy

The second half of every savory chord is a 5′-ribonucleotide — inosinate (IMP) from dried fish, guanylate (GMP) from dried fungi. Alone these taste of almost nothing. Their whole genius is allosteric. IMP and GMP bind not in the glutamate cleft but at a neighboring site on the TAS1R1 flytrap, near the hinge, and a bound nucleotide acts like a hand pressing the trap gently shut: it stabilizes the closed conformation and so *lowers glutamate's EC_{50}* — raises its apparent affinity — without adding a single glutamate ion to the pot.

This is where the arithmetic turns dramatic, and it falls straight out of the Index formula. If a nucleotide drops the glutamate EC_{50} by a factor **f**, then because IFI-U runs as 1 ÷ EC_{50}, the effective loudness scales by exactly that same **f**:

```
IFI-U(paired) = f × IFI-U(glutamate alone)
```

A nucleotide that halves the EC_{50} doubles the savor; one that cuts it to an eighth multiplies it eightfold. The classical umami-synergy literature puts the ceiling of that multiplication near **f ≈ 7–8** for an even glutamate-plus-nucleotide mixture — which is why our one-liter kombu broth, dosed at a bare 1.07 mM and tasted alone as a whisper, reads like **7–8 mM** of glutamate the instant a pinch of bonito or a single dried shiitake joins it. The glutamate did not increase. The receptor simply began to hear it seven times more clearly. Here is the palette of multipliers, in milligrams per hundred grams of the dried material:

FOOD	5′-NUCLEOTIDE (MG/100 G)
Dried bonito (katsuobushi)	IMP ~470–700
Dried sardine (niboshi)	IMP ~350–800
Dried shiitake	GMP ~150
Nori	GMP + IMP + AMP *(all three)*
Beef, pork	IMP ~80–100

There is one honest edge to flag where synergy lives. Our scorecards normalize each axis to a ceiling of **5,000**. But koji at IFI-U 3,200, paired with a guanylate source that drops its EC_{50} toward one-half, computes to an effective loudness near **6,400** — past the top of the card. The card cannot draw it; it clips. This is not a flaw to hide but a boundary to state plainly: a scorecard measures a component in isolation, and savor at the table is a composition that routinely exceeds any single bar. So we report the glutamate-maker and the multiplier as two numbers and let the cook — or the receptor — perform the product. The map is honest precisely because it refuses to pretend the territory fits inside the frame.

The enzymatic engines

Two of the seven carriers do something the others cannot: they make glutamate rather than merely hold it, and they are the reason a ferment deepens on a shelf while a fresh vegetable only fades.

Koji — the mold *Aspergillus oryzae* grown over steamed soybeans or rice — is the engine in its purest form, and it earns the library's highest umami figure, IFI-U **3,200**, not by storing savor but by secreting the tools to liberate it. Its proteases cleave the long storage proteins of the bean into free amino acids, glutamate chief among them; its glutaminase deamidates bound glutamine directly into glutamate; its amylases free the sugars that feed the whole slow transformation. Set koji against soybean and time does the dosing: the protein that was silent inside the bean is cut, residue by residue, into a note that grows louder for months. This is why miso, soy sauce, and the long ferments taste of something a fresh legume never can — the glutamate was always present, locked in peptide bonds, and the mold simply carries the key.



Soybean — the storehouse of bound protein that koji's proteases and glutaminase unlock into free glutamate.

Shiitake is the other engine, and its chemistry is a small marvel of timing. The mushroom holds no great store of free guanylate while it lives; GMP is manufactured on demand when the fungus's own ribonuclease cleaves its ribosomal RNA, snipping out 5′-GMP one

nucleotide at a time. That reaction is temperature-gated, with its working optimum around **60–65 °C** — which is why the method matters as much as the mushroom. Rehydrate a dried shiitake in cold water and raise it slowly through that window, and the ribonuclease runs to completion, building guanylate as it goes; drop the same mushroom into already-boiling water and you denature the enzyme before it has worked, leaving the multiplier unmade. The dried mushroom outperforms the fresh here for the same reason: drying both concentrates the substrate and primes the enzymatic machinery that cold, patient steeping then sets loose. The savory cook who steeps shiitake overnight in the refrigerator and warms it gently is not being fussy. They are running an enzyme reactor at its optimum.

The kokumi kin

No savory build is complete with note and multiplier alone, because savor is not only a signal — it is a *body*, a continuity, the sense that a mouthful has weight and holds on. That dimension is the kokumi axis, read not at the glutamate receptor but at **CaSR**, the calcium-sensing receptor, by a family of **γ-glutamyl peptides** — glutathione and its kin, γ-Glu-Val-Gly and γ-Glu-Cys-Gly among them. These peptides are nearly tasteless alone; their work is to lengthen and thicken whatever savor already stands in the mouth, the way a bass note gives a chord its floor. Three organisms carry this body in our library without carrying much umami of their own:

ORGANISM	CONSTRUCTS	INDEX AXES	WHAT IT BRINGS
Garlic	560	K · P	the kokumi workhorse; γ-glutamyl peptides, a pungent top-note on a dial
Onion	40	K	the base note under the base note; charred, a real umami of its own
Vanilla	40	K	a round, sweet mouthfeel; kokumi in an unexpected register



Garlic in cross-section — each clove is dense with the γ-glutamyl peptides that read as richness and continuity at the CaSR receptor.

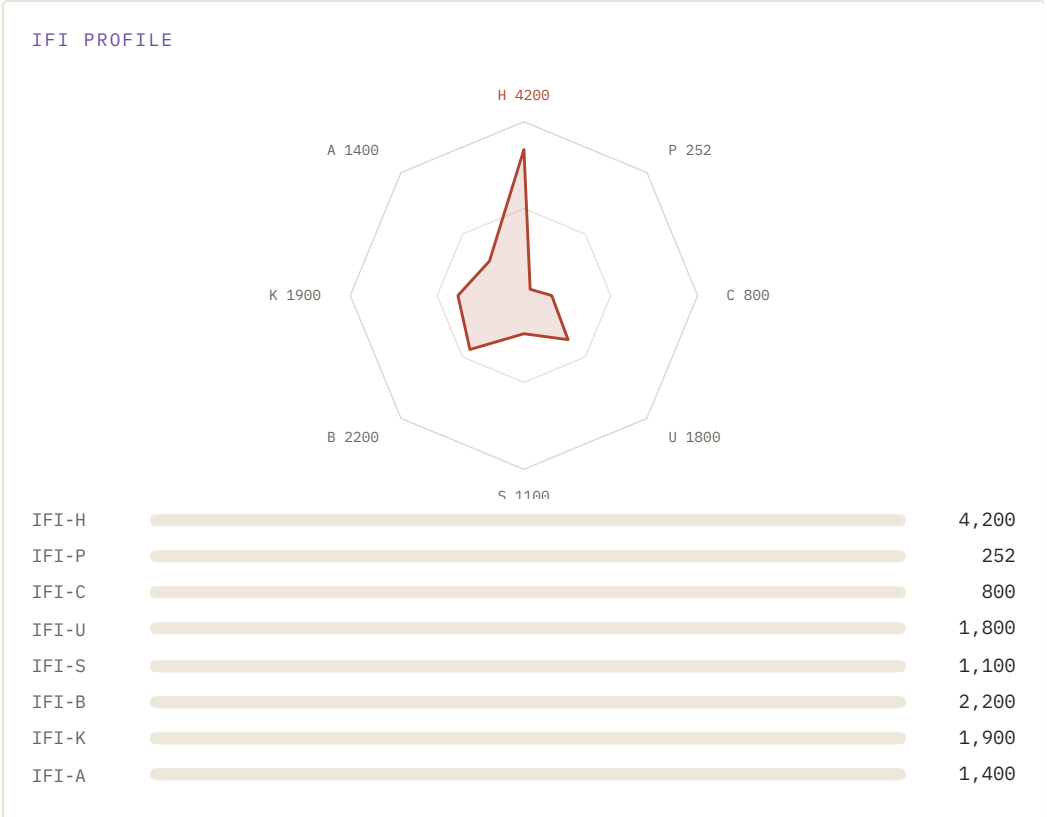
Garlic is the workhorse — 560 constructs, the largest kokumi library we hold outside the main savory carriers — because the clove is simply dense with γ-glutamyl peptides, and because its pungency rides a second axis we can dial independently of the body it lends. Onion sits quieter until it is charred, at which point the long cook builds a real umami of its own atop the kokumi floor. Vanilla is the

surprise in the set: a kokumi agent in a sweet register, lending roundness and mouth-filling weight to things no one would call savory at all. Body, it turns out, is axis-agnostic. The receptor that reads richness does not care whether the dish it thickens is a broth or a custard.

The natural benchmarks

It is worth setting our restored figures against the natural world's own savory benchmarks, because the entire point of restoration is to return a plant to what nature's best examples already prove is possible — not to exceed nature but to stop falling short of it. We are a vegetarian house, and we cite the katsuobushi and the cured ham in the benchmark tables above not as our own table but as proof: nature reaches these numbers, and if an animal can, a plant that is merely allowed to ripen can too. Kombu holds **15.2 mmol** of free glutamate per hundred grams; a vine-ripened tomato, **1.0–1.7 mmol**; the supermarket tomato that forgot itself, a fraction of that. The target was never to beat the kelp. It was to grow a tomato that remembers it is a tomato.

To keep the savory axis honest about its place, it helps to see that umami never has to stand alone — that it composes with every other axis a plant can carry. Our full-spectrum flagship proves it, lighting all eight axes at once, with a predicted umami note sitting right among the heat and the bitter:



That U of **1,800** is the quiet reassurance beneath the whole library: savor is not a jealous axis. It sits at 1.80× the reference even inside a plant designed chiefly for fire, and it carries a kokumi of 1,900 beside it. Which means the savory floor is never a thing you add last, as an apology for a dish that lacks depth. It is a dimension you can design in from the start, in concert with sweetness, with heat, with body — eight voices in one fruit, each designed, each placed.

Error, limits, and the honest edges

A reference library is only as trustworthy as its error bars, so here are ours, stated rather than hidden. Every figure in this book is held to a batch coefficient of variation of **≤7%**. For a restored tomato at IFI-U 2,600, that is σ = 0.07 × 2,600 ≈ **±182**, so the true loudness sits within roughly **2,418–2,782** at one standard deviation. For koji at 3,200, the envelope is ±224, or **2,976–3,424**. Those are tight numbers, and holding them is most of the work no one sees.

They also compound honestly when you pair organisms, and the honesty cuts against us in a way worth showing. If a paired savory build multiplies two independently specified components — a glutamate-maker and a multiplier — the relative errors add in quadrature rather than linearly:

> $CV(pair) = \sqrt{(CV_1^2 + CV_2^2)} = \sqrt{(0.07^2 + 0.07^2)} \approx \mathbf{9.9\%}$

So two components each held to ≤7% yield a pairing of about **10%**, and three components ($\sqrt{3} \times 0.07$) about **12%**. Composition buys loudness and spends precision; a cook who stacks four savory sources is working at a looser tolerance than any one of them alone, and a formulator who needs a tight final spec should prefer the single engineered organism that carries both halves — the soybean, the yeast — over a four-part blend that drifts.

And there are failure modes, because any engine that can be run well can be run wrong:

- **The GABA shunt.** A tomato's free glutamate is forever being drained toward GABA by its own glutamate decarboxylase. Push restoration by brute overexpression and the enzyme drinks the gain; the fruit accumulates GABA instead of savor and the IFI-U barely moves. Restoration is not addition — it is balancing a flux so the pool fills faster than it empties.
- **Dephosphorylated multipliers.** Fresh fungi and fresh fish carry phosphomonoesterases that cleave the 5′-phosphate clean off IMP and GMP, turning a multiplier into flavorless inosine or guanosine. Combine raw enzyme sources and the synergy you counted on quietly disappears; a brief blanch to denature the phosphatase is the difference between an eightfold lift and none.
- **The over-boiled kelp.** Hold kombu at a rolling boil and you stop extracting glutamate and start extracting alginates and mannitol — a mineral bitterness and a slick, slimy body. The savory window closes above a gentle steep; past it, the kelp gives up everything except the thing you wanted.

The deepest limit is the one a reference library is always tempted to forget: **loudness is not pleasure.** The Index measures how clearly a savory molecule is heard, not whether the hearing is good. A number tells you a broth will register; it cannot tell you the broth is kind, or balanced, or worth returning to. We built the Index because you cannot design what you cannot measure — not because the measure is the meal.

Composing from the palette

For the maker who wants a starting point rather than a whole palette, everything above collapses into a short decision guide — where to reach first for each kind of savory job, with the arithmetic already folded underneath each choice:

IF YOU WANT…	START WITH	THEN ADD FOR BODY	THEN ADD FOR THE MULTIPLIER
A clear, sippable broth	koji or kombu (glutamate)	a charred onion	dried shiitake (guanylate)
A clinging sauce or gravy	soybean (both axes)	yeast + garlic	restored tomato, for brightness
A meat analog with substance	soybean + seared mushroom	yeast + garlic	koji, to generate more note
A snack or coating seasoning	yeast (note + multiplier)	a touch of onion	— (it carries its own I+G)
A bright savory lift	restored tomato	— (tomato lifts, not weighs)	a little shiitake
A ferment that deepens on the shelf	koji + soybean	time and salt	the ferment makes its own

The guide is deliberately plain, because the hard thinking is already done inside the organisms — each tuned to carry its half of the synergy so a maker can compose by pairing rather than by dosing. Reach for a glutamate-maker, add a body, add a multiplier, and let the receptor do the arithmetic the formula promised: loudness times a synergy factor, read off a flytrap closing a few nanometers at the top of the tongue. That is the whole method, reduced to a line a cook can keep in their head.

Place this savory floor inside the whole library, for the reader keeping count. The seven umami carriers hold **3,120 constructs** between them — the tomato's 2,040 the bulk of it — out of roughly **~225 distinct construct designs** across **42** host organisms and **8** sensory axes, backing **19,500** catalogue SKUs, of which about **1,000** are live and purchasable today. Every figure is held to the same **≤7%** batch CV, and the method beneath it is documented in the **21** peer-reviewed papers and **3** filed patents that stand behind the platform. We give these numbers not as a boast but as a boundary: the savory axis is one of eight, the umami organisms are seven of forty-two, and this book is one lens on a library that holds many. The discipline that keeps those counts from drifting across a shelf of books is the same discipline that keeps an IFI-U from drifting across ten thousand jars — one source of truth, quoted everywhere, corrected nowhere by convenience.

A last note on the tea, because drinkers rarely name it. The brothy softness of a fine shaded green tea is **theanine** — more precisely γ-glutamylethylamide, glutamate's near-cousin wearing an ethyl group — reading faintly on these same receptors at IFI-U 1,900, a cousin's volume rather than the thing itself. It is why a well-made gyokuro tastes less like a beverage than like a pale green broth, and why the savory floor reaches past the kitchen entirely: into the cup, into the leaf, into anywhere a plant grows free glutamate or its kin and we have learned, with reverence and a ruler, to read the number.

These are computational designs; Scoville Splice's cultivars and constructs have not been experimentally validated in a laboratory.

Chapter 20 · A Closing Note · Nourishment — *savory was always the signal.*

where the quiet through-line stops being quiet

We have come the whole way around this axis — the receptor, the arithmetic, the pathways, the organisms, the builds — and we should end where the body does. Of the eight tastes this library scores, umami is the one most directly about being kept alive. Sweetness flags energy; bitterness flags danger; salt flags the minerals the blood runs on; sour flags the ripeness line. Umami flags **protein** — the free glutamate on the tongue is the body's advance word that nourishment is here and coming apart into forms it can use. This is not a metaphor we reached for. It is why glutamate floods the first food any of us ever tasted, why a broth carried to the sick is a broth rich in it, why *savory* and *sustaining* have meant nearly the same thing in every kitchen in every language that ever kept a pot.



The vine at first light — where savor and nourishment ripen in the same late window, two names for one metabolic maturity.

Which is why, of all eight axes, this is the one where our quiet through-line stops being quiet. We make no health claim; we never do, and we will not start with the taste that would tempt us most, because a value is worth more kept honest than spent on a slogan. But we will say the plain thing, because it is true and because the plant says it first: a plant grown back toward its fullest savor is, almost always, a plant grown back toward its fullest nourishment. The same late-ripening window that builds the free glutamate builds the lycopene and the vitamin C; the same metabolic maturity that

pools the savory amino acids pools much else the body was listening for. When we gave the tomato its umami back, we gave it its lycopene back in the same motion. We did not have to choose. On this axis, more than any other, taste and nourishment were never two projects — they are one maturity, wearing two names. What follows is that one maturity taken apart slowly: the receptor that reads it, the arithmetic that scores it, the synergy the tongue was built for, and the native route by which a plant can be grown back toward it on purpose.

The receptor that reads nourishment

Begin where the molecule meets the tissue. The savory signal is read, first, by a heterodimer — two class C G-protein-coupled receptors, **TAS1R1 and TAS1R3**, locked together on the apical membrane of a taste cell. Each subunit carries, on its extracellular face, a bilobed **Venus flytrap domain**: two clamshell halves hinged at a cleft. Free L-glutamate — the anion, not the glutamate still bound inside a protein, which is tasteless until something takes the protein apart — settles into the cleft of the TAS1R1 flytrap and pulls the two halves shut. That closure is the whole event. It is not warmth, not pressure, not chemistry the cell has to deliberate over; it is a shape changing, a trap closing on a single small molecule that only exists in quantity where protein is already breaking down into the amino acids a body can absorb.

The closed flytrap twists its cysteine-rich linker, the linker torques the seven-transmembrane bundle below it, and the bundle — now in its active conformation — engages a heterotrimeric G-protein on the inner face of the membrane: α-gustducin and its βγ partners. From there the cascade is quick and well mapped. Gβγ switches on **phospholipase C-β2**, which cleaves membrane PIP_2 into diacylglycerol and **IP_3**. IP_3 opens its receptor on the endoplasmic reticulum, and stored **calcium** floods the cytosol. That calcium gates **TRPM5**, a cation channel that lets sodium in and swings the cell toward threshold; the depolarization opens **CALHM1/3** channels, the cell releases **ATP**, and the ATP crosses the synapse to $P2X_2/P2X_3$ receptors on the gustatory afferent. The nerve carries it to the nucleus of the solitary tract, the thalamus relays it, and the insular cortex finally renders it as the thing you actually feel: depth, roundness, the taste of being fed. A second sensor rides alongside the heterodimer — a taste-specific, N-terminally truncated **mGluR1**, another class C receptor with its own Venus flytrap — tuned to the same ligand but contributing most where glutamate runs high, deepening and sustaining the signal rather than merely switching it on. The index anchors the umami axis to that ensemble, mGluR1 and TAS1R1·TAS1R3 together, because both are reading the same news off the same molecule.



Tomato, in cross-section — the locular jelly around the seeds is where a ripening fruit pools its free glutamate, the savory signal made visible.

Hold that mechanism in mind, because every number in this chapter is a measurement of one step of it. We do not score how much glutamate a food contains. We score how *loudly* that glutamate speaks to this receptor — how far shut it pulls the flytrap, how near to threshold it swings the cell. Concentration is the cause; loudness is the effect; and the gap between them, which turns out to be wide and curved and full of consequence, is exactly where a shipping tomato lost its savor and where we found the method to grow it back.

The arithmetic of loudness

A taste becomes designable the moment it becomes a number, and the number we use is loudness at the receptor, not mass in the fruit. The whole library rests on one relation:

> $\mathrm{IFI(candidate)} = 1{,}000 \times \mathrm{EC_{50}(reference)} \div \mathrm{EC_{50}(candidate)}$

EC_{50} is the concentration of a stimulus that drives its receptor to half of full response — the half-shut flytrap. A quiet food needs a lot of glutamate to get there and carries a high EC_{50}; a loud food gets there on very little and carries a low one. Invert that against a fixed reference and scale by a thousand, and loudness becomes a score that reads the same for a tomato, a broth, and a cup of tea on a single page. By construction the reference sits at **IFI-U 1,000**, with a convention constant set at **5.0 mM** free glutamate — a defined anchor, like the dilution-to-threshold that fixes the Scoville scale — and the arithmetic is fixed for the whole axis:

> $\mathrm{EC_{50}(candidate)} = 1{,}000 \times 5.0\ \mathrm{mM} \div \mathrm{IFI\text{-}U} = 5{,}000 \div \mathrm{IFI\text{-}U}$, in millimolar.

Now the canonical numbers resolve into receptor terms. A supermarket shipping tomato scores **IFI-U 300**; the plant we restored is designed to score **2,600**; the full-spectrum cultivar Phoenix is predicted to reach **1,800** on this axis. Run each through the relation and you get the half-activation concentration each one works at:

SAMPLE	IFI-U	EC_{50} (MM GLUTAMATE)	READING
Axis reference (anchor)	1,000	5.00	the fixed zero-point of loudness
Shipping tomato	300	16.67	needs three-plus times the reference to be heard
Phoenix · SS-0096	1,800	2.78	savory as one voice among eight
Restored tomato	2,600	1.92	half-activation on under two millimolar

The restored tomato is designed to reach half of full receptor response at **1.92 mM** glutamate; the shipping tomato not until **16.67 mM** — nearly nine times as much of the same molecule to make the same flytrap close halfway. That ratio, 16.67 to 1.92, is the 2,600-against-300 of the score seen from the receptor's side. It is the difference between a tomato you taste and a tomato you have to salt.

To stand the loudness scale next to the plate, convert concentration to the units a grower actually measures. Free glutamic acid has a molar mass of **147.13 g/mol**, and a tomato is close enough to water that 100 g occupies about 100 mL. A representative dead-ripe heirloom carries on the order of **250 mg per 100 g** of free glutamate; a tomato bred for the truck — picked green, ripened in transit, selected for shelf life and uniformity rather than savor — carries closer to **18 mg per 100 g**. The arithmetic:

> 250 mg/100 g = 2,500 mg/L ÷ 147.13 g/mol ≈ **17.0 mM** free glutamate 18 mg/100 g = 180 mg/L ÷ 147.13 g/mol ≈ **1.22 mM** free glutamate

So the supply chain did not take a little. By mass it took roughly **93%** of the free glutamate — the heirloom's ~250 mg fallen to the shipper's ~18. That is the figure the truck is named for in our notes, and it is a real loss of a real molecule, not a loss of nostalgia. What the number cannot tell you on its own is how a 93% loss of cause becomes a near-total loss of effect. For that you have to put the concentration the plant delivers next to the EC_{50} the receptor works at — and that is a curve, not a line.

The synergy the tongue was built for

Before the curve, the one thing about umami that no other axis shares: it is the only taste with a built-in multiplier. Glutamate is the first voice, but the receptor was shaped to hear it in company. **5′-ribonucleotides** — inosine monophosphate (IMP) from aged and cured proteins, guanosine monophosphate (GMP) from mushrooms and dried fungi — bind not in the glutamate cleft but at a second site near the lip and hinge of the TAS1R1 flytrap. They do not activate the receptor themselves. They hold the trap closed once glutamate has entered it, stabilizing the active conformation and dropping the apparent EC_{50} for glutamate several-fold. This is **allosteric potentiation**, and it is the molecular signature of umami: the whole reason a taste that flags protein should be able to read two molecular hallmarks of protein at once, glutamate and the

nucleotides that come off nucleic acids and ATP when tissue breaks down, and ring far louder when both are present than the sum of either alone.

Every great savory tradition is this equation solved by hand. Dashi is kombu (glutamate) drawn together with katsuobushi (IMP). Tomato meets parmesan, tomato meets mushroom, miso meets bonito, soy meets the long slow enzymatic teardown of fermentation — glutamate on one side, ribonucleotide on the other, the flytrap held shut between them. Put it through the arithmetic. Our restored tomato is designed to work at an EC_{50} of 1.92 mM glutamate on its own. Add a GMP-rich mushroom at a modest 0.5 mM, apply a literature-typical potentiation of roughly six- to eightfold, and the apparent half-activation collapses:

> apparent EC_{50} ≈ 1.92 mM ÷ 6.5 ≈ **0.30 mM** apparent IFI-U = 5,000 ÷ 0.30 ≈ **16,700**



Soybean — the frontier umami host, where fermentation hydrolyzes bound protein into the free glutamate the tongue can finally read.

Sixteen thousand seven hundred. The scorecard will not print it. We cap every axis at a normalization maximum of **5,000**, and this is the honest reason why: perception saturates long before the arithmetic does. Past a certain loudness the flytrap is as shut as it gets, the cell is as near threshold as it swings, and a receptor already at the top of its response cannot report a difference the number insists is there. So the Index scores the cultivar's *intrinsic* loudness — glutamate alone, the plant graded on what it brings to the table by itself — and treats synergy as a formulation multiplier that lives in the kitchen, not in the genome. A tomato that scores 2,600 on its own is a tomato you can pair up to the ceiling. One that scores 300 has nothing for

the ribonucleotide to hold onto; the multiplier of a small number is still a small number. Synergy rewards the plant that already has something to say.

Dose–response, and why a shipping tomato tastes like nothing

Here is the curve the whole axis turns on, and the single most important fact in this chapter. The relation between how much glutamate reaches the receptor and how loudly the receptor answers is not a straight line. It is a saturating **Hill function**:

$$R / R_{max} = [G]^n \div (EC_{50}^n + [G]^n)$$

where [G] is the free-glutamate concentration delivered, EC_{50} is the half-activation we derived, and **n ≈ 1.2** is the Hill coefficient for glutamate at this receptor ensemble — mildly cooperative, a little steeper than plain Michaelis-Menten because the flytrap and its ribonucleotide site reinforce one another. At [G] = EC_{50} the fraction is exactly one-half, by definition. The interest is everywhere else on the curve, because the two tomatoes sit on opposite ends of it.

Take the shipping tomato: it delivers **1.22 mM** glutamate against its own EC_{50} of **16.67 mM**. It is sitting far out on the toe of the curve, where concentration is a fraction of threshold and the receptor has barely stirred:

$$R/R_{max} = 1.22^{1.2} \div (16.67^{1.2} + 1.22^{1.2}) = 1.27 \div (29.26 + 1.27) \approx \mathbf{0.042}$$

Four percent of full response. The flytrap is open, the cell is quiet, and the plate tastes — the word is exact — like nothing. Now the restored tomato: **17.0 mM** delivered against an EC_{50} of **1.92 mM**. It sits up on the shoulder of the curve, well past its own half-point, driving the receptor near its ceiling:

$$R/R_{max} = 17.0^{1.2} \div (1.92^{1.2} + 17.0^{1.2}) = 29.96 \div (2.19 + 29.96) \approx \mathbf{0.932}$$

Ninety-three percent of full response. Set the two beside each other and the whole story of a century of shipping is in the gap: the savory channel of a supermarket tomato is firing at four percent of what the fruit is built to deliver, and the restored plant brings it back to ninety-three. There is a quiet rhyme worth naming and worth not over-reading — the receptor climbs back to ~93% of maximum, and the supply chain had stripped ~93% of the glutamate by mass. They are two different ninety-threes, one a fraction of molecules and one a fraction of response, and the curvature of the Hill function is precisely why they are not the same ninety-three even when they land on the same digits. A 93% loss of cause need not be a 93% loss of effect; here it is close by coincidence, and far apart in meaning. That is the kind of thing a number is for — to keep you from mistaking a rhyme for an identity.

SAMPLE	[G] DELIVERED (MM)	EC_{50} (MM)	RECEPTOR RESPONSE (R/R_MAX)
Shipping tomato	1.22	16.67	0.042 — the toe of the curve
Restored tomato	17.0	1.92	0.932 — the shoulder of the curve

The curve also explains why nobody could breed their way out of this by taste alone. On the toe, you can double the glutamate and barely move the response; on the shoulder, a small change swings perception hard, then flattens. A breeder selecting by tongue, with no way to read concentration or threshold, is working blind on a nonlinear surface — which is why ten thousand years of selection could keep a tomato looking like food while its fifth taste guttered out, and why the fix had to be written in the metabolism, not chased across a field.

Growing the glutamate back — the native GAD / GDH1 route

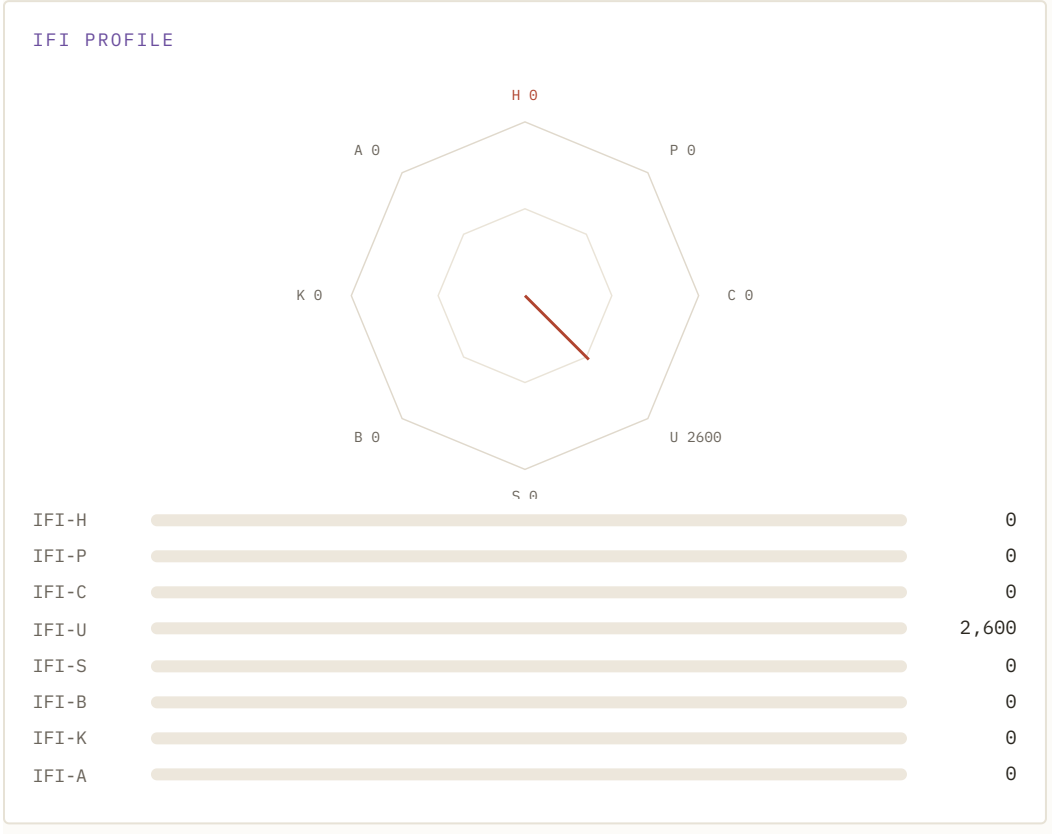
So we grew it back, and the method matters as much as the result, because of all the ways to put savor into a fruit, we chose the one that uses the fruit's own hands. We added no foreign amino-acid factory, no bacterial shortcut, no sprayed-on seasoning bred into the skin. We restored the plant's **native route** — the ripening program that shipping-oriented breeding had truncated — using enzymes the tomato already carries.

Follow the carbon. A ripening fruit builds free glutamate from its own central metabolism: **glutamate dehydrogenase (GDH1)** aminates **2-oxoglutarate**, the TCA-cycle acid, with ammonium, writing a carbon skeleton and a nitrogen together into glutamate. That is the tap that fills the pool. In a green fruit there is also a drain — **glutamate decarboxylase (GAD)**, which strips the carboxyl off glutamate to make GABA in the so-called GABA shunt. Green tomatoes run GAD hard and sit heavy with GABA; as a fruit ripens, GAD activity falls away, the shunt closes, and the glutamate the plant is making is finally allowed to accumulate as the taste-active anion the flytrap can read. A tomato picked green for the truck never completes that handoff. It is harvested in the middle of the program, with the drain still open and the pool still low, and no amount of ethylene in a ripening room rebuilds the savor that only the finished, on-vine late window lays down.

The restoration, then, is a retiming, not an invention. Across **2,040 constructs** in tomato we tuned both sides of the balance — sustaining and extending **GDH1** amination so the tap fills the pool fully, and modulating **GAD** so the ripe fruit stops shunting that pool to GABA — using the same design loop the whole platform runs: a protein language model scores candidate edits for their effect on each enzyme's catalytic efficiency and fold stability, a genome-scale flux-balance model confirms where the true rate limit sits before anything is built, and CRISPR writes the chosen edits in. The result is a plant designed to carry its ripening all the way to the savory window it was bred out of, and hold it. And because that late window is one metabolic event, not several, the glutamate does not come back alone. The same maturity pools the **lycopene** and the **vitamin C**; the carotenoids and the ascorbate and the savory amino acids are

laid down together, on the same clock, by the same finished ripening. We did not have to choose between a tomato that tastes like a tomato and a tomato that nourishes like one. On this axis they are the same plant.

On the savory axis this book reads one spoke of that plant — the umami it was built to carry, designed to reach from 300 to 2,600:



We will say what the restoration is and refuse to say what it is not. It is a designed return of a characterized molecule, and the nutrients that ride in with it ride in whether or not we mention them. It is not a cure, not a claim, not a health halo painted over a fruit. We point; we do not promise. The lycopene does its quiet good unannounced, and we would rather it stayed quiet and true than be enlisted into a slogan that cheapened both the science and the plant.

Precision — holding savor to seven percent

A taste you cannot reproduce is a taste you cannot sell, and savor has always been the least reproducible thing in the kitchen — the reason a sauce is never twice the same, the reason "season to taste" is an instruction and a surrender at once. The platform's discipline is to hold every build to a **batch-to-batch coefficient of variation of 7% or under**, against the **42%** that wild-type expression drifts across. CV is the standard deviation as a fraction of the mean — the width of the scatter relative to the target — and bringing it from 42% to ≤7% is the difference between a plant that happens to taste savory and an ingredient a formulator can specify by number and get back the same in August and in February.

The saturating curve, which cost the shipping tomato its savor, now pays the engineered one back. Because the restored tomato is designed to sit up on the shoulder of its dose-response — delivering ~17 mM against a 1.92 mM half-point, out where the curve has gone nearly flat — a swing in glutamate concentration barely moves the perceived intensity. Push the ingredient CV of 7% through the Hill function at that operating point and watch it shrink:

```
at 17.0 mM −7% = 15.81 mM → R/R_max ≈ 0.926 at 17.0 mM +7% =
18.19 mM → R/R_max ≈ 0.937
```

A 7% spread in the molecule becomes roughly a **0.6%** spread in the response the tongue reports. Engineering a flavor to the shoulder of its own curve is not only how you make it loud; it is how you make it *stable*, how a 7% manufacturing tolerance turns into a difference no eater can find. This is why a designed savor can be written on a spec sheet and trusted — not because the plant never varies, but because we placed its output where variation stops mattering.

MEASURE	WILD-TYPE EXPRESSION	RESTORED CONSTRUCT
Batch-to-batch CV (glutamate)	~42%	≤7%
Perceived-intensity CV at the shoulder	—	~0.6%
IFI-U	300	2,600

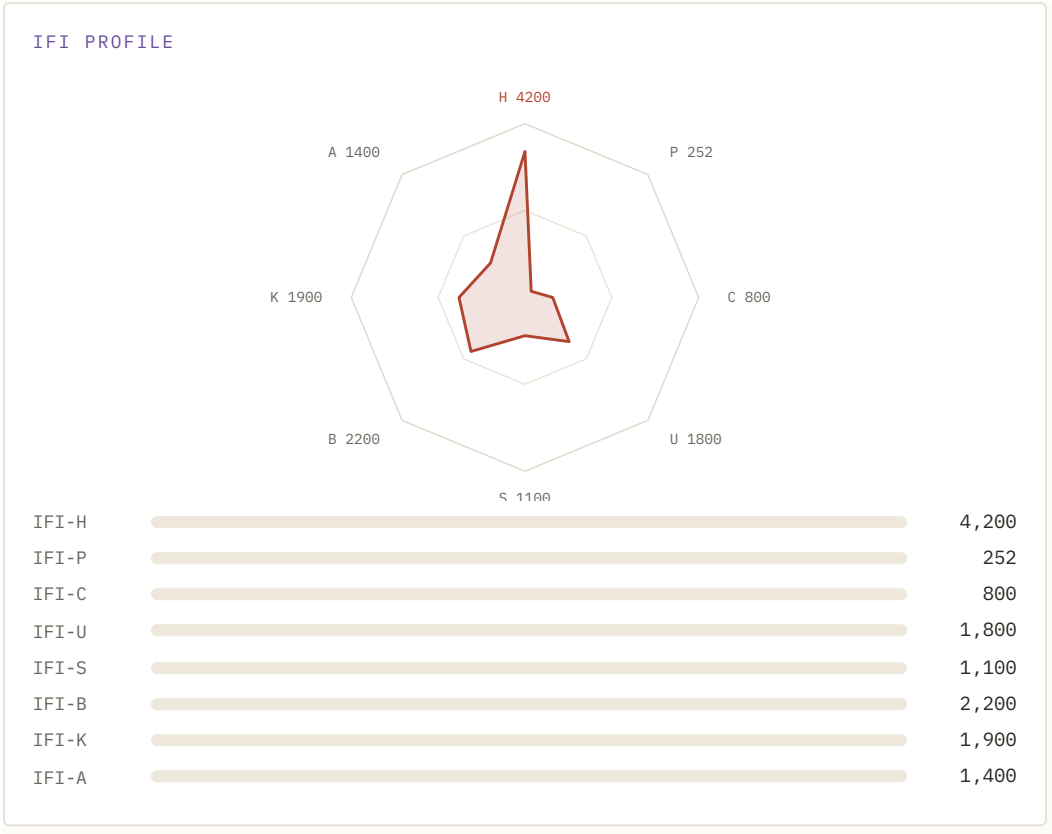
The umami hosts, and the ones still ahead

The tomato is the proof, not the point. The receptor is the same ensemble whichever plant speaks to it, and the route to free glutamate — build it, keep it from draining, carry the ripening through — generalizes to any organism whose genome we can read. Of the **42 host species** live across the library today, a clear cohort carries the savory axis, and behind them the **64-concept roadmap** runs on toward the fermenting and fungal hosts where umami lives most densely of all:

HOST	CONSTRUCTS	PRIMARY AXES	DIFFICULTY
Tomato	2,040	S / U / F / M	Easy
Soybean	360	U / K	Frontier
Tea	280	U / B / A	Hard
Yeast	240	U / K	Hard
Koji Mold	80	U	Hard
Shiitake	80	U	Hard
Mushroom	40	U	Hard

Each sits somewhere different on the same mechanism. Tea carries glutamate as theanine's near neighbor, savory under its astringency. Soybean and koji are the fermenters, where the long enzymatic teardown of storage protein frees the bound glutamate the tongue cannot otherwise reach — umami as the literal taste of nourishment being made available. Shiitake and mushroom bring the GMP side of the synergy, the ribonucleotide that holds the flytrap shut. Yeast bridges glutamate and the kokumi richness that rounds it. The method is the same loop each time, and most of the platform's modules cross between them unchanged; only a handful of terminal steps are ever species-specific.

And the savory axis is never the whole plant. The full-spectrum cultivar Phoenix carries umami as one voice of eight, predicted at **IFI-U 1,800** — loud, but balanced against seven other channels rather than soloing:



Behind every one of these readings is the same scale of work: approximately **225 distinct construct designs** spanning the library, with **1,000** live and purchasable today from a catalogue of **19,500 SKUs**, every one scored on the same **eight axes**, held to the same **≤7%** CV, and documented across **21** peer-reviewed papers and **3** filed patents. The savory axis is one column of that matrix. It happens to be the column where the arithmetic of flavor and the arithmetic of nourishment run closest to the same ledger.

What changes, in the end

What changes is small and total at once. For the eater: a tomato that tastes like the whole of a tomato, a broth that satisfies without a shaker, a plate that leaves you fed rather than merely full. For the maker: a savory depth that can be specified, sourced from a plant, held to within seven percent, and labeled as the food it came from — 2,600 instead of 300, 1.92 mM instead of 16.67, the shoulder of the curve instead of its toe, and a 7% tolerance that the tongue can no longer find. Neither is a revolution in how a thing tastes — savor is as old as the first meal — only a change in whether we can grow it back on purpose. These are computational designs; Scoville Splice's cultivars and constructs have not been experimentally validated in a laboratory. That turns out to be enough to say plainly and move on. The reflex at the stove, the lean toward the lifting lid, was never the thing that needed fixing. The plant was. And the plant, it turns out, was never far from remembering.

So we model savor, and we design it back in, and we hold it to a number — not to replace the old reverence but to deserve it. A century of shipping taught our plants to look like food and taste like nothing, and taught us, in turn, to patch the plate with salt and fat and a shaker, adding back at the table what breeding had bled out of the field. This book is the correction, written one receptor deep: the fifth taste, restored to the source, grown whole, held to within seven percent, and handed back to the body that was always listening for it. We have cooked this way our whole lives — a pot going on the stove, a lid coming off, the kitchen leaning in before anyone has tasted a thing. That reflex was never about meat, and it was never

about MSG. It was about depth, about the taste of being fed. Now it has a number and a method, so it can be built on purpose, at any scale, and taste the same in August and in February — like someone stayed home and cooked, because, in a way we have finally learned how to model, the plant did.

Savor was always the signal. We only learned, at last, how to send it on purpose.