Naturally-occurring Compounds which Create Unique Flavors in Wild-harvested Shrimp

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Executive Summary

In repeated evaluations throughout the world, taste panelists preferred marine animals which were described as having flavors such as *"shrimp-like," "sea-fresh," "sea-like," "ocean-like," "crab-like,"* or *"slightly iodine,"* and aromas best described as *"seashore-like."* By comparison, freshwater fish and shellfish were usually described as tasting bland to earthy. With the rapid growth of shrimp and salmon culture, recent sensory evaluations have revealed marked flavor differences between wild-harvested and cultured animals of the same species. Specifically, wild-harvested marine foods exhibited distinct *"ocean-like"* flavors while taste panelists described cultured shrimp as "bland-tasting."

Determining flavors unique to wild seafoods

This paper reviews a host of research undertaken to determine what complement of chemical compounds creates flavors in many marine foods that are unique from their freshwater counterparts. As research teams were attempting to learn why strong, iodine-like off flavors existed in a small percentage of wild-harvested shrimp, they concluded that bromophenols, naturally-occurring marine compounds, were responsible. Research teams from the U.S. and Australia also uncovered the fact that five bromophenol compounds were in fact responsible for the suite of flavor descriptors used by taste panelists to describe flavors of marine foods; notably, *"sea-fresh," "sea-like," "ocean-like," "crab-like,"* *"prawn-like,"* etc.

The next phase of worldwide research that focused on flavors in various seafoods was to learn the pathway by which bromophenol compounds came to reside in the muscle tissue of popular seafoods. The first study examined flavor changes in Pacific salmon, which undergo obvious dietary changes as they move from the open ocean to the headwaters of rivers and streams to spawn. Researchers documented that as these fish cease to feed on marine prey species which contain high levels of water-soluble bromophenols, these chemicals leach from the muscle tissue and result in the bland taste observed by the panelists. Researchers from the U.S., Australia, and Hong Kong all reached the same conclusion: bromophenol compounds found in muscle tissue result from dietary sources, not metabolic activity.

This research also demonstrated that because diet is the source of water-soluble bromophenols, the concentrations of these compounds in muscle tissue are in a constant state of flux. In other words, bromophenols increase when the animal eats, but decrease due to leaching from muscle tissue. Specifically, this is why shrimp from the same trawl can vary in flavor intensity. The few that have recently gorged themselves on marine worms just prior to capture often have an iodine taint, while the majority retain concentrations sufficient only to impart an enhanced *"shrimp-like," "briny"* flavor.

Amending commercial feeds with bromophenols

As shrimp culture expanded in the 1990s, clearly the next move was to add bromophenol compounds to commercial feeds thereby making cultured shrimp taste like their wild-harvested cousins. However, repeated trails have consistently demonstrated the inability of bromophenol amendments to create discernable flavor differences in cultured shrimp. Currently, there is both a technical and economic challenge that precludes cultured shrimp from having the suite of flavors characteristic of wild shrimp.

Next steps ...

The *"marine-like"* flavors created by consumption of natural diets are an intrinsic attribute of properly-handled, wild shrimp which cannot be duplicated in culture systems. This attribute is unique and scientifically verifiable. Therefore the unique suite of flavors inherent in wild shrimp should be a prominent element of all marketing efforts supported by the domestic shrimp industry aimed at creating a more informed constituency interested in making purchases based on real edibility differences between wild and cultured shrimp.

Naturally-occurring Compounds which Create Unique Flavors in Wild-harvested Shrimp

Introduction

For years culinary experts and consumers alike have recognized that certain seafoods, including shrimp, have **flavors** unique from freshwater organisms.[1] In taste tests throughout the world consumers preferred the marine animals which were described as having flavors reminiscent of *"shrimp-like," "sea-fresh," "sea-like," "ocean-like," "crab-like,"* or *"slightly iodine,"* and aromas best described as *"seashore-like."* By comparison, freshwater fish and shellfish were usually described as tasting bland to earthy.

With the explosion of shrimp and salmon culture worldwide, more recent sensory evaluations have revealed marked flavor differences between wild-harvested and cultured animals of the same species. In particular, the wild-harvested marine foods exhibited distinct *"ocean-like"* flavors while their cultured cousins were labeled as bland-tasting. This flavor difference is the result of naturally-occurring marine compounds consumed by many marine animals: **bromophenols!**

This paper traces the research which has documented (i) the role that bromophenols play in creating unique flavors in marine organisms, (ii) the interplay among various bromophenol compounds and the varied concentrations that result in different suites of flavors, and (iii) the origin of these naturally-occurring compounds in muscle tissue. It further discusses attempts to duplicate these flavors in marine species produced through culture systems by enhancing the formulated diets with various purified bromophenol chemicals as well as various by-products that result from seafood processing.

The Sensation of Flavor

Flavor is defined as the interplay of signals produced as a consequence of sensing tastes, aromas, irritating stimuli (such as hot peppers or peppermint), and texture or mouth feel from a foodstuff [4]. Taste is a component of the broader classification flavor, and is defined as the sensations resulting from the interaction of foods with sensory receptors on the tongue such as sweet, sour, salty, bitter, and more recently umami[2] (i.e., a measure of the intensities of existing flavors). In addition to taste, flavor also includes the volatile components sensed in the nose, both through the nostrils and inside the mouth. Actually, aroma is considered more important than taste in determining flavor because most individuals can perceive and memorize well over a thousand odors; often at thresholds 100 times lower than the most sophisticated analytical instruments [5].

1. Marine food products are harvested from waters containing 3.5 percent salt, so many feel that **saltiness** would be the predominant flavor in products harvested from the ocean. **This notion is incorrect!** Swimming seafoods like fish and crustaceans harvested from the marine environment are low-sodium foods! For example a 3.5 oz. (100 gm.) serving of chinook salmon contains 60 mg of sodium, a similar-sized portion of boiled shrimp contains 148 mg of sodium, and a 100 gm. serving of broiled T-bone contains 67 mg of sodium [1]. Comparing these three examples against the maximum Daily Reference Value (DRV) established for sodium of 2,400 mg indicates that (i) salmon would account for 1.6 percent of the maximum DRV, (ii) beef would contribute roughly 2 percent to the maximum DRV, and (iii) shrimp would contribute 6 percent to the maximum DRV [2]. Conversely, two frankfurters (100 gm.) contain 980 mg of sodium. Even without the addition of condiments (such as mustard and pickles) or a bun, this processed product would contribute 41 percent to the maximum DRV [3].

2. Umami, pronounced "ooh-MA-mi", is the fifth tongue taste sensation which detects free amino acids, specifically glutamic acid, which is the most abundant amino acid found in meat, seafoods, and aged cheeses such as Parmesan. The sensation has been described as meaty or savory and is most intense when combined with sodium; hence the development of the flavor enhancer monosodium glutamate (MSG) by Dr. Kikunae Ikeda in 1907.

Research to Assess the Sources of Flavors
Unique to Marine Food Products

Historically, most studies that addressed the origin of flavors in various seafood products focused on characteristic tastes and aromas that result from microbial degradation, lipid[3] oxidation, or volatile compounds generated when seafoods are heated. These researchers determined that **very fresh aromas** of uncooked fish and seafoods involved (i) a group of 6-, 8-, and 9-carbon aldehydes[4], (ii) ketones[5], and (iii) alcohols[6] that are produced by enzymatically breaking down long-chain, omega-3, polyunsaturated fatty acids[7] (PUFAs). The six-carbon aldehyde compounds provide green, *"plant-like"* aromas. Eight-carbon aldehyde compounds impart heavy, *"plant-like"* odors and *"metallic-like"* flavors, while the nine-carbon aldehyde compounds contribute fresh, *"cucumber-like"* odors and flavors [6].

Freshness of seafoods also has been closely linked to adenine nucleotides[8] which are enzymatically produced immediately following death. In particular, levels of adenosine monophosphate[9] and inosine monophosphate[10] rapidly change. However, their degradation is species specific. Rapid changes in the levels of these compounds explain why the flavor of some species of fish improve with time (up to a point) while others begin tasting flat almost immediately after death [7]. For example, the flavor of cooked cod is maximized after being held for two days in melting ice. Similarly, even fatty fish like salmon are judged as having improved aroma, taste, and texture after two days in ice [8]. It is important to recognize that enhancements to the aroma and taste occur only when products are properly handled and held under optimal storage conditions which, for fresh products, is surrounded by melting ice.

In a 1982 review paper addressing seafood flavors, the authors noted that relatively simple chemical compounds which were soluble in water (or in saliva when chewed) produced the characteristic flavors of a particular species.[11] Three broad classes of chemicals constitute the primary flavor compounds found in

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3. Lipid is a fat or oil formed by combining glycerol with any number of fatty acids.

4. Aldehyde is an organic compound incorporating a carbonyl group (C=O), in which one of the remaining two bonds is occupied by hydrogen. Two familiar aldehydes are benzaldehyde which is found in oil of almonds and vanillin found in the oil of vanilla beans. In seafoods hexan**al** provides green plant-like aromas whereas nonadien**al** produces a cucumber-like odor.

5. Ketone is an organic compound incorporating a carbonyl group (C=O), with each of the remaining two bonds attached to carbon atom. Octan**one** is responsible for mushroom flavoring**.**

6. Alcohol is an organic compound in which one of the hydrogens of methane is replaced by the functional hydroxyl group (-OH). Octen**ol** imparts plant-like odors.

7. Omega-3 polyunsaturated fatty acids (PUFAs) are found in the fats and oils in many seafoods, and which are beneficial to human health

8. Adenine nucleotides are chemicals found in the cell nucleus as well as its cytoplasm. In these nucleotides the base pair is a purine called adenine.

9. Adenosine monophosphate is a nucleotide found in RNA which results from the energy releasing breakdown of adenosine triphosphate (ATP). AMP has been approved by the USFDA as a "bitter blocker" additive to foods, making them appear sweeter.

10. Inosine monophosphate is a flavor compound formed from the enzymatic breakdown, or deanimation, of adenosine monophosphate which causes the release of ammonia.

11. Conversely, the more complex chemical compounds such as proteins, polysaccharides, pigments, and vitamins are seldom involved in flavor creation.

seafoods. These include: (i) low-molecular-weight, water-soluble components such as free amino acids[12], (ii) low-molecular-weight peptides[13], nucleotides[14], and organic bases[15], as well as (iii) non-nitrogenous substances such as organic acids[16], sugars, and inorganic constituents like salts.[17] Certain free amino acids like taurine, proline, alanine, and arginine are much more abundant in shrimp and crabs (crustaceans) and in scallops (mollusks) than they are in fish, and are thought to be responsible for the relatively sweeter taste of crustaceans and mollusks. While the authors speculated as to what combinations of these chemicals produced flavors unique to various fish and shellfish species, they **did not identify compounds distinctively found in wild, but not cultured, species**. In fact, their analyses of the above chemical classes in both wild and cultured red sea bream, yellowtail, and Ayu (sweet smelt) showed no significant differences in either composition or quantity of these compounds [7].

Determining the cause of "iodine-like," off-flavors in shrimp

In the mid 1980s, researchers in both the U.S. and Australia began searching for chemicals responsible for producing the "off" flavor of very strong iodine, which occasionally occurred in some wild shrimp [9][10]. They correctly identified the source as belonging to a group of compounds known as bromophenols. In particular, five bromophenol compounds were identified as providing unique flavors to certain seafoods. These include: (i) 2-bromophenol (Figure 1-A), (ii) 4-bromophenol (Figure 1-B), (iii) 2,4-dibromophenol (Figure 1-C), (iv) 2,6-dibromophenol (Figure 1-D), and (v) 2,4,6-tribromophenol (Figure 1-E). In determining the flavor threshold concentrations of these five bromophenol compounds in water (i.e., the minimum amount of the compound necessary to impart a discernable flavor), the most intensely flavored of these five compounds was 2,6-dibromophenol at 0.0005 parts per billion (ppb) followed by 2-bromophenol at 0.03 ppb; 2,4,6-tribromophenol at 0.6 ppb; 2,4-dibromophenol at 4 ppb; and 4-bromophenol at 23 ppb [9].[18]

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12. Amino acids are twenty one nitrogen-containing organic compounds which combine to form proteins. Proteins are made up of hundreds to thousands of individual amino acids linked together and folded. Free amino acids are those amino acids that are not bonded as part of a protein, but rather in a "free" state, ready to be instantaneously absorbed by cells in the body, including taste receptors on the tongue.

13. Peptides are two or more amino acids linked together through a chemical bond called a peptide bond. In general, if more than two amino acids are linked together the compound is called a polypeptide.

14. Nucleotides are a subunit of DNA or RNA comprised of a nitrogenous base, a sugar molecule , and a phosphate molecule. Thousands of these subunits join to form DNA or RNA.

15. Organic bases refer to chemical structures that can easily accept a proton. Many organic bases are carbon containing molecules that have nitrogen atoms which can accept protons. The purines and pyrimidines which form the base-pairs in DNA and RNA are examples of organic bases.

16. Organic acids are chemical structures that can easily lose a proton. Organic acids are carbon-containing molecules which contain carboxyl groups (-COOH). As a group, organic acids are much weaker than mineral acids like hydrochloric and sulfuric. Lactic acid found in yogurt and cheese is an organic acid.

17. A salt refers to the numerous combinations of any positive ion (except hydrogen) with any negative ion (except hydroxide and oxide). The combination of sodium ions (+) with chloride ions (-) produces common table salt. Sodium ions and sulfate ions produce the salt sodium sulfate. Numerous salts exist or can be produced from various combinations of positive and negative ions.

18. Even when measured in parts per billion, an extreme range in flavor intensity exists across these five compounds. The strongest of these five bromophenol compounds is 2,6-dibromophenol can be discerned at 0.0005 ppb while the flavor threshold of 4-bromophenol requires a concentration of 23 ppb. Thus, to create a discernable flavor difference when dissolved in water, the concentration of 4-bromophenol must be 46,000 times greater than 2,6-dibromophenol.

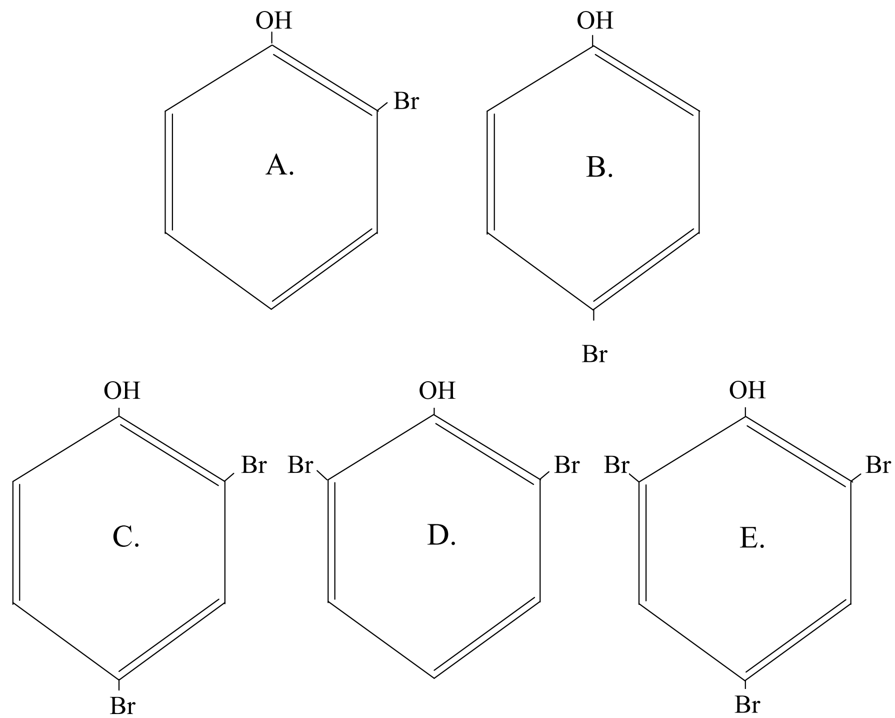

Figure 1. The five bromophenols most often associated with seafood flavors

When extremely low concentrations of these compounds are dissolved in water they impart a medicinal or iodine taste.[19] The Australian researchers theorized that when found in seafood – even at significantly higher concentrations – these compounds might contribute to distinct, recognizable *"marine-like"* and *"ocean-like"* flavors and may also enhance the intensities (umami) of existing flavors. Since previous taste tests had shown that shrimp with an *"off"* iodine flavor were more the exception than the rule, researchers chemically analyzed numerous shrimp and found that wild shrimp, on average, contained about 10 times less total bromophenols than those which were identified as having an iodine taint.[20] These findings lead to the next phase of the research which assessed how these lower, more typical concentrations of various bromophenol compounds might affect shrimp flavor [11].

Verifying the effects of varied bromophenol compounds and concentrations with taste panelists

<u>Adding purified bromophenols to otherwise bland-tasting freshwater products.</u> A research group at the University of Wisconsin-Madison (UW-Madison) led work that measured the bromophenol concentration which created *"shrimp-like"* as well as other seafood flavors. Using bland-tasting minced shrimp and surimi, these researchers added various concentrations of pure bromophenols to the chopped and reformed muscle, then cooked them as nuggets for tasting trials. This team also marinated Great Lakes whitefish fillets in various concentrations of these chemicals before cooking and subsequent taste tests. Finally, various concentrations of these bromophenol compounds were added directly to a vegetable oil and subjected to sensory evaluation.

19. For example, the active ingredient in some mouthwashes is phenol, while bromine is in the same chemical family as iodine.

20. Total bromophenols refer to the sum of all five bromophenol compounds in the entire animal, unless otherwise noted (e.g., in the edible portion, tail meat, etc.)

Depending on the bromophenol compound and the concentration used, treated samples in each of these tasting trials were distinguishable from controls (i.e., those samples with no added bromophenol compounds), and were described as having *"shrimp-like," "sea-like," "crab-like,"* and *"marine-like"* flavors. In particular, Great Lakes whitefish fillets which had been marinated in various bromophenols had significantly more overall *"seafood-like"* flavor intensity (e.g., *"saltwater fish," "iodine/sea salt," "shrimp-like"* flavor) than untreated fillets. Likewise, the bromophenol-enhanced vegetable oils resulted in a *"herring oil-like"* flavor [12]. Often the same chemical at a different concentration produced different flavors. For example, the shrimp nuggets which contained 4 ppb of 2,6-dibromophenol were described as having a strong *"crab-like"* and *"sea-like"* flavor. However, as the concentration was decreased the crab-like flavor diminished, and panelists described the resulting flavor as *"shore-like," "slightly-iodine,"* and *"fresh-shrimp."*

<u>Determining the source of bromophenols in seafoods.</u> With an understanding of the compounds responsible for the unique flavors in marine foods, researchers then questioned whether the bromophenol compounds were the result of metabolic activity or diet. Researchers from the U.S., Australia, and Hong Kong all reached the same conclusion: bromophenol compounds found in muscle tissue result from dietary sources. The first study took advantage of the anadromous Pacific salmon which is spawned in freshwater, matures in the open ocean, and ends it's life history by returning to the same stream where it originated to begin the cycle anew. Obviously, the diet of salmon changes as they move from seawater to freshwater.

A longstanding observation among salmon connoisseurs was that migrating salmon tasted bland once they entered freshwater compared to the same species of salmon caught offshore. Originally, the dramatic deterioration in flavor and texture of salmon as they migrate into freshwater to spawn had been thought to be due to loss of muscle lipids and carotenoid pigments.[21] However, through chemical analyses UW-Madison researchers concluded that as these fish cease to feed on marine prey species, which contain high levels of bromophenols, the water-soluble bromophenols leach from muscle tissue and result in the bland taste observed by the panelists. These conclusions were verified by the fact that hatchery-reared Pacific coho and chinook salmon that were stocked in landlocked, freshwater habitats lacked not only the pigmentation, but also the *"sea-like"* or *"shore-like"* flavor notes of their saltwater counterparts. Chemical analysis of their flesh showed either no, or very little, bromophenols [13].

UW-Madison researchers further verified that these chemicals originate via the marine food web by adding low concentrations of purified bromophenols into the manufactured diets of freshwater fish. After only three days, freshwater trout that consumed the bromophenol-enriched ration developed a *"rich, mild, marine-like"* flavor. Consumer taste panelists preferred these freshwater trout over the bland-tasting samples which had been fed the same diet, but without the purified bromophenol compounds added in the first treatment. Likewise, when freshwater crayfish were fed bromophenol-enhanced feed, consumers reported that the crayfish assumed a distinct *"lobster-like"* or *"crab-like"* flavor; again, depending on the types and amounts of bromophenol compounds incorporated into the feeds [14].

Another study found that taste panelists preferred Pacific salmon which had been fed a diet containing high levels of euphausid shrimp (i.e., krill)[22] because it imparted a *"wild-like"* flavor versus controls with no krill [15]. Likewise, in a similar taste test consumer panelists preferred wild, troll-caught chinook salmon over the same species of farmed salmon [16].

Researchers in Hong Kong found that the bromophenol content in many types of seafood (e.g., fish, clams, oysters, shrimp and crabs) varied with season, with the highest bromophenol levels coinciding with the

21. Carotenoid pigments are a group of natural fat-soluble pigments found in plants, algae, and photosynthetic bacteria where they play a vital role in photosynthesis. Animals appear to be incapable of synthesizing carotenoids, but often incorporate them from their diets.

22. Euphausid shrimp (krill) are free-swimming shrimp-like animals 1 to 2 inches long that filter feed on zooplankton. Many Antarctic species live in swarms which may contain up to 60,000 individuals per cubic meter of water.

seasonal growth of bromophenol-synthesizing seaweeds. They found much higher concentrations in the guts of finfish and the cephalothorax of shrimp and crabs, verifying that the chemicals were derived from the diet. The highest levels were found throughout the year in crabs, again reflecting a diet rich in bromophenol-laden polychaete worms [17].[23]

The Australian research group examined the bromophenol content of 32 species of ocean fish from Eastern Australia. With the exception of a single species, bromophenols were found in all **benthic**[24] **carnivores**[25] and **omnivores**[26], but were not detected in any of the five species of **pelagic**[27] carnivores. Separate analyses of gut and flesh showed the gut to contain much higher levels of bromophenols, which again supported the idea that a natural diet is the source of these chemical compounds. Although tasting trials were not performed on these fish, the authors commented that all of the benthic carnivores and omnivores analyzed are recognized by seafood connoisseurs as most likely to possess *"sea-salt-like," "iodine-like,"* or *"ocean-like"* flavors. Conversely, all of the pelagic carnivores tested have a reputation for flavors described as sweet, but bland [18].

How much bromophenol is required to affect flavor?

Researchers found that about 3 ppb is a "normal" total bromophenol level in the edible portion across all seafood products. However, among bottom-dwelling species such as shrimp and crabs and bottom-feeding fish, bromophenol concentrations are higher. Specifically, concentrations as high as 30 ppb in salmon, 38 ppb in herring, and 100 ppb in Gulf of Mexico shrimp have been reported. Comparing reported levels that range from 30 ppb to 100 ppb with the bromophenol flavor threshold concentrations in water which ranged from 0.0005 ppb to 23 ppb suggests that wild seafood might be considered tainted rather than flavor enhanced.

Two important conditions underscore why broad scale, sensory evaluations consistently herald bromophenol compounds for their flavor-enhancing ability rather than as a taint to existing flavors. First, bromophenols are soluble in water, and because of this do not bioaccumulate[28] to any significant degree. Thus, at any point in time the bromophenol concentration in muscle tissue reflects the balance between dietary intake and normal depuration. This constant flux between bromophenol concentrations increasing due to dietary intake and waning due to leaching from muscle tissue is why there is such a large range of bromophenols found in wild shrimp. In particular, this is why shrimp from the same trawl vary in flavor intensity. The few that have recently gorged themselves on marine worms just prior to capture often have an iodine taint, while the majority retain concentrations sufficient only to impart a *"shrimp-like," "briny"* flavor. Likewise, salmon that migrate up rivers to spawn in the headwaters were found to be bland-tasting in contrast to those harvested offshore which had *"marine-like"* flavors. Second, the effect these chemical compounds have on seafood flavor is actually moderated by the seafood itself. Specifically, for a shrimp to have an *"off"* taste of iodine, the concentration has to be 100 to 1,000 times higher than the same compound in water. For example, chemical analyses combined with sensory evaluations showed that the flavor threshold concentration of 2,6-dibromophenol was 0.06 ppb in shrimp, compared to 0.0005 ppb in water. Thus, this most intense-tasting

23. Polychaete worms are segmented worms bearing bristles called setae (= chaete).

24. Benthic refers to that part of an aquatic environment inhabited by organisms living permanently in or on the bottom

25. A carnivore is an animal that subsists primarily on animal rather than plant food.

26. An onmivore is an animal that consumes both plant and animal foods.

27. Pelagic is a primary division of an aquatic environment which includes the water column itself.

28. In toxicology, the LD50 (i.e., the abbreviation for "Lethal Dose, 50%") or median lethal dose of a toxic substance is the dose required to kill half the members of a tested population. LD50 figures are frequently used as a general indicator of acute toxicity for a substance. In assessing human bioaccumulation using rat LD50 data, it has been estimated that a human would have to consume between 8 million and 600 million shrimp in order to accumulate any toxic levels of bromophenols [19].

bromophenol had to be 120 times stronger in muscle tissue than in water in order to be discernable. However, at 32 ppb, this most potent bromophenol caused an undesirable iodine taint. Therefore, when comparing the lower bound of sensory detection in shrimp (0.06 ppb) with the concentration that led to a taint (32 ppb), the concentration of 2,6-dibromophenol would have to increase by 530 times above the 0.06 level to create an undesirable taste – a huge range and one into which the majority of wild-harvested shrimp fall [9].

The UW-Madison researchers determined that three of the five bromophenol compounds were actually responsible for the natural *"ocean-like"* or *"shrimp-like"* flavors, with the other two adding unique flavor tones. Based on their marinated Great Lakes whitefish study, the research team concluded that a minimum 10 ppb of 2-bromophenol resulted in a *"rich," "full,"* and *"sea-like"* flavor, whereas 2,6-dibromophenol of at least 0.1 ppb produced a *"crab-like"* or *"shrimp-like"* flavor. The 2,4,6-tribromophenol at 10 ppb resulted in "sea-salt" or "seafish-like" flavors. Both the American and Australian research groups showed that while a single bromophenol could impart a particular flavor, a combination of bromophenol compounds resulted in a suite of unique seafood flavors [12].

The Origin of Bromophenols

The first naturally-occurring marine organobromine compounds were isolated and characterized in 1909. Throughout the latter half of the twentieth century considerable work was devoted to isolating chemicals from marine plants and animals in search of new pharmaceuticals. By the turn of the twenty-first century some 1,690 bromine compounds had been identified. These are almost exclusively marine in origin with only a few from terrestrial sources. Bromophenols have been isolated in both warm and cold marine waters from around the globe. Although found in both pelagic and benthic organisms, the vast majority of the most potent bromophenols are produced by bottom dwellers [20].

About the time that the UW-Madison team was evaluating the effects of purified bromophenol compounds added to otherwise bland-tasting freshwater foods, on the other side of the world Australian researchers were determining the bromophenol content of several species of marine polychaete worms and speculating on the role these prey species played in the flavor of marine shrimp. This research team found that many species of marine shrimp landed off the Australian coast contained relatively high concentrations of total bromophenols in the edible flesh (2.2 to 312 ppb). When separate analyses of the gut showed that these bromophenol compounds were derived totally from the animals' diets, the research team concluded that polychaete worms – some of which contained up to 8.3 million ppb of bromophenol compounds – were the primary source of bromophenols in shrimp. In fact, the *"off"* iodine like flavors reported for the occasional shrimp were likely due to the animal having recently ingested a worm species with an exceptionally high bromophenol content [21].

Sixteen species (44 samples) of marine polychaetes from Australian waters were analyzed for all five bromophenol flavor-enhancing compounds. All five of the bromophenol compounds were found in 40 of the 44 polychaetes (91 percent), while the remaining samples contained at least three of the five compounds. One compound, 2,4,6-tribromophenol, was found in all samples. On a wet-weight basis, total bromophenol content ranged from 58 ppb to 8.3 million ppb.

Polychaetes from muddy sea-bottom environments consistently showed the highest levels of bromophenols regardless of species, with those from sandy and shelly substrates having the lowest levels. For example, 14 samples representing 10 species collected from mud or mud-sand bottoms had total bromophenol concentrations greater than 100,000 ppb while eight samples representing 2 species collected from beach sand or shell grit contained total bromophenol concentrations of less than 1,050 ppb [21]. Such information may have implications for the unique flavors associated with the various wild shrimp species harvested from different sea bottom types found across U.S. waters (e.g. brown shrimp from the mud bottom of the Western Gulf versus pink shrimp from the sandy bottom off Florida's west coast.)

Why do certain marine species produce Bromophenols?

Brominated organics of marine origin can be found in certain species of marine algae, polychaetes, hemichordates (acorn worms), molluscs, crustaceans, fish, sponges, corals, sea slugs and tunicates. Importantly though, only three groups actually produce the bromophenol compounds. These include polychaetes, acorn worms, and to a lesser extent various species of marine algae which may be consumed directly or after being incorporated into the marine food chain.[29] The other organisms mentioned above derive these compounds from their diet of polychaetes, acorn worms, and algae.

Conversely, analyses of various freshwater fishes such as cisco herring, whitefish, rainbow trout, northern pike, walleye and grass carp revealed a virtual absence of bromophenols. Also, recall that Pacific salmon which were hatchery reared and stocked in landlocked, freshwater habitats contained virtually no bromophenols[13].

Why do marine species feeding on natural diets have bromophenol concentrations sufficient to impart a discernable taste while freshwater species do not? Freshwater simply does not have either the phenol or bromine to allow synthesis of bromophenols for incorporation into freshwater food chains [25]. Specifically, freshwater contains virtually no bromine, whereas bromine is the ninth-most abundant element in seawater at a concentration of 65 parts per million.

Enhancing Feeds with Bromophenols to Create Similar *"Marine-like"* Tastes in Cultured Shrimp

If consumers prefer the shoreside aromas and unique flavors of wild-harvested shrimp, and if the chemical compounds responsible for producing this flavor are known, then why not simply add these compounds to the feeds of commercially-cultured shrimp? While the addition of bromophenols to commercial feeds seems like a straight-forward solution, actually amending feeds to create the flavors found in wild-caught marine shrimp has been problematic across two fronts. First, bromophenol compounds which have been added to commercial feeds have consistently failed to produce discernable flavor differences in the finished product. Second, repeated attempts to add bromophenols in commercial settings have resulted in significant cost overruns for feed, a major production expense even without the bromophenol complement.

With the rapid development in shrimp farming in the 1990s, particularly in southeast Asia, the Australian research group carried out extensive tests to develop a cost-effective method of incorporating bromophenols into the feeds for cultured shrimp. When researchers compared the diets of wild shrimp with those of cultured shrimp they found that wild shrimp consume a variety of polychaete species with a bromphenol concentration averaging well over 100,000 ppb. Commercial shrimp feeds, on the other hand, typically contained between 1 and 50 ppb bromophenols, with the source often being shrimp-head meal or some other by-product of crustacean processing. Further, bromophenols were detectable in commercial feeds only if the shrimp-head or crustacean by-product meal accounted for at least 10 percent of the final feed mixture. Often this percentage was not met since shrimp feed ingredients, like other animal feeds, are based largely on least-cost formulations. Chemical analyses showed that the bromophenol content in prepared diets were not only variable, but often contained less than 1 ppb of any bromophenol compound [26].

29. The function and ecological relevance of these compounds in marine worms still is not *totally* understood. Originally it was thought that they served as anti-predation and anti-microbial agents, since the compounds were concentrated in the most exposed portions of the worms' bodies, and also were shown to inhibit metabolism of certain bacterial species thought to be responsible for degrading the worms' burrow walls [22]. Also, early trials showed bromophenols to provide a negative recruitment cue, which inhibited settlement by other worms or bivalves which might compete for food [23]. New research, however, has cast some doubt on the anti-microbial aspect of this theory [24].

In an analysis of fourteen commercial shrimp feeds, researchers reported that all but one contained under 100 ppb total bromophenols. Taste tests confirmed that shrimp raised on these feeds – while acceptably sweet – were bland and lacked the characteristic flavor of wild shrimp. The single feed sample with over 100 ppb total bromophenols produced shrimp with a *"slightly sea-salt"* and *"iodine-like"* flavor. However, even these shrimp contained only 9.7 ppb of total bromophenol compounds. By contrast, the wild, tiger shrimp used as a control in this study contained a total bromophenol concentration of 180 ppb [26].

Thus, the low bromophenol content of farmed shrimp is primarily a result of low bromophenol concentrations in commercial feeds. In addition, the availability of bromophenols in these feeds for incorporation into shrimp flesh is further reduced by one or both of the following conditions: (i) the bonding of these compounds to other components in the feeds during fabrication, or (ii) being lost during steam or extrusion pelletization. For example, shrimp fed an artificial diet enriched to a level of 18,932 ppb bromophenols using polychaetes, retained only 22.2 ppb of the bromophenol compounds in the muscle, suggesting that the bromophenol compounds added to the feed were either chemically unavailable for absorption by the animal or lost during manufacturing. The authors concluded that a finishing feed required to produce a desirable *"ocean-like"* flavor would (i) have to contain enough shrimp-head meal to produce a bromophenol abundance of at least 100 ppb in the feed and (ii) have to be fed to shrimp for at least eight weeks prior to harvest. This is not economically practical, nor is the bromophenol content of shrimp-head meal sufficiently consistent to produce a reliable bromophenol-enriched feed; particularly if the meal comes from cultured shrimp since the ultimate source of bromophenol compounds is a natural diet from the marine environment. Even when purified bromophenol chemicals were incorporated into feeds at concentrations ranging from 30 ppb to 378 ppb, shrimp that were fed these rations retained less than 10 ppb total bromophenol in the muscle tissue. Sensory panelists judged these shrimp as sweet-tasting, but otherwise bland [26]. Robert Lindsay, one of the UW-Madison flavor chemists, summarized their work by stating:

> *"while farmed shrimp can have the texture of wild shrimp, they have a very bland taste, largely because of their diet which consists of low-cost, terrestrially-produced protein"* [27].

Additional research which compared flavors found in wild and cultured shrimp

The Australian research team analyzed nine species of wild shrimp found off the Australian coast, along with two species of cultured shrimp. Wild shrimp ranged between 9.5 to 1,114 ppb in total bromophenol (head and tail) depending on species, location, and time of year they were caught. In just the tail muscle though, values ranged from 2.2 to 312 ppb, with most falling between 5 and 30 ppb. Seventeen of the wild samples contained all five of the bromophenol compounds, while eleven contained four of the five compounds, and two samples contained just two of the five bromophenol compounds. Across the nine species of wild shrimp, the ratio of these flavor compounds in the head/gut versus the tail muscle varied widely from 1.3 to 25. The concentrations of flavor compounds in the head/gut compared with the tail muscle also varied within the same species. This condition supports the idea that bromophenol concentration reflects how recently the animal had eaten; attesting again to the source of bromophenols being dietary, not metabolic [11].

In contrast, analysis of cultured shrimp showed a total bromophenol concentration in the head and tail of less than 1 ppb with only one tail-meat sample containing all five bromophenols, and the remainder having only two or three compounds present, but generally in **concentrations 100 times less than their wild counterparts** [11]. In consumer taste tests using these same shrimp, all of the wild-shrimp samples were rated as having *"prawn-like"* flavors, with six of the samples also rated as having an *"ocean-like"* flavor. Only one of the five cultured samples was described as having **some** *"prawn-flavor,"* and none of them were described as having an *"ocean-like"* flavor. Both wild and pond shrimp were described as being sweet.

Thus, wild shrimp were perceived to have sweet, *"prawn-like"* and *"ocean-like"* flavors, whereas cultured shrimp were regarded as sweet, but otherwise bland [11]. These flavors were directly correlated with prey items found in the digestive systems of shrimp. Specifically, certain species of polychaete worms as well as algae growing on organic-rich detritus contain high levels of bromophenol compounds which leads to *"shrimp-like"* and *"ocean-like"* flavors. In contrast to these natural diets, which include certain polychaetes that contain as much as 8 million ppb of bromophenol compounds, commercial shrimp feeds generally contain less than 2 ppb, more than a million times less [11].

Researchers at Texas A&M University found that a significant number of taste panelists could differentiate between wild and pond-raised shrimp in each of four taste tests, with 55 percent of the panelists preferring wild over farmed shrimp. The wild shrimp were rated as having a more *"shrimp-like"* flavor [28]. In earlier research at Texas A&M, taste panelists rated shrimp from higher salinity waters as being "sweeter" than those from low salinity water. Researchers attributed this difference in flavor to the higher concentration of free amino acids found in the higher salinity shrimp which they produced in response to the greater osmotic gradient [29][30]. Assuming the cultured shrimp used in the taste tests reported in this paper were from ponds with near ocean salinity, it would explain why these shrimp were usually described as tasting bland, **but sweet.**

Summary and Conclusions

This paper has traced research across the globe that sought to determine the chemicals responsible for the suite of unique flavors found in many seafoods. It also reviewed the approaches that created flavor distinctions in otherwise bland-tasting freshwater species through the addition of purified bromophenol chemicals so that the impressions of taste panelists could be pinpointed. Beginning with a longstanding observation that the flavor of salmon changes once they enter rivers to spawn, this paper also traced the work which documented that the source of bromophenol compounds in muscle tissue results from the natural diet of bottom feeders and bottom dwellers.

The flavor of any foodstuff is the sum of all aroma, taste, and intensity sensations experienced by consumers. Indeed, every bite generally invokes an entire suite of aromas and tastes which can be quite broad. Thus, while bromophenols are not entirely responsible for the particular flavor of a given seafood, they are the major class of chemicals which distinguishes saltwater from freshwater species. More specifically, bromophenols create the flavors found in wild-harvested seafoods that are not found in cultured animals of the same species. *"Marine-like"* and *"ocean-like"* flavors should not to be confused with *"fishy"* odors or *"off"* tastes which result from microbial spoilage. For this reason wild seafoods must be carefully handled on board the vessel as well as in the processing plant to prevent these unique ocean-like natural flavors from being masked by spoilage flavors and odors which tend to be strong.

Currently it appears as though there is both a technical and economic challenge that prevents incorporation of bromophenol compounds into the prepared diets for cultured species in sufficient quantities to impart a *"marine-like," "ocean-like,"* or *"iodine-like"* flavor characteristic of wild seafoods. A variety of studies collectively verifies the often anecdotal observations that there are discernable differences in flavor between wild and cultured seafoods, particularly bottom-feeding species like shrimp. Research has also documented that the compounds responsible for such flavors are derived from a natural diet.

The *"marine-like"* and *"ocean-like"* flavors created by consumption of natural diets are an intrinsic (i.e., *"built-in"*) attribute of properly-handled, wild shrimp. As the domestic shrimp industry and the trade associations that represent producers and processors continue their pursuit of creating a more informed constituency interested in the differences between wild and cultured shrimp, the findings in this paper suggest that flavor is a scientifically-verified attribute and should be included in all marketing and promotion programs.

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