Replacing CO2-Intensive Food Refrigeration with Storage Under Pressure

Geoff Graham, March 24, 2022

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Abstract

The refrigeration of food contributes significantly to global CO2 emissions, and this contribution seems likely to increase. The need to preserve food while emitting less CO2 has led scientists to search for alternatives to refrigeration, the most important of which is the treatment of food with high hydrostatic pressure. Although preservation of food using high hydrostatic pressures emits far less CO2 than refrigeration does and preserves nutrients, it requires the use of very high pressures—which are dangerous and have other drawbacks.

Fortunately, biological agents can magnify the effects of pressure on contaminating microbes, the main contributors to food spoilage. This could greatly lower the hydrostatic pressure required to preserve food. This review discusses ten such agents: lysozymes, chitinases, ribosome-inactivating proteins, bacteriophage lysins, bacteriocins, antimicrobial peptides, microbial signaling compounds, essential oils/spices, and pressure-dependent enzymes.

In addition, another contributor to food spoilage, the action of enzymes endogenous to the food, may be blocked by genetically engineering of those enzymes to become pressure sensitive.

A. CO2 reductions with less pain

Society needs to reduce CO2 emissions, and the need is urgent. However, there is no working consensus on measures needed to decrease emissions. Attaining such a consensus is likely to be easier if the necessary measures are less painful and involve changes in in how goods and services provided, rather than curtailment of those goods and services. This article describes ongoing work on one method to reduce CO2 emissions, along with suggestions that have not yet been tried.

As I write this, world oil markets are in crisis in response to the Russian invasion of Ukraine. The price of fossil fuels is rising steadily and pressure to maximize the output from American and other reserves may soon become overwhelming. If so, and production is increased, how will we meet our CO2 emissions targets?

Actually, there are a number of steps we can take to lower our CO2 emissions without reducing people's material well-being. In the coming weeks, I will describe several of them [A1]. This review explores work being done to replace CO2-intensive refrigeration of food with CO2-moderate food storage under high hydrostatic pressure, along with other research that could greatly reduce the enormous pressures required.

B. Food is stored and distributed via the "cold chain"

Immediate refrigeration of raw food ingredients, just after harvest, fishing or butchery, and their storage and transportation at low temperature are required to provide food products that are safe, appetizing, and nutritious [B1]. The arrangements made to refrigerate food from the food's origin to its consumption are collectively called the "cold chain" [B2].

Although cold chains are important, a review published in 2012 states that less than 10% of perishable foodstuffs are refrigerated, and that 30% of the world food supply is lost as a result [B3]. Unless an alternative method of food preservation is devised, the demand for adequate cold chains is likely to increase as global temperatures rise and as people in developing countries either demand higher more healthful and appetizing food or realize that they cannot afford to waste over 200 million tons of perishable food [B3].

C. The cold chain's cost in energy consumed and CO2 emitted

About 50% of the energy consumed by the food preparation industry is spent on refrigeration [C1]. It has been estimated that 1% of global CO2 emissions are produced by food refrigeration [C2].

D. Alternatives to the cold chain

There are several traditional alternatives to the cold chain. These include canning of food, dehydration (drying), freezing, lowering of the food's water activity by addition of salt or sugar, addition of smoke as a preservative, and addition of spices or essential oils to the food. However, these methods are generally unsuitable for foods preserved by a cold chain. Such foods include

cheese, cottage cheese, yogurt, ice cream, pudding, fresh fruit and vegetables of any kind, fresh meat, fruit juices and other beverages: essentially every food in in the frozen foods or chilled foods section of a supermarket. Although spices and essential oils have strong antimicrobial activity and preserve foods as well as disguise the tastes and odors of food decomposition, they change in undesirable ways the taste and odor of foods that they are added to.

Newer techniques of food preservation include use of a cold plasma [D1], use of ultrasound [D2], use of pulsed electric fields [D3] and use of high hydrostatic pressure. The high hydrostatic pressure can be applied either as pulses or as a constant force. As noted above, this article focuses on high hydrostatic pressure and on how molecular biology might improve its effectiveness.

E. The Alvin incident and food storage under high hydrostatic pressure

In 1968, edible food was recovered from the research submarine Alvin, which had been sunk for 10 months in the ocean at a depth of 1540 meters. Sandwiches, bouillon, and apples, which had been maintained at a temperature of 3-4 ºC and a pressure of 15 megapascals, were practically untouched by decay when retrieved. However, when they were later kept under refrigeration at atmospheric pressure, they were spoiled in a few weeks [E1].

This demonstration that high pressures can preserve foods has suggested to many scientists and engineers that high-pressure storage might replace refrigeration for many foods, and much research has been devoted to this. In most cases, the pressures would be generated by specialized compression machinery; however, in a few cases discussed at the end of this article, such machinery might not be required.

F. The advantages of preserving food using high hydrostatic pressure

Preservation of food using high hydrostatic pressures has several advantages. The most important advantage from our point of view is that it is usually much less expensive in terms of energy used and carbon dioxide emitted into the atmosphere than is refrigeration. Unlike a cooled state, pressure within a container does not dissipate over time, or dissipates only very slowly. One study concluded that the cost in energy to preserve fruit juice for a period of 2 weeks was 1/26 the cost of refrigeration [F1] [F2].

A second advantage of pressure storage is that it is less damaging to nutrients in food and to the organoleptic properties of food than are canning and other forms of heating [F3] [F4]. Moreover, pressure is probably distributed more evenly

throughout a portion of food than are heat, microwaves or gamma-rays [F5] [F6] [F7] [F8], although pressure distribution can also be uneven [F8a].

The hydrostatic pressures needed to preserve food vary depending on the identity of the food, whether pressure is applied continuously until the food is consumed (as opposed to brief pressure shocks), and whether the food is expected to remain appetizing for a limited period or indefinitely. However, useful pressure regimes can involve pressures as low as 10 megapascals (98.7 atmospheres, 1450 pounds per square inch) or even, on one report, as low as 1.5 atmospheres [F9].

One report stated that 25 megapascals of pressure preserved strawberry juice at room temperature for 15 days and reduced the microbial load below detectable amounts [F10]. Treatment of watermelon juice, which is highly perishable, at 100 megapascals for 8 hours caused large drops in contaminating microbes, with the concentration of molds falling below detectable limits [F11], After the 8 hours of pressure at 100 MPa, the concentration of viable microbes remained constant for up to 60 hours [F12]. Microbial growth in melon juice was not affected by storage at 25 megapascals but was inhibited somewhat at 50-75 megapascals (the inhibition exceeded the inhibition under refrigeration) and was inhibited even more by storage at 100-150 megapascals. At 150 megapascals, microbes were not merely inhibited but were also killed [F13].

Pressures that are insufficient to kill contaminating microbes may nevertheless interfere with their growth and activity. One analysis gives the range where this happens as being between 10 and 100 megapascals.

Treatment of food using high hydrostatic pressures has unique features that can be either advantageous or disadvantageous. Under very high pressures, water undergoes anomalous phase transitions that can both kill microbial parasites and damage food microstructure [F14].

High pressure lowers the freezing point of water. This might be an advantage if it were desirable to store food below 0 ºC without freezing it [F15] [F16].

The effects of pressure on foods are more likely to be reversible than are the effects of heat, at least in the range of 100 to 400 megapascals. This is probably due to reversible changes in protein folding and protein subunit association/dissociation [F17], and because non-covalent bonds are much more likely to be affected by high pressure than are covalent bonds [F18]. This can be both an advantage and a disadvantage: food macromolecules are less likely to be irreversibly denatured by high pressure than by heat but contaminating microbes may be more likely to recover when the pressure or heat is removed. However, storage at high pressure for 15 days has been reported to prevent subsequent microbial growth for at least two weeks at 5 ºC [F19].

G. The disadvantages of preserving food using high hydrostatic pressure

The most serious disadvantage of high hydrostatic pressure as a food preservative, is that dangerously high pressures are required in many cases. Bacterial spores stoutly resist high hydrostatic pressure, and pressures as high as 1200 megapascals (11,843 atmospheres or 174,045 pounds per square inch) may be needed to kill them [G1]. The deep-sea bacterium Shewanella is a normal component of the surface flora of fish and contributes to fish spoilage but resists very high pressures [G2]. A container holding food at very high hydrostatic pressure could be dangerous to transport or to open if it contained a highly compressible material such as air. Most portable containers cannot maintain ultrahigh pressures on the materials they contain and, even if the containers do not burst open, will fail to keep the foods adequately pressurized. As result of this, preservation of food that is transported may require that high pressure be removed and reapplied repeatedly, with interludes of lower pressure or even normal pressure.

A second problem is that equipment needed to subject foods to high hydrostatic pressures is more expensive than is equipment needed to refrigerate foods [G4], and the amounts of food that can be processed at any given time may be small, at least in developing countries [G5]. Presumably, this problem becomes more difficult as the pressures required increase.

A third problem that may also, in some cases, be an advantage is that compression of compressible materials increases their temperature. Even water is somewhat compressible, and its temperature rises 3 ºC for every 100 megapascal increase in its temperature [G6]. Other components of foods are more compressible than water and get hotter under pressure; for example, extracted beef fat and soybean oil both increase in temperature by 6.3 ºC per 100 megapascals [G7]. Most foods are mixtures of different components, and many foods consist mostly of water. As one example, fresh salmon muscle showed compression heating values closer to water (3 ºC per 100 MPa), because it contained 68% water and about 4.9% fat [G8]. The possible advantage to this heating is that pressure and heat in combination can be more effective in preserving foods than either is alone; however, if the heat is unwanted, then it's a problem.

Compression of air or other gasses within or around food causes much greater heating than does compression of liquids or solids. Hence, air might have to be removed or included in the pressurized food depending on whether heating was desired.

A fourth problem is that pressure can have unexpected effects on chemical reactions within foods. High pressures drive equilibrium reactions such as association/dissociation of acids toward the most compact (associated) state. Moreover, high pressures can accelerate reactions if the reaction intermediates are smaller than the combined reactants and decelerate reactions if the intermediates are larger than the combined reactants [G9]. Since some chemical reactions contribute to spoilage of food, this can be important.

The incidence of food allergies is increasing in society, as is their severity and the range of foods involved [G10] [G11]. As with heat, pressure and the chemical changes caused by pressure may erase some epitopes that provoke food allergies but may also create others [G11].

A fifth potential problem is that bacteria vary in their vulnerability to high pressure. In general, Gram-negative bacteria are more vulnerable to pressure than are Gram-positive bacteria, but there are large differences in pressure resistance among various strains of the same species. This suggests that resistance to pressure might be a selectable trait in bacteria that, over time, could reduce the ability of pressure to sterilize food [G12].

Because of these problems, foods processed under high pressure are likely to face regulatory hurdles in both the United States and in the European Union [G13].

H. The causes and prevention of food spoilage

There are several important causes of food spoilage. One cause is microbial activity. A second cause is the action of oxygen from the air on food components. A third cause is the action of enzymes within the food. These, along with the action of light and unwanted humidity, are the main causes of food deterioration [H1].

Microbes can be defeated by measures that will inactivate or kill them, as discussed throughout this article. Damage to food by oxygen can be prevented by excluding air from the food to be preserved and by the use of oxygen absorbers within the food packaging [H2]. Enzymes within the food can be inactivated by heat, drying, or high hydrostatic pressure, or can be retarded in their action by cold storage. Light and unwanted humidity can be excluded from the food by appropriate choices of processing conditions, packaging, transport, and storage.

Spoilage by enzyme action deserves additional comment. Enzymes are proteins present in foods that typically transform one molecule or macromolecule into another. Some enzymes have a measurable effect on bulk substances present in food. Enzymes cause the ripening process in fruits and vegetables. They cause texture, color, and flavor changes. For example, as a banana turns from green to yellow to brown, not only does the color change, but there is also a change in the

fruit's texture. The enzymes of respiration contribute greatly to food spoilage [H1] [H3].

Many of the problems that accompany food preservation by high hydrostatic pressure could be lessened if less pressure were needed. The main subject of this article is how molecular biology might lessen the pressures needed.

I. Biological stresses have additive effects

Life can exist only where environmental conditions permit it to. Moreover, among permissive conditions, only some conditions are optimal, allowing organisms to grow and reproduce unimpeded. We can define a "stress" as any deviation from optimal conditions for a given organism that imposes significant difficulties that the organism must overcome. Stresses might include temperatures that are so low or so high that the organism must struggle to thrive, non-optimal pH values of the environment, very high or very low environmental salinity, the presence of chemical toxins or harmful radiation, scarcity of nutrients, etc.

In most cases, biological stresses are loosely additive. For example, if the pH of the environment is too low and the salinity of the environment is too high, a given microbe will be unable to survive and grow even if it could survive and grow at the same pH and benign salinity, or at the same salinity but a benign pH. This generalization has been termed the "hurdle concept" [I1], and there are many examples of it.

The combination of high pressure and heat kills more than twice as many bacterial spores as does heat alone [I2] and is used in pasteurization [I3].
High pressure increases the antimicrobial effects of gamma radiation [I4] (as does heat [I5]), and of alternating electric current [I6]. The combination of ultrasound and high pressure completely inactivated the mold Rhodotorola rubra, but neither treatment alone was effective [I7].

Food additives such as potassium sorbate and sodium benzoate increase the effectiveness of heat in killing the fungus Aspergillus flavus. A. flavus produces a very toxic and carcinogenic food poison, aflatoxin [I8]. The combination of heat (52 ºC), low pH, and either potassium sorbate or sodium benzoate was even more effective [I9]. Studies on 12 strains of yeast showed similar synergistic effects in 10 strains [I10]. Sorbate plus low pH was more effective in suppressing aflatoxin production than was either treatment alone [I11] and the same was true of propionate [I11] although the pH-dependence was less [I11]. Both sorbate and propionate are more effective against spores that have already been injured [I12]. The food additives butylated hydroxyanisole and tertiary butylhydroquinone also suppress fungal growth and have an additive effect [I13].

Most microbes are more sensitive to high pressures at lower pH [I14] [I15].

The combination of heat and γ-irradiation was more effective in killing spores (conidia) of Aspergillus flavus than was either treatment alone [I16].

Pairs of medical antimicrobial agents also may have roughly additive effects (nikkomycin Z plus caspofungin) [I17] and micafungin plus amphotericin B [I18].

At least in some cases, the hurdle concept also applies to enzymes. High hydrostatic pressures can be combined with carbon dioxide gas to kill microbes and to inactivate enzymes in food [I19] and combined with both carbon dioxide and heat to inactivate stubborn enzymes [I20]. High pressure combined with citric acid was used to completely inactivate the enzyme polyphenol oxidase in potato cubes [I21]; polyphenol oxidase causes unwanted browning of potatoes and of several fruits [I22].

J. The use of lysozyme

Lysozyme is a broad-spectrum antibacterial protein. Lysozyme kills bacteria by cleaving the peptidoglycan component of their cell walls [J1]. Common sources of lysozyme variants include milk and the whites of hen's eggs. However, lysozyme variants are also present in vertebrate animals, invertebrate animals, plants, bacteria and viruses [J2] [J3].

High pressure and lysozyme complement each other because Gram-positive bacteria are more resistant to high pressure than are Gram-negative bacteria, but Gram-positive bacteria are also more sensitive to lysozyme.

Bacteria of various species were subjected to high hydrostatic pressures. The Gram-positive bacteria Lactobacillus helveticus, Lactobacillus plantarum, and Listeria monocytogenes resisted very high pressures. In contrast, the Gram-positive bacteria Bacillus subtilis and Staphylococcus aureus and the Gram-negative bacteria Pseudomonas putida, Salmonella typhimurium, Salmonella enteritidis, and Proteus vulgaris were very vulnerable to the hyperbaric treatment.

The presence of lysozyme increased the lethality of high pressure toward almost all of the tested bacterial species [J4]. Presumably, the damage inflicted by lysozyme renders the bacteria more sensitive to high pressure but, alternatively, there is some evidence that the high pressure instead renders the lysozyme more active [J4]. However, the enzyme lactoperoxidase also increases the vulnerability of bacteria to high pressure, which favors the explanation that injuring the bacteria increases their vulnerability to pressure [J4].

The lysozyme that is a natural component of milk is more active on Gram-positive bacteria than on Gram-negative bacteria, but some Gram-negative bacteria are vulnerable to it [J5]. Gram-positive bacteria are more susceptible to the action of lysozyme because their cell wall contains up to 90% peptidoglycan, whereas

Gram-negative bacteria are more resistant because of the smaller amount of peptidoglycan in their cell wall [J5].

Lysozyme is effective against Gram-positive bacteria such as Micrococcus, Sarcina, Lactobacillus, Bacillus, Clostridium botulinum and Listeria monocytogenes and against Gram-negative bacteria such as Salmonella, Pseudomonas, Aeromonas, and Escherichia. Lysozyme also inhibits some viruses, parasites, and fungi [J5].

K. Plant defense proteins: chitinase, ribosome-inactivating protein and others

Many plants make proteins that attack fungi and other parasitic microbes. The choice of plant sources poses an interesting dilemma. Is it better to use proteins from plant food sources that are Generally Recognized As Safe (GRAS), in hopes of getting quick regulatory approval but with the risk that overuse will eventually select for resistant pathogens that could not only evade the food protections but might also attack the plant food source? Or is it better to start with defenses from a wide variety of plants of no economic value, such as weeds? At least so far, researchers have overwhelmingly chosen the first option.

Ribosome-inactivating protein from Zea mays, the maize or corn plant, inactivates fungal ribosomes but not Zea mays ribosomes [K1] [K2]. Zeamatin, also from Zea mays, attacks fungi, perhaps by permeabilizing their membranes [K3].

A third protein from Zea mays, the enzyme chitinase, is produced by 7 different genes in the Zea mays genome. Chitinase attacks the fibrous polysaccharide chitin in the cell walls of fungi (and exoskeletons of insects) [K4]. Chitinases fall into two broad categories: endochitinases and exochitinases, which are most effective in combination [K5]. Chitinases are found in many types of organisms including microbes, plants, and animals—any of which might supply suitable enzymes to attack fungal contaminants in food.

Multiple distinct versions (isozymes) of chitinase working together may afford greater protection against fungi than does a single version. As one example, a recently described strain of the bacterium Streptomyces exfoliates secretes six distinct chitinase isozymes [K6].

β-D-Glucans, like chitin, are polysaccharides present in the cell walls of fungi. Barley (Hordeum vulgare) produces a β-1,3-glucanase as one of its defenses against invading fungi [K8]. Resistance proteins, like other antimicrobial defenses, can have loosely additive effects. Ribosome Inactivation Protein and β-1,3-glucanase are more effective together than either is alone. The same is true of β-1,3-glucanase and chitinase [K8] [K9] [K10].

It is likely that many inedible plants also produce antimicrobial proteins, some of which might be quite useful in protecting foods, and which might avoid the risk of a generalized resistance emerging that could endanger crops or food supplies. However, most of these would probably not be Generally Recognized As Safe, and hence would probably need to earn regulatory approval. In addition, many antifungal proteins are produced by fungi themselves, but probably are not Generally Recognized As Safe [K11]. Bacteria also produce potentially useful antifungal proteins [K12].

L. The use of bacteriophage lysins

As mentioned previously in this article, Gram-negative bacteria are generally more sensitive to high pressure than are Gram-positive bacteria [L1]. One potential method to kill Gram-positive bacteria is to use lysins. Lysins are bacteriophage proteins that selectively lyse specific bacterial strains. A good review of lysins as antibacterial agents can be found here: [L2]

In nature, lysins are produced by bacteriophage and lyse infected bacteria from the inside. However, because they can directly access the cell walls of Gram-positive bacteria, they can also lyse those bacteria when applied from the outside. In contrast to this, an outer membrane blocks their access to Gram-negative bacterial walls [L3].

Lysins are very active enzymes. Nanogram to sub-microgram amounts of lysins can sterilize a 10E7 colony-forming-unit (cfu) bacterial suspension in seconds. Except for lysins, only chemical agents can kill bacteria this quickly; other biological agents cannot [L4].

Most bacteria have bacteriophages and, hence, are vulnerable to lysins, except that Gram-negative bacteria (as noted above) are more resistant targets [L5]. Lysins bind their substrate as tightly as immunoglobulins bind their targets, making them single-use enzymes [L6].

Lysins have been proposed for use as medical agents to supplement antibiotics. Lysins that are adopted for this purpose might be withheld from use in food preservation to avoid selection for resistant mutants. However, lysin proteins are probably large enough to provoke an immune response when given to humans or animals and might fail as medical agents for that reason. The molecular mass of lysins is typically 25-40 kilodaltons [L7]. If amino acids have an average mass of 110 Daltons, a 25 kilodalton protein would consist of about 225 amino acid residues, which is large enough to provoke an immune response. One attempt to inactivate a lysin with rabbit antiserum failed [L8].

Attempts to isolate lysin-resistant bacterial mutants have failed, perhaps because lysins bind cell wall molecules that are indispensable to the bacteria [L9].

Lysins are extremely selective in their killing. They typically kill only the bacterial species that supported the bacteriophage that produced the lysin, and sometimes kill only a subset of subspecies [L10]. They are harmless to humans and animals [L11].

Among the dangerous Gram-positive bacteria that can be killed by lysins are Streptococcus pyogenes, Streptococcus pneumoniae, Staphlyococcus aureus, Enterococcus faecalis and Enterococcus faecium, Bacillus anthracis, Bacillus cereus, Clostridium difficile, and Clostridium perfringens [L12].

M. The use of bacteriocins.

Bacteriocins are toxic proteins or peptides produced by bacteria to inhibit the growth of similar or closely related bacterial strains [M1]. Because they kill only a limited range of bacteria, many bacteriocins might be used to preserve foods without the danger of killing the consumer's intestinal bacteria.

N. The use of antimicrobial peptides

Proteins are chains of amino acids. Proteins whose length is between 2 and about 50 amino acids are usually referred to as "peptides" [N1]. Antimicrobial peptides are a huge class of peptides that kill or disable microbes such as bacteria, viruses, fungi, trypanosomes, malaria parasites, and so on. An excellent review of antimicrobial peptides has been published recently [N2].

Short peptides are very common among living things; many short peptides function as signals and have no antimicrobial activity. However, the subset of short peptides that kill or incapacitate microbes is still enormous.

The human body makes many types of antimicrobial peptide, and these function as natural antibiotics. Although the number of antimicrobial peptides in nature is huge, they may kill microbes via a much smaller number of mechanisms, and many antimicrobial peptides may share the same mechanism. This raises the possibility that if a strain of microbe develops resistance to one antimicrobial peptide, it may also become resistant to many others.

If antimicrobial peptides are overused or misused in medicine or used for non-medical purposes such as preserving food or fattening livestock, they may spawn dangerous microbial pathogens which can overcome some of the human body's natural defenses. This danger was noted almost 20 years ago [N3]. The possibility that antimicrobial peptides or other antimicrobial agents may be used to fatten

livestock in the same way that antibiotics are (mis)used for that purpose is significant: the antimicrobial enzyme lysozyme, discussed above, is added to swine feed to promote growth, in lieu of antibiotics [N4].

Many antimicrobial peptides also have signaling activities in humans. These include influencing inflammation, stimulating cell proliferation, promoting wound healing, and recruiting cells. It would be better to avoid using peptides with biological activity in food [N5].

Most peptides are easily hydrolyzed by proteases present in the human body, a characteristic that favors their use in foods [N6] [N7]. Moreover, some antimicrobial or similar peptides are active only at certain pH values (e.g., certain anti-cancer peptides [N8]). Hence, it might be possible to inactivate such peptides simply by mixing food with a sauce that will change the food's pH [N9].

As of August 24, 2020, more than 3,240 antimicrobial peptides were reported in the antimicrobial peptide database (APD31) [N10]. The peptides form a diverse group, and it is still uncertain which ones, if any, would be safe to use against microbes in food.

Antimicrobial peptides are expensive to synthesize chemically and then purify [N11] [N12]. Biological synthesis in yeast or other industrial microbes would ordinarily be easier, but some synthesized antimicrobial peptides might kill their own microbial producers. This problem could be overcome by synthesizing antimicrobial peptides as inactive precursor molecules from which the active antimicrobial peptide could then be liberated by proteolysis. Alternatively, and better, antimicrobial peptides might be used that are inactive at normal pressures, but active against microbes at high pressures. As discussed below, deep sea organisms might be a convenient source of such antimicrobial peptides.

A small number of antimicrobial peptides have already been approved as food additives. These include poly-L-lysine [N13] [N14] and the bacteriocins nisin [N15], pediocin PA-1 [N16] , enterocin AS-48, and enterocin CCM4231. Bacteriocins are discussed above. It seems plausible that antimicrobial peptides that are already present in common human foods (e.g. snakin in potato tubers) [N17] might also be cleared for use in preserving foods.

O. Exploiting microbial communications

One of the most promising methods of preventing microbial growth or toxin production is to exploit the chemical signals that microbes use to communicate with other members of their species, or with members of other species, or (in the case of large fungal colonies) with themselves. It is not yet clear how many of

these chemical signals are safe and palatable, but some of them are volatile [O1] and might be removed from food by a brief period of heating or being allowed to sit in the open air for a few minutes.

Conidia (spores) of the fungus Neurospora crassa are prevented from germinating before they are dispersed by the presence of auto-inhibitory molecules that detect conidia overcrowding and prevent germination. Following dispersal of the conidia, the concentration of the inhibitory molecules drops to a level that allows the conidia to germinate [O2] [O3]. Although very high densities inhibit conidial germination, lower densities accelerate it (compared to single conidia) [O4]. Other fungal species have different controls on conidial germination, but which still involve diffusible signals [O5]. Manipulation of these chemical signals might be exploited to suppress germination of any fungal spores that might be present.

After the conidia have germinated, germling cells recognize each other and communicate bidirectionally using extracellular signaling molecules, resulting in germling fusion. These fusions are important for network formation [O6] [O7] [O8] [O9]. In some cases, conidia of different species will grow toward each other, suggesting that the species use similar chemical signals [O10].

Similar chemotrophic interactions and cell fusion events occur between mature hyphae in the interior of the colony to maintain the interconnectedness as the mycelium develops [O11]. As the colony grows, hyphae at the leading edge use self-avoidance mechanisms to maximize outward, exploratory growth of the colony [O12] [O13] [O14] [O15]. Moreover, asexual development (conidiation) is regulated by light and is subject to circadian rhythms; unknown signaling mechanism coordinate conidiation throughout the colony [O16].

In some cases, fungi secrete chemicals that prevent colonies of two different species from growing into each other [O17]. Moreover, inhibitors produced by the fungus Aspergillus niger downregulate production of aflatoxin (described above) by Aspergillus flavus. [O18].

The aflatoxin-producing fungus Aspergillus flavus is sensitive to 6-pentyl-α-pyrone (also termed 6-pentyl-2-pyrone and 6-Amyl-α-pyrone) [O18a] [O19], a volatile substance with a coconut aroma that is present in animal foods, peach (Prunus persica), and heated beef [O20].

As with other inhibitors of microbial growth, chemicals produced by fungi (which may be signals, toxins, or both) sometimes act cooperatively [O21]. Interspecies communication between bacteria, particularly communication that can simulate or affect quorum sensing, might also be used to control bacterial growth, metabolism, and toxin production [O22]

An understanding of the many biochemicals that may inhibit the growth or metabolism of likely contaminants of preserved food could allow us to magnify the

effects of high hydrostatic pressures. This research is still in its infancy and, clearly, deserves strong public support.

P. Essential oils and spices can disable or kill microbes

"Essential oils" are oils extracted from plants. Spices are pungent substances extracted from plants. Many essential oils and many spices have antimicrobial activity. A review article published in 2004 stated that about 3000 essential oils are known, of which about 300 are economically important [P1].

Essential oils and spices have the disadvantage that they change the taste and texture of foods in ways that may be undesirable (although not always). Essential oils have the additional disadvantage that they vary in antimicrobial activity depending on the procedure used to extract them, how they are stored, the time in the development of the plant when they are obtained, the part of the plant extracted, and the geographical location of their origin. For this reason, attempts to use essential oils to preserve foods often instead use chemically pure components isolated from native essential oils [P2]. Further, some essential oils decompose at temperatures (e.g., 60 ºC) useful in killing contaminating microbes [P3] and may lose activity after one week of refrigeration [P4].

Combinations of essential oils may be more effective against microbes than the individual oils are alone. For example, a mixture of cinnamaldehyde and eugenol at 250 and 500 μg/ml respectively inhibited growth of Staphylococcus sp., Micrococcus sp. Bacillus sp. and Enterobacter sp. for more than 30 days completely, whereas the substrates applied individually did not inhibit growth (ref) [P5].

Essential oils and other stresses can have additive effects against microbes. Sodium chloride and mint oil act additively against Salmonella enteritidis and Listeria monocytogenes [P6]. In addition, the combination of 2-3% sodium chloride and 0.5% clove powder totally prevents growth and histamine production by Enterobacter aerogenes [P6]. Carvacrol and p-cymene, both being components of several essential oils, work additively against Bacillus cereus in rice [P7].

Another example of the additive effects of biological extracts and other stresses is the combination of oregano oil and nitrate, which inhibits the growth of Clostridium botulinum in broth [P8]. Other examples include the natural food preservative nisin + carvacrol [P9], nisin + thymol [P9], nisin + carvacrol + heat [P10], carvone + heat [P11], thymol + high pressure [P12], and carvacrol + high pressure [P12]. Yet another example is the effect of aqueous extracts from the combination of dried fruits of Xylopia aethiopica (an evergreen, aromatic tree) and dried seeds of Piper guineense (West African pepper), which completely inhibited the growth of the toxic fungus Aspergillus flavus [P13] [P14]. Still another

example is a mixture of the organic acids acetic, caproic, formic, propionic, butyric and n-valeric acids [P15].

Trans-anethole is a component of several essential oils and is used as a flavoring. Trans-anethole is active against yeasts and other fungi. However, it is more effective when combined with polygodial (a natural compound from some peppers), nagilactone E (a compound isolated from a gymnosperm), and n-dodecanol (a hydrogenated derivative of compounds within palm kernel oil or cocoanut oil) [P16] [P17].

The natural and non-toxic fungus inhibitor strobilurin is more effective when combined with the food additives vanillyl acetone, vanillin, veratraldehyde, or cinnamic acid [P18] [P19].

When oxygen is absent or nearly absent, the antimicrobial activity of essential oils is usually increased [P20]. The antimicrobial activity of essential oils is also increased by low pH of the environment [P21].

Bacteria also produce antifungal metabolites that might be useful in preserving foods, e.g., [P22].

Q. Constant or episodic stresses

Antimicrobial stresses can be either constant or episodic. Stresses applied constantly have the obvious advantage of not allowing targeted microbes the opportunity to recover but have the disadvantage that applying a physical stress such as high hydrostatic pressure, heat, ultrasound, or alternating current to a food from the beginning of processing to its consumption may be very inconvenient and costly in energy. Chemical or biochemical stresses, on the other hand, are easy to leave in the food once they have been added. If a physical stress such as high hydrostatic pressure cannot be maintained indefinitely, it might be administered episodically and strengthened with chemical or biochemical stresses.

A study on pulsed high hydrostatic pressure (compression to 600 megapascals or 5921 atmospheres) [Q1] concluded that 3 pulses of 60 seconds each were more effective against microbes than a single pulse of 180 seconds was and that the multiple pulses also inactivated enzymes that would otherwise shorten the food's duration of palatability [Q2].

Compression and decompression are not instantaneous in this system and the shape of the pulse (e.g., sinusoidal) can affect its lethality to microbes [Q3] [Q4]. At least over the temperature range of 2 – 50 ºC, higher temperatures were more effective [Q5].

The lowest effective pressure in this system was 200 megapascals. If lower pressures could be made effective by the addition of additional stresses, perhaps

other advantages such as greater throughput and cheaper pressurization machinery might be achievable.

Pulsed high hydrostatic pressures act roughly additively with heat in killing microbes (e.g., bacterial spores) [Q6].

R. Retarding enzyme action

The activity of endogenous enzymes within food contributes to the loss of that food's palatability. Inhibition of the enzymes might extend the period that the food remains palatable. However, because the enzymes of interest are sequestered within cells, physical treatments such as freezing are more realistic inhibitors of endogenous enzymes than are chemical treatments—although some peptides and some other macromolecules [R1] can cross cell membranes and inhibit enzymes. However, as noted above, freezing is expensive in energy and CO2 emissions. In addition, freezing and thawing are said to produce superoxide radicals [R2] which are generally unhealthful.

High hydrostatic pressures can inhibit the activity of enzymes within food by changing their shapes. Pectin methylesterase lowers the viscosity of fruits and adversely affects their texture. Pectin methylesterase is sensitive to pressures above 400 megapascals, although quick inactivation requires pressures above 500 megapascals (4935 atmospheres) (reviewed in [R3]). These pressures are orders of magnitude too high to preserve the cellular structure of fruits, and so can be applied only to fruit juices [R4].

Pectinesterase reduces the quality of citrus juices by destabilizing clouds. Inactivation of this enzyme requires either heat (75 ºC) or high pressure (600 to 700 megapascals) [R5].

Polygalacturonase softens and spoils plant-based foods. Depending upon the plant species that the polygalacturonase is present in, pressures of between 200 and 800 megapascals are required to inactivate the enzyme [R6].

Polyphenoloxidase and peroxidase cause loss of color and flavor in various plant-derived foods. Complete inactivation of polyphenoloxidase from grapes is not possible even at 900 megapascals, although limited inactivation occurs above 300 megapascals [R7]. Peroxidase can be inactivated by a pressure of 600 megapascals.

Papain is a protease derived from the papaya and mountain papaya plants. Papain can be partly inactivated by a pressure of 800 megapascals [R8].

As the above examples show, very high pressures can be necessary to inactivate unwanted enzymes in plant-based foods. Inactivation of enzymes in meat can also require enormous pressures. A pressure of 900 megapascals was required to partly

inactivate the enzymes superoxide dismutase and glutathione peroxidase in dry-cured ham [R9].

It might become possible to genetically engineer enzymes in plants and animals to be more sensitive to high pressures. These enzymes would function normally in plants and animals living at normal pressures but would cease to operate at some pressure that might be as low as 5 megapascals. Although this might be done to livestock such as cows and pigs, there would likely be fewer complications and less chance of cruelty to animals if it were done to cultured muscle tissue from those animals.

Production of meat from livestock has many costs. The entire process requires large inputs of corn or other plant-based food, which in turn requires large inputs of fresh water, artificial fertilizer, herbicides and pesticides. The livestock produce greenhouse gases such as methane and CO2 along with animal wastes and the byproducts of butchering. Because most livestock are treated with antibiotics to promote their growth, they become incubators for antibiotic-resistant pathogens that can be transferred directly to humans, or which can donate their antibiotic-resistance genes to human pathogens. Added to the fact that livestock are treated with much cruelty, and it seems likely that public pressure will grow to produce meat some other way. Society may choose plant-based meat substitutes, but the muscle tissue itself could also be cultured and consumed.

Enzymes are proteins and, as with all proteins, their stabilities depend on their amino acid sequences. The relationship between protein sequence and stability is incompletely understood, but one classic way to destabilize a functioning enzyme is to bury a hydrophobic cavity within it [R15]. These hydrophobic (water-repelling) cavities are formed by the presence of amino acids with hydrophobic side chains that are clustered within the folded protein in a manner that produces a cavity.

The natural enzyme urate oxidase is a homotetramer. Each of the four identical subunits contains within it a hydrophobic cavity that is predicted to rigidify under pressure and prevent the enzyme from functioning [R10].

The wild-type lysozyme protein of bacteriophage T4 has a mutated variant L99A (leucine to alanine at amino acid residue no.99) that has a cavity [R11] [R12] [R13]. This cavity destabilizes the protein to pressure because less-folded derivatives of the folded L99A lysozyme protein occupy less space [R14] [R17]

The protein RalGDS unfolds near hydrophobic cavities when subjected to high hydrostatic pressure [R16].

Studies on a series of 10 different cavity-containing variants of Staphylococcal nuclease show that pressure unfolds proteins in a way that eliminates cavities and thus reduces the volume of the protein [R17].

It has also been reported that very modest increases in atmospheric pressure can retard spoilage of some foods. In one study, breaker-stage tomatoes were subjected to pressures of 2 to 9 atmospheres for periods up to 15 days. Pressures of between 3 and 7 atmospheres slowed the ripening process in several ways: the respiration rate, rate of color change and acidification were lowered, while the lycopene content, tomato weight, and firmness were better maintained [R18]. (The breaker stage in tomato ripening is when the pink color first becomes noticeable. These tomatoes are physiologically mature and will develop their tomato-red color naturally. These breaker-stage tomatoes can be handled and shipped with less damage than those that are more mature when picked [R19].)

From the above, we see that endogenous enzymes in foods can be genetically engineered to increased their sensitivity to pressure, and that in some foods (breaker-stage tomatoes) slightly elevated pressures can retard post-harvest changes. Hence, genetic engineering might convert some pressure-insensitive foods into foods that can be preserved by modest hydrostatic pressures.

S. Pressure-dependent enzymes

It might be possible to discover or create enzymes that function only at high hydrostatic pressures. This could allow genetic engineers or food processors to add antimicrobial enzymes to food that would otherwise be too harsh because they would injure the consumer or the consumer's intestinal microflora or the engineered food organism.

Pressure-dependent variants of known enzymes could probably be created, with effort, but there may be an easier way. Some enzymes from deep sea organisms can not only withstand high pressures but depend on such pressures.

As one example, genes for dihydrofolate reductase enzymes were isolated from deep sea bacteria. Of more than 10 enzymes isolated, 3 were not only pressure-resistant, but were also pressure-dependent. In addition, one pressure-dependent mutant (D27E) of E. coli's dihydrofolate reductase is also known [S1].

It might be possible to use these deep-sea enzymes directly in food. However, a more likely prospect is that they could guide protein engineers in the modification of existing, well-characterized enzymes to pressure-dependence.

Deep-sea organisms might also be a source of pressure-dependent antimicrobial peptides.

T. The storage of meat under pressure

Meat is frequently stored either frozen or at a temperature slightly above the freezing point. Unlike many other foods, unground meat cannot be mixed with antimicrobial agents such as lysozyme. However, it might be possible to engineer meat-producing animals to express antimicrobial proteins or peptides that only become active at high pressures.

Many animal proteins such as laminin (a component of the extracellular matrix) produce biologically active peptides as they degrade. Examples are laminin-γ3 domain IV/F5 peptide [T1] and YIGSR peptide [T2]. Other proteins also produce biologically active peptides as they degrade; an example is the multifunctional protein vitronectin [T3], which produces the bone formation-promoting peptide VP-16 [T4]. With these examples as a guide, we might genetically implant proteins within the skeletal muscle of meat-producing animals that would degrade and release pressure-dependent antimicrobial peptides when the animal is slaughtered.

The same strategy could be used on cultured ("test-tube") meat.

U. Temporary drying of food under pressure

Another way to retard microbial growth and activity in a food is to dry or partly dry the food. As water is removed and the concentration of dissolved solids in the remaining water increases, stress on microbes that are present also increases. If enough water is removed, microbes cannot grow or function—although microbes differ in the amount of drying they can tolerate.

It might be possible to engineer a protein that soaks up water at high pressures but releases that water at low pressures. One way to do this is to find or create a protein with a buried hydrophobic cavity that resists the presence of water but will accept it under high hydrostatic pressure. If the protein were edible and did not degrade the organoleptic qualities of the food, enough protein could be added remove significant amounts of water at high pressure. After the food was depressurized, it might be necessary to allow it to sit for a while, perhaps with stirring, to mix the newly released water with the rest of the food.

Although most proteins with hydrophobic internal cavities unfold at high hydrostatic pressures in ways that destroy the cavities, it may be possible to engineer a protein that retains a hydrophobic cavity at high hydrostatic pressure. [U6]. The L99A variant of lysozyme from bacteriophage T4 has a hydrophobic

pocket within its C-terminal domain that excludes water molecules at ambient pressure [U1], but has been argued to accept water at 2.5 kilobars (250 megapascals, 2467 atmospheres) [U2] [U3]; however, there are other interpretations of what happens to this protein under high hydrostatic pressure. Proteins containing hydrophobic pockets might be engineered to allow penetration of water into those pockets at specified pressures [U4].

One possible problem is that while osmotic drying combined with pressure rapidly removes fluid from the surface of foods, the rate decreases away from the surface [U5]. Hence removal of water uniformly from food might require that it be kept under pressure for hours or days during which time microbes deep inside the food might spoil the food.

V. Selection for proteins that absorb water at high pressure

If an expression host could be found that is very sensitive to ice crystals, but which also resists high hydrostatic pressures, it might be possible to select for proteins that absorb water at high hydrostatic pressure.

Genetic constructs engineered to express and export a starting protein would be mutagenized and transformed into a population of the expression host. The population would then be mixed with growth medium that contained ions that were significantly larger than water molecules. The growth medium would also contain agar or some other gelling compound that would impede the flow of liquid. The transformed cells would be allowed to grow until small colonies formed. The mixture would then be subjected to a combination of high pressure and low temperature that allowed ice crystals to form, but not in areas where the ion concentration was significantly increased. If water molecules under high pressure could enter the protein, but larger ions could not, the increased ionic concentration would prevent ice crystals from forming locally and spare the expression host, while other expression hosts would be killed. Surviving expression hosts could then be isolated by plating on nutrient agar or some other method.

W. Storage of food in deep water

The preservation of food in the sunken submarine Alvin, described above, suggests that some island or coastal communities might be able to store food in deep water if they had equipment to lower and retrieve the food. Both the cold and the pressure of the deep ocean would tend to preserve the food.

Abandoned mineshafts or wells that have been made impermeable to water might be filled with water and used to store food. However, these would probably lack the advantage of low temperatures at the bottom of the shafts.

X. Combining food storage with renewable energy storage

It might be possible to combine food preservation with storage of renewable energy. One scheme to store renewable energy involves lifting very heavy weights against gravity, and then making use of that gravitational energy when the sun is not shining, or the wind is not blowing [X1]. If there were times when one or more of those heavy weights were lying on the ground, the weights might provide the pressure needed to inactivate the microbes and enzymes within foods. This ability to perform this service might increase the value of energy storage machinery and make the economics of renewable energy storage more favorable. When energy reserves were high, an electric compressor could be used to pressurize food; when energy reserves were low, the heavy weights could be used.

Y. A Final word

The USA and other developed nations currently face a shortage of fossil fuels and consequent very high energy prices. Although these nations hope to replace fossil fuels with renewable energy, times of energy scarcity are also times of capital scarcity. If modern events follow the same path as events in the 1970s and 1980s, many promising renewable energy projects and projects to conserve energy will never be fully funded and will never mature. In the 1980s and 1980s, big American companies could not respond to market signals to conserve energy or develop renewable energy because they were too short of capital. They were like starving elephants: big, but weak.

Faced with these circumstances, it might be wisest for the USA and other fossil-fuel-rich nations to increase fossil fuel production enough to moderate inflation and instead find other ways to reduce energy demand and CO2 emissions. I believe that there are a number of effective ways to do this that are not being pursued but are within reach of modern technology (one such idea is described in this review). In the near future (today is March 16, 2022), I will describe a several of these on Substack: https://geoffreyjgraham.substack.com

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