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Cover Story

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Reining In Ripening

Researchers are learning to control key biochemical processes that affect the quality of fruits and vegetables

Sarah Everts

FAST-FORWARD to a dreary winter day. The gorgeous red tomato that you've just bitten into may not taste as good as it looks. Or maybe its consistency is wrong. It's simply not as good as the tomatoes you've grown in your garden in past summers or bought fresh at farmers' markets. Ditto for apples, strawberries, and peaches.


Gettyimages

Produce Panorama The fruit and vegetable industry stocks shelves with ripe produce 12 months a year, regardless of the season.

In your frustration, you might blame the growers or distributors. After all, the global multi-billion-dollar fruit industry wants to disarm the reality of the seasons to make ripe fruit available anywhere, anytime. To achieve this goal, most produce has been bred for shelf life, yield, and shipping durability—so that jet-lagged fruit looks good to the buyer even after extensive travel. While trying to optimize a fruit's suitability for globe-trekking, flavor and aroma often get left behind.

But plenty of fault for that tasteless tomato lies with consumers. The produce industry is selling its wares to a population that demands exotic seasonal fruit like oranges, papayas, and avocados year-round. And providing a constant supply of fresh produce requires interfering with the complex metabolism that leads to a tasty fruit.

Plant-science groups all over the world are now teasing out the chemistry and biology of fruit ripening and other key plant processes. They hope that the fruit of this labor will be an ability to control the ripening process, while optimizing flavor and other sensory qualities to make the best possible produce available to consumers.

Fruit ripening, like human puberty, is a complicated affair: Hormones ignite an explosion of ripening biochemistry, the downstream physiological effects of which result in a mature seed's distribution. Thereafter, the fruit body begins its slow, post-ripening decline toward death, euphemistically called senescence in plant circles.

Plants may be a sedentary life form, but they nevertheless burn a lot of energy to produce an appealing fruit. The effort pays off when birds and mammals eat and then expel the fruit-embedded seeds, delivering the plant's genetic offspring far afield.

To get animal buy-in, ripening hormones activate genes for enzymes that build pigmentation and aromatic volatiles, convert starch to sugar to sweeten up the flesh, and break down cellulose and pectin to soften it. If the fruit fails to attract a mobile seed distributor, senescence and decay becomes "escape plan B for the seed," says James J. Giovannoni, a Department of Agriculture plant geneticist at Cornell University.

The oversoft flesh of a dying, past-ripe fruit increases its susceptibility to microbial infection. If animals have not complied with the plant's genetic travel plans, the microbes can decompose fruit to release the seeds locally.

In a perfectly controlled produce world, fruits would be picked hard so they could better survive transport. Ripening would be timed to exactly complement placement on a grocery store shelf, and senescence would be staved off indefinitely, all while maintaining an odor and flavor profile of a garden-fresh crop.

UNFORTUNATELY, long shelf life and aroma are currently incompatible, says Jean-Claude Pech, a plant biochemist a the Ecole Nationale Supérieure Agronomique in Toulouse, France. "But ultimately we want to make a fruit with good taste, aroma, and high texture that can survive longer and ship better."

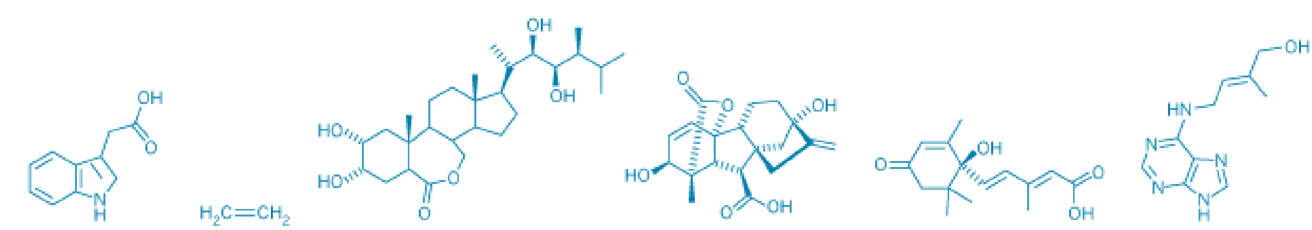
This target is still far off, but plant biochemists and geneticists are on their way to reaching it. They are beginning to untangle the networks of hormones, genes, and proteins that control fruit ripening. And they are finding out new ways t delay ripening while teasing apart why this often comes at the cost of flavor.

This research will aid the worldwide produce industry, which exports some 65 million tons of fresh fruits and vegetables annually. The U.S. retail produce industry alone made some $56 billion in sales in 2006.

Although a growing list of plant hormones—including auxin, brassinosteroids, cytokinins, gibberellin, and abscisic acid—play a powerful role in the ripening and senescence of different fruits and vegetables, the czar of produce ripening is actually the humble molecule ethylene.

"It's mind-boggling that two carbons and four hydrogens can have such a profound effect on fruit development," says Harry J. Klee, a plant biochemist at the University of Florida. In orange musk melons, ethylene is responsible for turning on biosynthesis of thioether ester volatiles that give these fruits the characteristic odor that discerning melon buyers often use for quality control. This simple hormone can also take credit for turning on the production of lycopene, a tomato's brilliant pigment, and polygalacturonase, which renders its unripe, unyielding flesh mouth-wateringly juicy.

Specifically, ethylene triggers ripening in so-called climacteric fruit, categorized as such when ripening is linked to a dramatic increase in respiration and ethylene production. When these climacteric fruit detect the ethylene, they quickly make more, creating a hormonal chain reaction. Climacteric fruits such as tomatoes, apples, and bananas account for "a majority of high-value produce," Klee says.



Auxin (indole-3-acetic acid) | Ethylene | Brassinolide | Gibberellic acid | Abscisic acid | Cytokinin (zeatin)

Plant Puberty The top six ripening hormones.

In practice, these fruits are often picked green off the tree or plant so they can withstand shipping before they get too soft. Ethylene is such a powerful hormone that if a single banana is ripening in transit from the tropics, the gas can spu a mutiny of ripening among its compadres. Bananas are therefore shipped in containers that contain chemicals such as potassium permanganate that can absorb or inactivate ethylene and prevent any ripening disasters. The green banana are then exposed to ethylene gas when they are safely ensconced in a local warehouse, a few days before their planned delivery to nearby store shelves.

A big player in ripening control is the ethylene receptor, which detects and transduces the signal that triggers its metamorphosis. Although the ethylene receptor has not had its structure solved by X-ray crystallography—it's a recalcitrant, membrane-embedded protein—extensive genetics and biochemical studies have unraveled key characteristics that give plant biologists a modicum of control over how it responds to ethylene.

The receptor exists as a dimer held together by disulfide bonds. A single copper ion is shared between the monomer units, and it mediates ethylene binding to the dimer. "We think that when ethylene binds, there is a conformational change that occurs in the protein," Klee says. The conformation change kicks off a phosphorylation signal transduction cascade that stabilizes ripening transcription factors so they can do their job.

As early as the 1970s, researchers began to tease out the receptor mechanism and to take control of this important protein. 2,5-Norbornadiene was found to compete with ethylene for a seat in the receptor. But the molecule isn't great a keeping ethylene out of the way, and it is far too stinky to be useful in commercial food applications. So Edward C. Sisler, a biochemist at North Carolina State University, Raleigh, set about to find something better. After years of trial and error, he figured out that the best ethylene receptor inhibitors are strained cyclic compounds with at least one double bond.

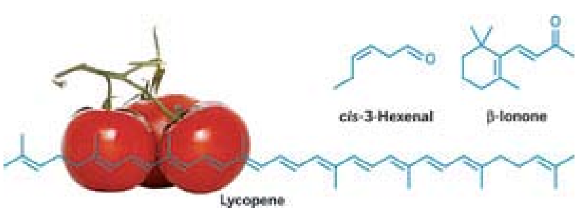
By the mid-'90s, the most promising compound, 1-methylcyclopropene (1-MCP), was being tested in a variety of plants says Margrethe Serek, a horticultural scientist at the Gottfreid Wilhelm Liebniz University Hannover (formerly the University of Hannover), in Germany, who did some of the testing and invented the code name for the project (Sis-X) used in scientific papers prior to patent approval.

IN 2002, 1-MCP was approved by the Environmental Protection Agency for use in the U.S. as a tool for delaying ripening. The molecule has since been approved in 27 countries, including some in the European Union, for fruits as diverse as apples, kiwis, avocados, and persimmons.

The gaseous ripening inhibitor is enclosed in an α-cyclodextrin cage and distributed as a powder, explains John Buckley, president of AgroFresh, the Spring House, Pa.-based company that sells 1-MCP. When the powder is exposed to water, 1-MCP is kicked out of the cage and diffuses out of solution and into the air. Fruits are treated in an enclosed room for several hours, and the treatment blocks the receptor for up to 12 days.

Pech says that "1-MCP is something of a magical molecule. It has been very useful for some tropical fruits like papaya that have a very fast ripening rate and cannot be stored in cold to decrease ripening metabolism because they get chilling injuries."

The molecule has also been particularly successful in apples—in 2006, it received two gold medals from French apple industry trade groups.



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Tasty Tomatoes *cis*-3-Hexenal and β-ionone are the basis for a tomato's most important aromas, and lycopene provides its ruby red color.

In bananas, however, this molecule's impact can sometimes be misleading, Giovannoni says. 1-MCP may prevent banana skin from undergoing ripening-induced yellowing, but it doesn't always stop ripening of the fruit inside, possibly because it can't pass beyond the skin. The consequence is a fruit that seems to have had ripening staved off but is actually mature on the inside. 1-MCP also faces a formidable challenge in fruits such as peaches, which continuously degrade and build new ethylene receptors that need to be newly blocked. A peach would have to be continuously exposed to 1-MCP to keep ripening at bay, which is simply not commercially feasible.

A liquid formulation of 1-MCP that's in the works will permit the ripening inhibitor to be applied directly to live crops. The idea is to modulate ripening in the field so that fruit pickers have enough time to collect all the crops, Buckley says.

"Unfortunately, you just can't put an orchard in an enclosed room," Sisler says. A liquid formulation would sidestep the inevitable loss of the gas formulation in the field.

On the front end of ripening, there's been extensive research on how ethylene is produced by the plant. Shang Fa Yang, a biochemist from the University of California, Davis, who passed away earlier this year, is largely responsible fo figuring out the biosynthesis: The amino acid methionine is converted into ethylene through S-adenosylmethionine and 1-aminocyclopropane-1-carboxylic acid intermediates.

Ethylene biosynthesis can be blocked by 1-aminoethoxyvinylglycine, for example, but in practice, such inhibitors are no commercially useful for delaying ripening in edible fruits. Even if a fruit can't make its own ethylene, it can still detect ethylene produced by nearby ripening fruits or in the atmosphere. Ethylene synthesis inhibitors have been handy in the lab, however, as a tool to separate the impact of self-made and externally sourced ethylene on fruit ripening.

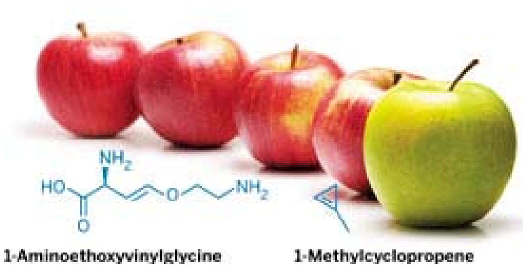
The intricacies of ethylene reception have been primarily untangled in the tomato, "the mouse of ripening research," Klee says. This is for several reasons. Tomatoes take home the biggest percentage of produce sales in the U.S., taking 9% of the market. Berries, bananas, and apples follow close behind. But being the product of a plant that can go from seed to fully mature organism much faster than a tree, tomatoes have a leg up on bananas and apples as a model organism. Giovannoni currently coheads a tomato genome project that he and his coworkers plan to complete in the next two years.

So if so much is known about the tomato, why do so many on grocery store shelves taste banal compared with their garden-picked counterparts?

Simple genetics, Klee says. A common tomato found on many produce shelves has a debilitating, naturally occurring mutation in a transcription factor required to activate the ethylene biosynthesis needed for ripening. The mutation occurs in one of two genes for the transcription factor. So the ripening signal is never more than half as strong as that c a normal fruit. Softening is slowed down for easier shipping, but so is flavor biosynthesis. "These tomatoes never have a chance to taste good," Klee says. The signal is simply too weak.

In an effort to figure out how to make a tastier tomato, Klee has been tabulating tomato flavor volatiles. He's found that the biggest component of tomato aroma—to human noses at least—is *cis*-3-hexenal, a fatty-acid-derived volatile present at 12,000 parts per billion in a garden-fresh tomato. The second most important volatile is β-ionone, an apocarotenoid present at only 4 ppb. The low concentration of this volatile is compensated by our incredible ability to sense it.

With the aromatic components of a tasty tomato tabulated, Klee has turned his attention toward boosting tomato flavors and adding new ones. His team recently spliced into tomatoes a gene for an enzyme that converts salicylic acid into methyl salicylate, giving the fruit a basil-like, minty flavor, Klee says. "The next step is to add a gene for mozzarella flavoring, and presto, you've engineered a caprese salad into a tomato." Other researchers like Efraim Lewinsohn, a plant geneticist at Newe Ya'ar Research Center. in Israel, are engineering tomatoes to produce geraniol, which carries a rose scent. Enzymes in the tomato then convert the geraniol into a lemon aroma.



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Impeding Ethylene 1-Aminoethoxyvinylglycine blocks the biosynthesis of ethylene, whereas 1-methylcyclopropene (1-MCP) interrupts the fruit's ability to detect it.

Of course, all this flavor genetic engineering could be a moot point: Even though genetically engineered legumes like soybeans are commonplace in the U.S., most companies are simply not interested in investing in genetically engineered fresh vegetables, Klee says. In fact, he points out, one of the first genetically engineered products on the market was actually a tomato called Flavr Savr, which used antisense technology to block polygalacturonase, which slowed down softening while leaving the other features of ripening alone. But this product was taken off the shelves in the early 2000s due to strong negative public reaction.

Some Geneticists remain hopeful that genetically engineered tomatoes and other crops will eventually find their way back onto store shelves, particularly if they can be made to taste good. But many plant biologists say that for the foreseeable future, the genetic insights on flavor they obtain in the lab will not likely be applied to develop transgenic crops but instead will provide traditional breeders with genetic markers they can use to check whether their new varieties contain optimal mutations.

For example, Arcadia Biosciences, a Davis, Calif.-based company, is developing so-called tilling technology to provide breeders with a high-throughput screening platform that evaluates whether genetic mutants developed through traditional means (such as exposure to ultraviolet light and chemical mutagens) contain the mutation the breeder is after. "Plants can be screened genetically for changes in target genes, thereby dramatically reducing the number of plants that need to be screened based on phenotype [physical appearance]," chief executive officer Eric Rey says. The company is currently applying the technology under a Department of Defense contract, the goal of which is to increase the shelf life of a ripe tomato, Rey tells C&EN. "We have prototype tomato lines that, once ripe, have shelf life of more than three months with taste and physical attributes essentially the same as when first ripe."

Although the tomato is a high-value crop and its mechanism of ripening is similar to that of other climacteric fruit, research insights about the way it ripens cannot be applied to nonclimacteric fruits. As such, the ripening of high-value nonclimacteric fruits like strawberries and grapes is studied independently.

In strawberries, the hormone auxin is thought to control ripening by acting as a dam that prevents ripening until the seeds are mature. The immature seeds pump out auxin, and when they stop, a flood of ripening gene transcription begins.

In the past two years, the molecular understanding of auxin's action has come to light, first with the identification of its protein receptor, which had remained elusive for decades, and then with the subsequent announcement of the receptor's crystal structure by Mark Estelle, a plant biologist at Indiana University, Bloomington, and Ning Zheng, a biochemist at the University of Washington, Seattle. "For a long time, auxin's action in a plant cell was a complete mystery," Estelle says. Some elements of auxin's action, such as how plant cells synthesize it, remain shrouded, but in many other ways "our understanding of auxin is starting to catch up with that of ethylene."

THE AUXIN RECEPTOR structure is a boon to a strawberry research community trying to understand the wild berry's enticing flavor features. "The problem with supermarket strawberries is that they have been bred for size, not flavor," says Wilfried Schwab, a food chemist at the Technical University of Munich. Flavor in strawberries is a surface-area-to-volume consideration because most of the aroma compounds are produced in the skin. The bigger the berry, the more money growers get, but the more dilute the berry flavor is, he says. Schwab and coworkers are trying to determine the connection between color, flavor, and berry integrity in strawberry ripening. His team has found that inactivating a gene involved in anthocyanin pigment formation during ripening can divert the enzyme's reactants onto a pathway that produces aromatic phenols, yielding albino berries.

In grapes, the most important hormone involved in ripening is abscisic acid (ABA), says Steven Lund of the University of British Columbia's Wine Research Center. Unlike ethylene in tomatoes, bananas, and apples, "ABA is 'a' master switch, not 'the' master switch," he says. Last year, brassinosteroids were reported to play a part in advanced softening of wine grapes. Hexose sugars may also act to instigate some aspects of ripening. "There's not one single signal barreling down to activate ripening in grapes; it's more of a coordination of several hormones," Lund explains.

Still, some California vineyards are testing ABA sprays to look at their impact on ripening, says Grant R. Cramer, a plar biologist at the University of Nevada, Reno.

Earlier this year, an Italian and French research consortium published the complete genome of the pinot noir grape varietal, the first of any fruit-bearing plant (*Nature* **2007,** *449*, 463). In their discussion, the authors point out the "striking" feature that the grape genome has more than twice the number of terpene synthase genes—genes involved i the production of aroma volatiles—than any other sequenced plant genome.

This and other grape genome projects (chardonnay and cabernet sauvignon are also in the works) will lead "to an explosion of research" that connects volatile biosynthesis and hormones, Cramer says. But he adds that many of the lovely volatiles found in wine begin as grape amino and fatty acids and then are processed by yeast enzymes—driving home the idea that when wine connoisseurs refer to "complexity," they are being literal.

Keeping Flowers Fresh

AntiAging Research

If the flowers in the bouquet you just received look a little past their prime, ethylene may be the culprit. The hormone makes flowers such as orchids, petunias, and carnations wilt and lose their petals, a process known as abscission. Sometimes ethylene prevents buds from opening.

"For many flowers, ethylene is a like a vandal," says David Clark, a flower geneticist at the University of Florida. "It comes in like a graffiti artist, it marks its place, and then it's gone."

Thousands of genes are activated in a flower when it is treated with ethylene, including those responsible for hydrolytic enzymes that degrade pectin in the cells at the interface between the petals and the stem, causing dissociation of the petals, says Ernst Woltering, who studies flowers at the Agrotechnology & Food Sciences Group, in Wageningen, the Netherlands.

In this industry, scientists have tried myriad techniques to deactivate the sensitivity of ethylene receptors. They've soaked fresh flowers in baths of silver thiosulfate, which is thought to replace an important copper atom in the ethylene receptor, thereby seizing the transduction machinery. But the heavy-metal liquid waste has led the U.S. and some European countries to ban the practice.

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The ethylene receptor inhibitor 1-methylcyclopropene (1-MCP) has also been used with some success in flower preservation. Although it effectively blocks the ethylene receptor in some flowers for about one week, the antiaging product must be applied as a gas. This requires producers to invest in airtight infrastructure, complementing the familia liquid-bath treatment facilities.

Although interfering with ethylene reception can help preserve a subsection of cut flowers, the senescence of roses and tulips-two of the most commercially important flower species-is not coupled to ethylene. So the floral industry must look to factors beyond hormones to postpone wilting in these flowers. Microbes, it turns out, have a propensity for setting up shop in a flower's water-conducting vessels. As such, soaking flowers in antimicrobial baths is another common way florists keep flowers fresh.

The characteristics producers desire in wine grapes don't necessarily extend to table grapes or juice-making varieties. For wine making, varieties that yield small fruit are preferred, because a high ratio of skin to berry enhances concentration of flavor and color compounds found in the skin. On the other hand, Thompson seedless grapes used to make juice are sprayed with gibberellins, because this hormone increases the grape's girth. Normally, the seed would supply this hormone, but the seeds in falsely named seedless grapes are so small they don't produce enough to get the grape as big as desired.

Although ethylene doesn't turn on ripening in nonclimacteric fruit, the hormone can bring about some of the characteristics that one associates with a mature fruit, such as pigmentation. For example, externally applied ethylene spurs anthocyanin production in grapes and cherries, deepening their plum-red color. Although the mechanism is not well-understood, it is widely applied. In citrus fruits, ethylene increases degradation of chlorophyll, which brings out the orange color. Some produce outlets expose green citrus fruits to ethylene "to 'orange-up' the rind," Giovannoni says. "It's used to make fruit look salable."

COMPARED WITH research on fruit ripening, vegetable ripening research is a poor cousin. In many cases, the biology of vegetable ripening has not been worked out because the genetics of vegetable plants is too complicated; edible parts of the plant (such as leaves, roots, or stems) have not been as well-studied as the fruits and seeds; or the crop has not been valued highly by research funding agencies. In fact, the term "ripe," although commonly used to describe vegetables, is not entirely accurate because the timing of vegetable edibility doesn't necessarily correspond to seed production.

An exception to the tendency to sidestep vegetable research is found in Susheng Gan, a plant geneticist at Cornell University. He studies a family of vegetables that includes broccoli, cauliflower, and bok choy. In these veggies, the hormone cytokinin blocks flesh senescence. Peering into the genome of these plants, Gan's group found a section of the genome that is activated when these veggies verge past their prime. It occurred to Gan that if he and his coworkers could turn on the production of cytokinin when the veggies start their decline, they might be able to thwart it. So they inserted into plants a gene that codes for isopentenyl transferase, an enzyme involved in the biosynthesis of cytokinin.

When senescence is activated in these plants, the genetic engineering causes cytokinin to be produced. But cytokinin, in turn, is a hormone that turns off senescence in leafy vegetables. This feedback mechanism has successfully delayed senescence by four days in broccoli and by about a week in lettuce at room temperature. Gan and coworkers haven't taken the research much further than the proof-of-principle stage because of the strong public reaction against genetically engineered fresh crops.

Chemically preventing senescence by directly applying cytokinin hasn't been successful and is not fiscally feasible for the industry. The cost of the hormone is simply too expensive, Gan says.

Currently, much of the vegetable industry relies entirely on modified environments low in oxygen and high in carbon dioxide to keep their wares fresh. The idea is that during ripening and senescence, the plant is doing more respiration than photosynthesis. By reducing the amount of oxygen available, respiration and other metabolic processes can be slowed down. Likewise, cold storerooms slow down the action of enzymes involved in degradation.

Indeed, a fruit's or vegetable's environment, be it during postpicking or preripening phases, cannot be ignored. As plant researchers tease apart the role of plant hormones on ripening, an even greater challenge remains: Figuring out how environmental factors such as sunlight, heat, and drought push ripening metabolism pathways in one direction or another. For example, production of phytoene, a precursor for the beautiful array of carotenoid pigments that give tomatoes their red color, can be initiated by both ethylene and light.

"Pretty much what we know is this: Without ethylene, nothing happens, and without light, some things don't happen," Klee says. A group led by Peter Bramley, a biochemist at Royal Holloway University of London, is developing sophisticated tools to uncouple the relative effects of light and hormones in ripening pigmentation of tomato, but the work is too new to deliver conclusive answers yet.

A continent away in Vancouver, British Columbia, Lund is trying to figure out how sunlight and temperature intertwine with grape hormones to impact production of volatiles. He believes the plant biology field is poised to completely untangle the complicated networks that make fruit tasty, sweet, attractive, and textured.

The past decade has seen many ripening processes come to light, such as identification of receptors for most of the major hormones and the structure of some of these receptors. Klee thinks that other hormones that play a role in ripening will be discovered and that these may have been bypassed because plant biologists have rarely collaborated with analytical chemists. The tools to detect even well-known hormones such as gibberellins have been slow in coming he tells C&EN.

According to Giovannoni, another major unanswered question is, "How does fruit know it should begin ripening?" What is the signal that turns on the biosynthesis of ripening hormones, and is there a master switch that activates ripening more universally among both climacteric and nonclimacteric fruit?

As researchers unravel the complex hormonal networks that lead to fruit ripening and figure out how to tame the process, they will eventually reach the limit of antiaging research. Just how long can any fruit be kept from spoiling? Ripening hormones blocked to the nth degree don't negate the fact that fruits are a complicated mélange of compounds, capable of breaking down to less savory products by the sheer force of time or by an army of endogenous or microbial enzymes willing to lend a hand. Breaking these additional barriers would have applications far beyond the global produce industry.

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