Review

Significance of Antioxidants for Seafood Safety and Human Health

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ABSTRACT: The demand for high-quality seafood products is constantly growing worldwide. Nevertheless, seafood is susceptible to rapid rancidity mainly due to lipid oxidation and microbiological spoilage. Thus, treatment with antioxidants offers a preservation technique that can prolong the shelf life of seafood. However, because of food safety and health concerns about the use of synthetic antioxidants, there is growing interest in the application of natural antioxidants, mainly plant extracts and compounds, as an alternate means of confronting the problem of lipid oxidation. In this review, up-to-date information and recent discoveries about different naturally occurring antioxidants on the oxidation progress, synthetic antioxidants and their health concerns, health benefits of antioxidants, antioxidants used for seafood, and food safety concerns are addressed. The antibacterial effects of natural antioxidants are also reviewed. Finally, the most effective methods for analyzing a wide range of antioxidants in plants are described.

KEYWORDS: seafood, antioxidants, food safety, rancidity

IMPORTANCE OF SEAFOOD

The importance of seafood is growing significantly every year,[1] mostly due to the fact that seafood contains many beneficial healthy substances. The most important of these is the fat, which usually contains high amounts of omega-3 fatty acids, mainly α-linolenic acid, eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA). The omega-3 fatty acids have numerous beneficial impacts on human health. They include decreasing the risk of myocardial infarction,[2] lowering blood pressure and triglyceride concentration in blood,[3−5] enhancing the immune system,[6] and sustaining proper brain function.[7] They also protect against various psychological disorders, depression[8] and attention deficit hyperactivity disorder (ADHD) in particular,[9] and cancer.[10−12] However, fatty acids are not the only important nutrients in seafood. It is also a good source of easily digestible proteins, and its amino acid profile usually contains most of the essential amino acids. Seafood is rich in fat-soluble and B-group vitamins.[13,14] It is also widely appreciated for its contents of antioxidants, of which carotenoids, flavonoids, polyphenols, and tocopherols are the most prominent.[15] Marine fish contain antioxidant enzymes, including catalase superoxides, dismutase, and glutathione peroxides.[16] A large body of literature suggests that populations which consume high amounts of seafood are protected against cardiovascular diseases.[17]

SEAFOOD SPOILAGE AND LIPID OXIDATION

Although the above information clearly indicates that consumption of marine products is beneficial for human health, one must not forget about a major disadvantage of seafood. It has a short shelf life compared to chicken and red meat. The spoilage of seafood is very high and estimated at 10−12 million tons per year (around 10% of whole-fish harvests).[18]

There are three main causes of seafood spoilage: autolysis, microbiological spoilage, and lipid oxidation. The first of these is usually due to improper handling or processing immediately after harvest and is caused by enzymes that occur naturally in seafood. The usual effects of autolysis are softening of tissue, belly bursting, and production of hypoxanthine and lactic acid, which changes the pH of seafood.[19] Furthermore, Hansen et al.[20] showed that postharvest autolysis was responsible just for the texture changes but did not affect the production of off-odors and off-flavors.

Microbiological spoilage is caused mainly by microorganisms present in the water environment in which the fish live. The most important bacteria species involved in seafood spoilage are *Pseudomonas* spp., *Aeromonas* spp., *Vibrio* spp., certain species of psychrotolerant Enterobacteriaceae, which are usually found in iced freshwater fish, and *Shewanella putrafaciens*-like bacteria, mainly *S. putrafaciens*, *Shewanella algae*, and *Shewanella baltica*, which are commonly found in iced marine fish. *Photobacterium phosphoreum* is also responsible for spoilage in seafood packed in modified atmosphere with CO_2.[21]

Microorganisms present in seafood generate a wide array of compounds that contribute to the whole fish spoilage and produce many off-odor and off-flavor compounds such as hydroxylamine, biogenic amines, ketones, aldehydes, alcohols, and organic acids.[19] The specific ammonic odor in fish is caused mostly by trimethylamine, which is produced by bacteria from trimethylamine oxide present in high quantities in marine base seafood.[21] Chinivasagam et al.[22] found 21 malodorous compounds produced by *S. putrafaciens* and *Pseudomonas fragi* in frozen shrimp.

As mentioned above, one of the best bioactive and abundant ingredients in seafood is polyunsaturated fatty acids, mainly the omega-3 fatty acids. Because of this, however, seafood is

especially susceptible to the third main reason for food spoilage, namely, lipid oxidation. It has been proven that oxidation in seafood can cause the deterioration of color, flavor, texture, and nutritive values.[23]

The oxidation of lipids by molecular oxygen generates free radicals and can be accelerated by light, heat, or metal ions, forming free radicals. The peroxides formed in these reactions can, in turn, react with other lipids, fatty acids in particular, to form new kinds of peroxides. This second stage of lipid oxidation, referred to as the propagation phase, persists until the final termination stage, which occurs when two free radicals combine.[24]

In seafood, autoxidation can be caused by both nonenzymatic and enzymatic means. It has been proven that thorough bleeding of fish can improve seafood shelf life. After autoxidation, hemoglobin produces active heme and iron, which serve as catalysts for lipid oxidation. Richards and Hultin[25] showed that the reduction of erythrocyte lysis decreased autoxidation in fish muscle, possibly due to retention of hemoglobin inside erythrocytes. The research clearly showed that the quality of seafood depends highly on the concentration and type of hemoglobin present inside the muscle. Myoglobin in meat has shown pro-oxidation tendency, especially at pH 5.5.[26]

ANTIOXIDANTS

Due to problems with rapid postharvest lipid oxidation, the seafood industry has begun to look for solutions, of which one of the most promising and effective interventions is the application of antioxidants.

Antioxidants are compounds that can inhibit oxygen-dependent lipid oxidation, usually by scavenging and thereby neutralizing free radicals. This chain-breaking process can occur in either the initiation or propagation phase of lipid oxidation. Some free radical quenching chain-breaking antioxidants act by forming molecular complexes with free radicals that are too unreactive to propagate further oxidation. Russo et al.[27] described the main structural features of effective radical scavenging substances. First, they possess two or more hydroxyl groups, which are attached to an aromatic ring in the *o*-dihydroxy conformation. Such compounds can create numerous radicals due to the high reactivity of their OH groups. Second, the compound should provide a planar surface that is readily accessible for conjugation and electronic delocalization. Additionally, the presence of certain functional groups such as C=O or carbon–carbon double bonds increases free radical scavenging effectiveness. Heim[28] has argued that the potent scavenging ability of flavonoids is related to their ability to form polymerization products. Another way certain antioxidants, including flavonoids, inhibit lipid oxidation is by producing a stable complex with chelating metals which when free would be able to catalyze lipid oxidation.[29,30]

Antioxidants can also exert their protective effects vis-à-vis lipid oxidation by inhibiting pro-oxidative enzymes such as glutathione reductase (GSH-RD) and xanthine oxidase.[31] Cos et al.[32] observed that the hydroxyl groups at C-5 and C-7 and the double bond between C-2 and C-3 were crucial for a high inhibitory activity on xanthine oxidase. For instance, the absence of a hydroxyl group at C-3 enhances slightly the inhibitory effect on xanthine oxidase because the structural difference influences the inhibitory effect on xanthine oxidase.

Two or more antioxidants acting together can enhance the antioxidant activity of one or both of the antioxidants in a phenomenon known as synergism. One of the mechanisms by which such synergism can occur between two or more free radical scavengers is when steric hindrance of interactions between antioxidant and free radicals accelerates free radical scavenging reactions. Alternatively, synergism can also occur between two antioxidants when one of them binds the free radical and is then itself regenerated by the other antioxidant. This type of synergism is exemplified by ascorbic acid and α-tocopherol when ascorbic acid, due to its lower reduction potential, converts the tocopheroxyl radical back into α-tocopherol, which is a primary antioxidant.[33,34] Another effective synergistic combination obtains when a free radical scavenger sequesters a pro-oxidant metal. In this instance, the metal chelator inhibits the production of free radicals by trapping the metallic oxidation catalyst while pre-existing free radicals are inactivated by the free radical scavenger.

SYNTHETIC ANTIOXIDANTS AND THEIR HEALTH CONCERNS

Synthetic antioxidants were used commonly to prevent products from spoiling, not only in the food industry but also in other industries. For instance, butylated hydroxytoluene is widely used in the petroleum industry to preserve the shelf life of biodiesel.[35] The most important antioxidant compounds used in food processing are butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), octyl gallate (OG), propyl gallate (PG), *tert*-butylhydroquinone (TBHQ), and nordihydroguaiaretic acid (NDGA).[36] Their advantages, which made them so popular among food producers, are mainly lower production cost and higher antioxidant capabilities when compared to extracts of natural spices such as rosemary (approximately 4−5 times weaker). On the other hand, pure antioxidants derived from natural spices tend to have more powerful scavenging abilities, such as rosmarinic acid, which has twice the antioxidant capacity of PG.[37] Synthetic antioxidants are also less polar than their natural equivalents and therefore more soluble in lipids.

In recent years many studies have shown that a high intake of synthetic antioxidants can be hazardous for health. Ito et al.[38] clearly demonstrated the procarcinogenic effect of some synthetic antioxidants. BHA has been implicated in stomach cancer and urinary bladder cancer. BHT has been linked to urinary bladder cancer and thyroid cancer. Moreover, in a study conducted in rats BHT was shown to cause hemorrhage and death.[39] Because of health issues, the use of synthetic antioxidants in seafood should be carefully monitored and regulated. Holaas and colleagues[40] performed a study on Atlantic salmon fed a fodder containing BHT for 12 weeks. After 12 weeks, the salmon were subjected to 2 weeks of depuration. Depuration is a procedure commonly used in fish farming to remove all residues of antibiotics and other potentially hazardous substances from the fish before slaughtering and processing for consumption. BHT accumulates in fish liver and in theory should be removed from the fish during the depuration period. In practice, however, only 8−13% of BHT accumulated in salmon liver is removed by this procedure; this level of residual synthetic antioxidant can pose a toxicological threat to the consumer.

Other studies have shown that synthetic antioxidants may interact with each other, thus enhancing hazard. Groten et al.[41] showed that BHT, when applied together with PG, caused joint pathology and liver enlargement. They recommended that the levels of PG and BHT in foods should be limited when both are present in the same product.

NATURALLY OCCURRING ANTIOXIDANTS AND THEIR SOURCES

Because of the health concerns discussed above, industries have begun to consider the use of antioxidants that occur naturally in

Table 1. Important Antioxidants, Their Sources, and Antioxidant Value[a]

Name	Structure	Antioxidant value (TEAC [mMTrolox])	Main spice sources
Caffeic acid		1,3	mint, oregano, rosemary, thyme, nutmeg, coriander, parsley, green cardamom,
Rosmarinic acid		3,7	mint, sweet basil, oregano, rosemary, sage, thyme
Catechin		3,2	sweet basil, nutmeg, dill
Carvacrol		0,9	sweet basil, oregano, rosemary
Carnosic acid		5,7	sage, rosemary
Gallic acid		3,6	clove, thyme
Quercetin		4,7	broccoli, red grapes, tea, onion

Name	Structure	Antioxidant value (TEAC [mMTrolox])	Main spice sources
p-Coumaric acid		2,2	oregano, sage, thyme, sweet basil
Eugenol		1,85	sweet basil, clove
Thymol		1	thyme
Sesamol		2,4	sesame
Ascorbic acid		1	
α-tocopherol		0,9	
Linalool			thyme, basil, rice paddy herb,
p-Cymene			Thyme, basil

[a]According to refs 85−93.

living organisms such as plants and seafood. Due to their natural origin they are regarded as safe and have good acceptance among consumers. Furthermore, they can also act as colorants and preservatives or even add a specific and favorable odor or taste to the product.[30]

However, certain studies indicate that even natural antioxidants can be also harmful to human health when consumed in high quantities. For example, Butler[42] showed that some enzymes are inactivated by polyphenolic compounds. The problem, as clearly pointed out by Pokorny,[30] is that most of the research on natural antioxidants has focused on their benefits for health, whereas the research on artificial antioxidants has mostly emphasized their negative aspects. Thus, more studies regarding the health safety of natural antioxidants, especially whole plant extracts, which can contain other healthful substances in addition to antioxidants, should be encouraged.

Currently, much is known about the chemistry and biological functions and properties of natural antioxidants, just as their number continues to grow. The most popular ones presently used in the food industry are ascorbic acid, α-tocopherol, a wide range of polyphenols (e.g., flavonoids and catehins), and many plant or vegetable extracts from spices that are well-established as a rich source of these compounds. Some of the most

important natural antioxidants present in plants and spices can be found in Table 1.

Because extracting and purifying the antioxidants listed in Table 1 would be costly and time-consuming, and thus not profitable, the food industry usually uses extracts from whole plant such as rosemary extract or clove extract,[37] or sometimes the whole spice is powdered and added to minced product.[43]

■ HEALTH BENEFITS OF ANTIOXIDANTS

Antioxidants, both natural and synthetic, have been shown to have many health benefits for humans, the most important of which are indicated in Table 2.

However, the list of the beneficial effects of natural antioxidants is quite impressive and contains only the most important ones, and the mechanisms by which they exert their efforts in biological system are in most cases still unknown.

Food antioxidants have been reported to be effective in lowering the risk of cardiovascular diseases such as athero-sclerosis prevention by inhibiting the oxidation of low-density lipoproteins (LDL). Development of atherosclerosis in humans has been shown to be slowed by natural antioxidants present in black and green tea, almonds, strawberries, tomatoes, garlic, and red wine.[30,44] In other studies, apple flavonoids were associated with reduced mortality from cardiovascular diseases. Food

Table 2. Most Important Health Benefits of Antioxidants Together with Foods Found To Be Responsible[a]

disease prevention	dietary source
cardiovascular diseases	red wine, white wine, apple, garlic, black tea, green tea
cancer	apple, onion, garlic, BHT, BHA, α-tocopherol
atherosclerosis	BHT, probucol, red wine
diabetes type II	apple peel
asthma	apple, pear
skin aging	green tea

[a]Data according to refs 30, 38, 44−47, and 94−98.

antioxidants may reduce the probability of certain cancers. Apples and onions, for example, contain large amounts of flavonoids, mostly quercetin and its conjugates, which have been shown to be effective in lung cancer prevention. Apple peel has also proven to be effective against liver and colon cancer.[45] Garlic was shown to prevent colon, mammary, cervical, stomach, and other types of cancers through a variety of mechanisms including one that involves free radical scavenging.[46]

Food antioxidants are also effective against skin aging, through inhibition of collagen cross-linking (green tea catechins). Combinations of vitamins C and E improve asthma,[47] whereas apple and pear flavonoids have proven effective in treating type II diabetes.[45]

ENDOGENOUS ANTIOXIDANTS IN SEAFOOD

Seafood itself contains some intrinsic means of protecting the endogenous lipids from rapid lipid oxidation. Various proteins and amino acids present in seafood have a capacity to scavenge free radicals by virtue of their ability to chelate metals, thereby serving as synergists with other antioxidants.[15,48] Moreover, fish muscle is also a rich source of antioxidative enzymes such as catalase, which is present in high quantities in saithe muscle and which converts H_2O_2 (hydrogen peroxide) into O_2. Oxygen superoxide dismutase and glutathione peroxidase have also been documented in seafood.[49]

Of course, due to the high content of PUFA in fish products, fish lipids can act as natural antioxidants. In fact, some processers are extracting oil from fish byproducts for this purpose.[50] Jaczynski et al.[51] showed that the antioxidant-scavenging abilities of oil extracted from Antarctic krill were 0.009−0.012 mM Trolox equiv/mL oil. However, this value is quite small when compared to the antioxidant content of spice extracts such as that of rosemary. Trolox equivalent antioxidant capacity (TEAC) measures the antioxidant capacity of a given substance, as compared to the standard (Trolox).

Tocopherols comprise a family of antioxidants that can be found in high levels in the flesh of a variety of seafood. Tocopherols present in fish consist mostly of α-tocopherol, β-tocopherol, γ-tocopherol, and δ-tocopherol; α-tocopherol is the form usually found in the highest amount.[52] The antioxidant activity of these tocopherols is as follows: α-tocopherol > β-tocopherol > γ-tocopherol, δ-tocopherol. Moreover, as described earlier, the antioxidant activity of α-tocopherol can be enhanced by the presence of ascorbic acid; however, the ascorbic acid content of fish muscle is usually very low.[53]

Carotenoids are present in high levels in many different seafoods, especially salmon, trout, shrimp, and krill. The antioxidant activity of carotenoids consists of free radical scavenging and prevention of photosensitized oxidation (oxidation catalyzed by visible light

and sensitizer). Astaxanthin is the dominant carotenoid in most fish species, including salmon, tilapia, and Atlantic krill.[53−55] Other antioxidants present in seafood include flavonoids or polyphenols. Polyphenols act as metal chelators and synergists, which enhance the activity of other antioxidants such as α-tocopherol, BHT, BHA, and TBHQ.[15,53]

Many seafood species also contain significant amounts of chlorophyll *a*, purpurin, and bromophenols, which have antioxidant activity. The fish species that contain chlorophyll are those which feed on seaweed and algae that are photosynthetic organisms.[53]

ANTIOXIDANTS USED IN SEAFOOD PROCESSING

Although processing does not negatively affect the antioxidative capacity of seafood (shrimps),[15] this capacity is still too small to meet the needs of the industry. For this reason, much research in recent years has been conducted regarding the supplementation of seafood products with natural antioxidants during processing.

One of the most commonly used is the extract from rosemary. Özogul et al.[56] prepared rosemary solution by immersing 20 g of powdered rosemary extract in 1 L of distilled water. In this solution, fillets of sea bream were immersed for 1−2 min right after processing. After that, fillets were cooked using three different thermal processing methods (frying, baking, and grilling) and then stored at −18 °C. They found slower oxidation of fatty acids in samples treated with rosemary extract.

Studies established by Vareltzis et al.[57] showed extensive inhibition of malondialdehyde and TVB-N levels and significantly slower degradation of PUFA for up to 50 days of storage in minced and filleted samples of horse mackerel and Mediterranean hake treated with rosemary extract. Rosemary extract was applied either by soaking fillets in a solution containing 800 mg rosemary extract/L water or by mixing minced samples with 400 mg rosemary extract/kg mince. Samples were then vacuum-packed and stored in a freezer (−18 °C) for 120 days.

A study conducted by Tokur and Ozyurt[58] demonstrated the advantageous effects of rosemary on the proteins. In this study, the rosemary extracts were added to fillets of Alaska pollock by means of immersion in a solution containing 20 g of rosemary extract in 1 L of distilled water for 1−2 min and followed by frying, oven-baking, or grilling. The samples were then stored at −18 °C and analyzed for changes in protein oxidation every month. The study showed that the addition of rosemary extract inhibited the degradation of salt-soluble proteins and weakening of protein bands. However, rosemary extract protected against protein carbonyl production after cooking.

Perez-Mateos et al.[59] performed a study on surimi from Alaska pollock fortified with n-3 fatty acids, to which the antioxidant extracts from rosemary and green tea were added. The antioxidants were added to the lipid phase, before its application to the surimi. The content of the antioxidants was stabilized at the rate of 0.6 g/kg of the final product for the green tea extract and at 0.75g/kg of the final product for the rosemary extract. The surimi was frozen at −40 °C right after extrusion and cooking and then stored at −18 °C for another 9 months. A sensory evaluation, conducted by a six-member trained sensory panel, assessed the changes in aroma, flavor, and after-taste. At the same time, the levels of lipid oxidation volatiles were also determined using the solid-phase micro-extraction (SPME) method with volatiles being quantified by gas chromatography (GC). They found that the addition of antioxidants masked the fishy taste and odor created by the

Table 3. Summary of All Effects of Antioxidants on Seafood

antioxidant used	fish	main results	shelf life extension (days)	ref
rosemary extract	sea bream fillets	inhibited oxidation of fatty acids during storage	unknown	56
rosemary extract	fillets and minced samples of horse mackerel	inhibited degradation of PUFA until 50 days of storage; inhibited significantly formation of TVB-N and MDA	25−70 days of storage in −18 °C (according to sensory analysis)	57
rosemary extract	fillets and minced samples from Mediterranean hake	inhibited degradation of PUFA until 50 day of storage; inhibited significantly formation of TVB-N and MDA	25−70 days of storage in −18 °C (according to sensory analysis)	57
rosemary extract	Alaska pollock	inhibited degradation of salt soluble proteins and weakening of protein bands.; did not affect protein carbonyl formation after cooking	unknown	58
rosemary extract	surimi from Alaska pollock, fortified with n-3 PUFA	reduced the formation of fishy off-flavors and odors; increased the yellowness of the surimi; did not affect the formation of volatile compounds	unknown	59
rosemary extract	sardine fillets	inhibited the PV and TBA value and formation of FFA and TVB-N; slight antibacterial effect; higher concentrations of antioxidants gave unpleasant bitter taste of product	unknown	23
rosemary extract with combination of low-UV lighting storage	sea bream fillets	combination of two different preservation techniques proved to be more powerful than control groups using only one of the methods, according to TBARS and sensory analysis; addition of rosemary extract had stronger antioxidative effect than addition of ascorbic acid	around 6 days of storage in 1 °C (according to sensory analysis)	73
rosemary essential oil	minced chub mackerel	inhibited the formation of MDA; slightly reduced the formation of FFA and PV; did not affect the content of TVB-N and TMA-N	30 days of storage in −20 °C (according to sensory analysis)	62
α-tocopherol with rosemary extract	coho salmon	antioxidant extract added to fish fodder while the fish were still alive; fish fed fodder containing natural antioxidants proved more effective in inhibition of TVB-N formation during frozen storage than the control fodder and fodder with synthetic antioxidants (BHT and EQ)		75
green tea extract	surimi from Alaska pollock, fortified with n-3 PUFA	reduced the formation of fishy off-flavors and odors; increased the redness of the surimi; did not affect the formation of volatile compounds	unknown	59
green tea extract	blood, muscle, and skin of rainbow trout	catechins present in extract (mostly epigallocatechingallate) showed inhibitory effect on metalloproteinase activity, thus preventing the fish from texture detorioration; extracts from bilberry, ginkgo, apple, and grape did not inhibit the gelatinase activity	not aplicable	68
tungting oolong tea extract (semifermented tea)	skin and muscle of blue sprat	inhibition of PV and CV; discovered the correlation between the total catechin (especially EGCG) content in tea extract and the antioxidative power but no connection between total polyphenol content and antioxidative power; strength to inhibit oxidation is as follows: tungting oolong tea > green tea > black tea	5 days of prolonging in 6 days storage at 5 °C (according to PV)	69

Table 3. continued

antioxidant used	fish	main results	shelf life extension (days)	ref
green tea extract	minced horse mackerel	inhibition of TBARS, formation of propanal and total volatiles; antioxidant ability is mostly due to presence of catechins; antioxidative power was higher than those of BHT and α-tocopherol	6 days of storage in 4 °C (according to formation of total volatiles)	70
green tea extract	common kilka	inhibition of PV, FFA, and TBA formation; three concentrations have been used: 200, 400, and 600 ppm of green tea polyphenols; concentration of 200 ppm showed the best antioxidant effects while not changing the sensory quality	24 h of ice storage (according to TBA analysis)	65
annato seed extract compared with coriander extract	hake meatballs	significant inhibition of EPA and DHA degradation; small inhibition of TBARS formation; research conducted during 120 days of frozen storage	unknown	64
marjoram extract	slices of yellowfin tuna	inhibition of TBAR products and PV and reduction of microorganisms growth, especially in concentrations of antioxidant at 750 and 925 ppm	at storage in 0 °C for 5 days in concentration of 925 ppm (according to TVC analysis)	60
soy protein isolate extract	minced trout	slightly pro-oxidative effect when used in high doses (1000−4000 ppm); it is predicted that small doses (300−900 ppm) should have opposite effect	none at examined doses	61
soybean meal extract	minced trout	slight reduction in TBARS; high free radical scavenging abilites	unknown	61
bay leaf essential oil	minced chub mackerel	best of all eight compared essential oils; inhibited PV, formation of MDA, TVB-N, TMA-N, and FFA	60 days of storage in −20 °C (according to sensory analysis)	62
thyme essential oil	minced chub mackerel	inhibited PV, formation of MDA, TVB-N, and FFA; did not affect the formation of TMA-N	30 days of storage in −20 °C (according to sensory analysis)	62
thyme essential oil	swordfish fillets	inhibition of TBA, TMA-N, and TVB-N; combination of thyme oil addition with modified packaging proved to be the most efficient method of protecting against oxidation; microbial growth has also been noted	3 days of storage in 5 °C for samples with just thyme essential oils and 9 days for sample with combination of antioxidant and modified packaging (according to TVC analysis)	71
black seed essential oil	minced chub mackerel	inhibited PV, formation of MDA, TVB-N, and FFA; did not affect the formation of TMA-N	30 days of storage in −20 °C (according to sensory analysis)	62
shallot extract	rainbow trout fillets	significant reduction of TBA and bacterial growth	5 days of 4 °C storage (according to sensory analysis)	76
sage essential oil	minced chub mackerel	inhibited PV, formation of MDA and FFA; did not affect the formation of TMA-N; did not affect or sometimes even increased the formation of TVB-N	30 days of storage in −20 °C (according to sensory analysis)	62
grape antioxidant dietary fiber	minced horse mackerel	during first 3 months of storage inhibited the formation of MDA, dienes, and trienes	unknown	63
grape seed essential oil	minced chub mackerel	inhibited PV, formation of MDA and FFA; did not affect the formation of TMA-N; did not affect or sometimes even increased the formation of TVB-N	60 days of storage in −20 °C (according to sensory analysis)	62

Table 3. continued

antioxidant used	fish	main results	shelf life extension (days)	ref
flaxseed essential oil	minced chub mackerel	inhibited PV, formation of MDA, FFA, and TMA-N; did not affect the formation of TVB-N	60 days of storage in −20 °C (according to sensory analysis)	62
lemon essential oil	minced chub mackerel	inhibited formation of MDA, TVB-N, TMA-N, and FFA; slightly reduced PV	30 days of storage in −20 °C (according to sensory analysis)	62
hydroxycinnamic acids	minced horse mackerel	oxidative power is as follows: caffeic acid > ferulic acid = cholorogenic acid > o-coumaric acid; all above inhibited the TBARS and PV values (10 ppm) except o-coumaric acid, which was almost completely uneffective	unknown	66
catechins	minced horse mackerel	oxidative power is as follows: catechin > gallocatechin = gallocatechin gallate > catechin gallate; only catechin showed the ability to significantly inhibit TBARS and PV values (10 ppm); there was no correlation between the amount of hydroxyl groups and antioxidative power of compound	unknown	66
catechins	minced horse mackerel	antioxidant power was as follows: EGCG = ECG = TBHQ > EGC ≫ EC; BHT and α-tocopherol did not show relevant antioxidant effect similarly to EC	>6 days for EGCG for storage in 4 °C (according to total volatile formation)	70
quercetin	surimi from blue whiting	quercetin increased the yellowness of product, while not affecting the texture, rheological properties, and water-holding capacities	not applicable	99
combination of sodium ascorbate and sodium tripolyphosphaste	fillets from Atlantic mackerel	filleting the fish submerged in antioxidant solution or rinsing with it right after improved the sensory quality, TBARS, and PV value.	3 days of storage in 2 °C (according to sensory and TBARS analysis)	25
β-carotene in combination with ascorbic acid	common kilka	inhibited formation of PV, FFA, and TBA; three concentrations have been used: 100, 200, and 300 ppm of β-carotene combined with 0.1, 0.2, and 0.3 g of ascorbic acid, respectively; the best antioxidant effect without affecting sensory quality has been shown by 100 ppm of β-carotene and 0.1 g of ascorbic acid	24 h of ice storage (according to TBA analysis)	65
ascorbic acid with combination of low-UV lighting storage	sea bream fillets	combination of two different preservation techniques proved to be more powerful than control groups using only one of the methods, according to TBARS and sensory analysis; the addition of ascorbic acid had a lower antoxidative effect than the addition of rosemary extract	around 5 days of storage in 1 °C (according to sensory analysis)	73
witch hazel extract	minced Atlantic mackerel	thermal processing increased the antioxidative power of witch hazel extract (PV and TBARS); effect was especially visible during long-term heating	unknown	74

addition of n-3 PUFA. Color changes were also documented. Rosemary extract caused an increase in yellowness, whereas the green tea extract increased the redness of surimi. The study also showed that the addition of antioxidants did not affect the production of volatile compounds. These findings indicate that antioxidant extracts can be successfully used as colorings and for reducing the fishy taste and odor caused by fortification with essential fatty acids.

Özogul et al.[23] carried out studies on frozen sardine fillets treated with 1 and 2% of rosemary extract in distilled water. The fishes were immersed in both solutions for 2 min and frozen at −18 °C. Thirty fillets for each concentration of

rosemary extract were used. The results confirmed significant positive effects of rosemary on the formation of malondialdehyde (MDA), free fatty acids (FFA), TVB-N, and peroxide value (PV). As expected, the higher antioxidant effects were observed in the fillets exposed to 2% rosemary extract. The sensory analysis conducted simultaneously on the same fillets showed that the best overall quality of the fillets was displayed by those dipped in the 1% rosemary extract. Fillets treated with 2% rosemary had a bitter taste.

Rosemary extract, despite being one of the most popular natural antioxidants, is not the only one used to inhibit oxidation of seafood. Studies conducted with *Majorana syriaca* extract also have promising results.[60] The marjoram extract was prepared from dry leaves, which were then minced (5 mm sieve) and extracted in ethyl acetate in a Soxhlet apparatus until they were free of color. The extract was then diluted in refined corn oil and homogenized with fish samples. The final concentrations of marjoram extract in fish were in the 0−925 ppm range. The samples were packed aerobically and stored for 15 days at 0 °C. The results showed a significant reduction in PV and TBARS formation as well as high inhibition of microbiological growth, especially with concentrations of antioxidant at the 750 and 925 ppm range.

Research conducted on soy protein isolate and soybean meal[61] showed that the latter has better antioxidant properties than the former. In this study, minced trout was mixed with 1000 or 4000 ppm of aqueous and methanolic extracts of both soy protein isolate and soybean meal. The samples were kept in a refrigerator at 4 °C for 14 days. Surprisingly, soy protein isolate in both aqueous and methanolic extracts not only failed to inhibit oxidation but showed pro-oxidative effect abilities, increasing the TBARS value throughout the storage period. The authors suggested that lower concentrations of soy protein extract (e.g., 300−900 ppm) might provide a beneficial effect on oxidative process. Soybean meal, on the other hand, showed a slight reduction in TBARS value and a higher free radical scavenging capacity.

Erkan and Bilen[62] examined changes in chub mackerel fillets during frozen storage following treatment with essential oil antioxidants (eight different plants: bay leaf, thyme, rosemary, black seed, sage, grape seed, flaxseed, and lemon). The amount of essential oil applied was 1% of the fillet weight. Fillets were homogenized and stored in −20 °C for 11 months. Every month the sample was monitored for changes in sensory attributes and lipid oxidation. All eight of the essential oils inhibited lipid oxidation. The TVB-N and TMA-N contents differed only slightly in the samples with antioxidants, whereas the PV, FFA, and TBA decreased significantly. Of the eight different essential oils, black thyme oil proved to be most effective in inhibiting the oxidation of fillets. It is worth mentioning, however, that even the control group, without the addition of antioxidants, did not manifest the levels of deterioration that would indicate spoilage after 11 months. In addition to various analyses, a sensory analysis of all samples was also conducted to check for deterioration of odor, taste, and texture. All of the essential oils improved the shelf life of the minced fillets by at least 1 month (black thyme, rosemary, black seed, sage, and lemon) or 2 months (flaxseed, grape seed, and bay leaf). The deterioration of the control samples, according to sensory analysis, appeared after 6−7 months of storage. These results indicated that most of the lipid oxidation parameters, such as PV and TVB-N, did not reveal the true condition of the stored seafood, and sensory analysis should be

conducted by industry before the final product is released to the market.

In shorter periods of storage, the grape antioxidant dietary fiber (GADF) was also found to be very useful.[63] GADF was obtained from red grape seeds and skin and added to minced muscle from horse mackerel at concentrations of 2 and 4%. The samples were then stored at −20 °C for 6 months. The results showed a very good protection against increases in the TBA value, dienes and trienes, but only for the first 3 months of storage. The authors suggested that after 3 months, all of the antioxidant capacity of GADF was used to protect against lipid oxidation of minced fish.

There is research indicating that the use of two different plant extracts may enhance one another's antioxidant activity. Such synergism was documented for annatto and coriander extracts in a study by Sancho and associates.[64] Both plant extracts have been added to hake meatballs, stored for 120 days at −18 °C. Although each extract alone exhibited the capacity to reduce TBARS and inhibit losses of EPA and DHA, their effect was more pronounced when they were applied in combination. The combination of β-carotene (100 ppm) and ascorbic acid (0.1 g) when applied to 3 kg of common kilka meat stored in ice for 41 h inhibited the levels of PV, FFA, and TBA when compared to the control.[65]

The use of pure antioxidants has been thoroughly examined by a number of researchers. Medina et al.[66] compared the antioxidant effects of two groups of phenolic compounds, hydroxycinnamic acids and catechins, in minced horse mackerel. The mince was obtained from white muscle of fresh horse mackerel, which was homogenized and supplemented with streptomycin sulfate to inhibit microbial growth (200 ppm). The antioxidants added to the samples were caffeic acid, *o*-coumaric acid, ferulic acid, and chlorogenic acid from the group of hydroxycinnamic acids and catechin, gallocatechin, catechin gallate, and gallocatechin gallate from the group of catechins. The concentrations of these compounds in fish samples ranged from 10 to 200 ppm. According to this study, hydroxycinnamic acids had, in general, higher antioxidant potency than catechins, with the exception of *o*-coumaric acid, which appeared to have almost no effect on oxidation inhibition (as measured by TBARS and peroxide levels). In most cases, as little as 10 ppm was enough to inhibit rancidity. The overall oxidative power of hydroxycinnamic acids was as follows: caffeic acid > ferulic acid = chlorogenic acid > *o*-coumaric acid. The antioxidant power of caffeic acid was compared to that of the artificial PGs. Among the catechins, only catechin itself showed to be a good antioxidant at 10 ppm. Interestingly, the amount of hydroxyl groups in these compounds did not affect the antioxidative efficiency of catechines. The antioxidative power of catechins was as follows: catechin > gallocatechin = gallocatechin gallate > catechin gallate. Medina et al.[66] concluded that the high antioxidative power of hydroxycinnamic acids was most probably correlated to the electron donor abilities, whereas the metal chelating properties and distribution between aqueous and oily phases were not correlated to oxidation inhibition in fish muscle.

As previously shown in studies with rosemary extract, the pure antioxidants can also be added to food to change the color or texture. Studies on fish surimi from blue whiting with addition of pure quercetin (1.05 g/kg surimi) and fish or sunflower oil (83 g/kg surimi) showed that the addition of antioxidant has improved the yellowness of product while not

affecting the overall rheological values, texture, or water-holding capacities. Changes in oxidation had not been noted, but it is suspected that oxidation was already inhibited in the control groups by the addition of industrial oils which contain high doses of antioxidants.[67]

A study by Saito et al.[68] found that catechin-related antioxidants inhibited the activity of metalloproteinases in fish muscle. The research conducted on fresh skin, muscle, and blood of rainbow trout examined the inhibitory effect of natural polyphenols present in green tea, ginkgo, bilberry, grape, and apple on gelatinase activity. They found that only antioxidants present in green tea deserve attention in terms of preventing texture deterioration of fish. They concluded that the highest contribution to this inhibitory effect was attributable to epigallocatechin gallate (EGCG), which showed the same metalloproteinase-inhibitory effect as ethylenediaminetetra-acetic acid (EDTA).

Seto et al.[69] showed that a correlation exists between the amount of total catechins in tea extracts, especially EGCG, and the oxidative protection of the extracts. On the other hand, there was at best only a weak relationship between the antioxidant power of the various tea extracts and total polyphenol content. The study conducted on blue sprat, which is a small fish in which rancidity appears very rapidly, examined the impact of semifermented oolong tea extract on the oxidation progress. The extracts were prepared by adding 3 g of tea leaves into 100 mL of water at 97 °C for 3 min followed by filtration. Extracts (110 mL) were used to treat 100 g of fish (5 °C for 30 min), and without further drying, the samples were stored for 6 days at 5 °C. They found that the oolong tea extract significantly inhibited the PV and carbonyl value (CV). The study also showed that tunting oolong tea, which contains the largest amount of total catechins, had higher oxidative power than the extracts from green tea or black tea, which contained much more total polyphenols, but less EGCG, suggesting that this exact antioxidant could be used in the future as the most powerful from the catechin family. Other studies also suggest that EGCG might be the most effective antioxidant in the catechin family in inhibiting rancidity in seafood. He and Shahidi[70] showed that pure catechins and green tea extract alike are very effective antioxidants. In this study, where the antioxidants have been added to minced horse mackerel followed by the storage of samples for 7 days in 4 °C, the antioxidative power fell between those of natural and artificial antioxidants. The overall strength was as follows: EGCG = epicatechin gallate (ECG) = TBHQ > epigalloca-techin (EGC). BHT, α-tocopherol, and epicatechin (EC) showed such weak antioxidative ability as to be unsuitable for use in seafood.

Although the type of antioxidant in extension of the shelf life of seafood is an important factor, the method of application and time after processing also make a big difference in efficiency. A study conducted by Richards et al.[25] showed that the antioxidative power of the same antioxidant solution depended on how it was applied. Their investigation was conducted on Atlantic mackerel in three different stages of rigor. Fish were stored until reaching proper rigor stage and then filleted in the presence of the antioxidant solution, which consisted of 0.2% of sodium ascorbate and 0.2% sodiumtripolyphosphate. The fish were either filleted in air and rinsed in water or filleted in air and rinsed in antioxidant solution for 10 s or filleted while submerged in antioxidant solution for 30 s. Fillets were then stored either at 2 °C for 9 days or for 9 weeks at −20 °C. The

use of antioxidants increased the shelf life of stored fish the most both in rinsed and cut under submerging; the latter, however, showed even higher inhibition in the oxidation process. The determination of shelf life involved the sensory, TBARS, and PV analyses. Interestingly, even filleting under pure water increased the shelf life when compared to fillets cut in air. The method of rinsing and submerging the fillets could prove to be very valuable, except for a few problems identified by Richards et al.[25] First of all, using the antioxidants when rinsing the fillets would increase the overall cost of production, thus making it unsuitable for production of low-cost fishes (such as the Atlantic mackerel used by Richards et al.[25]). Filleting fish when submerged in water can also cause a serious engineering problem. Moreover, the blood accumulated on the skin of fish would quickly contaminate the solution, thus requiring frequent changes and cleanings. The research also showed big differences in the quality of fish stored in frozen conditions held for longer than 1 min before rinsing or dipping into an antioxidant solution, which would indicate that some important changes are taking place during this first minute after filleting. The author suggested that the best option (from both economical and engineering points of view) would be to rinse the fillets immediately after cutting in water, remove most of the blood, and then dip the fillets into the antioxidant solution to inhibit oxidation.

The combination of different preservation methods is also an active aim of research. The combination of modified atmosphere packaging (MAP) and antioxidant addition (e.g., thyme essential oil) in swordfish fillets, as conducted by Savvaidis et al.,[71] was very effective in inhibiting both oxidation and microbial growth. De Abrauand and colleagues[72] demonstrated a novel approach to antioxidant application to seafood by coating MAP or ordinary film packing with antioxidants. Instead of dipping the fish in a solution of antioxidant, they dissolved the antioxidant extract from barley husk in methanol and then dispersed it on the film. After drying, the film was stored in the dark. The film was used to pack hake fillets, which were then stored at −20 °C for 12 months. The investigators observed inhibition of FFA oxidation and the concentration of TBARS. This innovative approach to preserving seafood and lengthening shelf life could prove very useful for the industry due to its simplicity. Fish fillet manufacturers could order specially prepared film coated with antioxidants directly from the film producer or elect to produce special film in their own seafood-processing plant. A study conducted by Gimenez et al.[73] found that the antioxidant effects of both rosemary extract and ascorbic acid solution were enhanced when they were stored under lighting with low UV. The samples were stored under three different lighting conditions: darkness, standard supermarket fluorescent light, and low-UV lamp at 1 °C.

The choice of antioxidant, when applied to seafood, should strongly depend on the type of product one wishes to produce. Gonzalez et al.[74] demonstrated that minced muscle from Atlantic mackerel treated with witch hazel extract and stored at 4 °C increased its antioxidant power after thermal processing. The polyphenols present in witch hazel consisted mostly of hydrolyzable tannins but little in the way of gallic acid (5−10 units), hamamelitannin, and proanthocyanidins. The hydro-lyzable tannins, when treated at high temperature (110 °C for 2 h), created pentagallolyl glucose, which in turn increased significantly the antioxidant power of product. The addition of witch hazel extract after thermal processing also reduced oxidation of the fish but not as much as did the addition of the

Table 4. Summary of Antioxidant Effects on Bacterial Growth in Seafood and in Vitro

antioxidant used	product	bacteria	effect	ref
rosemary extract	sardine fillets	TVC	slight inhibition of growth in concentrations of 1 and 2%	23
combination of rosemary extract and low-UV lighting	sea bream fillets	PTC	slight inhibition of growth comparing both to control and with only one preservation method group	73
shallot extract	rainbow trout fillets	TVC, PTC, Enterobacteriaceae	significant inhibition of TVC, slight inhibition of PTC and Enterobacteriaceae; two methods of antioxidant application used − soaking fillets and live fish in antioxidant solution; both proved effective although there were no clearly visible differences in effectiveness of both methods	76
majorana (Majorana syriaca) extract	yellowfin tuna slices	TVC, Pseudomonas, lactic bacteria	reduction of growth at levels of 43% for TVC, 60% for Pseudomonas, and 65% for lactic bacteria at concentrations of antioxidant of 750 and 925 ppm	60
thyme essential oil (Thymus longicaulis)	in vitro	E. coli, S. enteritidis, S aureus, L. monocytogenes, B. cereus	growth inhibition of 23% for E. coli, 24% for S. eteritidis, 31% for S. aureus, 22% for L. monocytogenes, and 21% for B. cereus	78
thyme essential oil and combination of thyme essential oil with modified atmosphere packaging	swordfish fillets	TVC, H₂S producing Pseudomonas, Enteriobacteriaceae lactic bacteria	addition of only antioxidant inhibited only slightly the growth of bacteria; best results acquired were for samples with combination of modified atmosphere packaging and antioxidant addition, which inhibited significantly the growth of TVC (increased the shelf life of fillets stored in 5 °C by 9 days), Pseudomonas, H₂S producing bacteria, including S. putrafaciens and lactic bacteria; Enterobacteriaceae count was not altered in significant way by any modification	71
savory essential oil (Satureja spinosa)	in vitro	E. coli, S. enteritidis, S aureus, L. monocytogenes, B. cereus	growth inhibition of 32% for E. coli, 28% for S. eteritidis, 56% for S. aureus, 39% for L. monocytogenes, and 27% for B. cereus; savory essential oil showed highest inhibitory strength from all compared by Chorianopoluos et al. essential oils	78
oregano (Origanum vulgare) essential oil	in vitro	E. coli, S. enteritidis, S aureus, L. monocytogenes, B. cereus	growth inhibition of 28% for E. coli, 33% for S. eteritidis, 34% for S. aureus, 33% for L. monocytogenes, and 34% for B. cereus	78
mixture of oregano (Origanum vulgare) and cranberry (Vaccinium macrocarpon) extracts	cod fillets shrimps	V. parahemolyticus	mixture of both extracts was significantly more efficient than control (difference of around 5 log cfu/g) and singular extracts (difference of 2 log cfu/g); further addition of lactic acid to the mixture inhibited totally the growth of bacteria	77
carvacrol	in vitro	E. coli, S. enteritidis, S aureus, L. monocytogenes, B. cereus	growth inhibition of 7% for E. coli, 7% for S. eteritidis, 8% for S. aureus, 7% for L. monocytogenes, and 8% for B. cereus; inhibitory power of antioxidant alone was significantly lower than of the whole plant essential oil	78

Table 4. continued

antioxidant used	product	bacteria	effect	ref
thymol	in vitro	E. coli, S. enteritidis, S. aureus, L. monocytogenes, B. cereus	growth inhibition of 10% for E. coli, 9% for S. eritidis, 12% for S. aureus, 10% for L. monocytogenes, and 11% for B. cereus; inhibitory power of antioxidant alone was significantly lower than of the whole plant essential oil	78
combination of ascorbic acid and low-UV lighting	sea bream fillets	aerobic psychrotrophic bacteria	slight inhibition of growth comparing both to control and with only one preservation method groups	73

antioxidant solution before heating. Furthermore, the addition of witch hazel at 30 ppm did not change the sensory quality of fish muscle.

The antioxidant extracts may also be added to fish fodder, while the fish are still alive. Ortiz et al.[75] compared the efficiency of natural antioxidants (α-tocopherol and α-tocopherol with rosemary extract) added to salmon fodders with the efficiency of synthetic ones (BHT and ethoxyquin (EQ)). Every 3 months five fish from each diet were killed and analyzed for spoilage indicators. The diet with both α-tocopherol and rosemary extract was most efficient in inhibiting the formation of TVB-N, which, according to the authors, is probably due to the antimicrobial effects of the rosemary extract.

Collectively, all of these above-mentioned studies, including the shelf life extension, are summarized in Table 3.

INHIBITION OF BACTERIAL GROWTH BY ADDING ANTIOXIDANTS

The addition of antioxidants can also offer additional protection against spoilage beyond that of inhibition of rapid oxidation. Microbiological spoilage is also an important problem with seafood. By addition of antioxidants to seafood, the industry could eliminate two major problems at the same time: rapid oxidation and microbiological spoilage. Oreopoulou et al.[60] showed that bacteria growth reduction achieved by the addition of marjoram extract at concentrations of 750 and 920 ppm was 43% for total viable count (TVC), 60% for *Pseudomonas*, and 65% for lactic acid bacteria. Rosemary extract also had a slight antibacterial effect and inhibited growth of TVC bacteria; the higher the concentration of antioxidant, the greater the inhibition.[23]

Pezeshk and co-workers[76] compared the antiseptic abilities of shallot extract on rainbow trout. One sample was soaked in antioxidant solution (1.5%) for 30 min following killing and gutting. The fish samples were then vacuum-packed and stored for 20 days at 4 °C. In the other group, the fish was dipped for 30 min into the same antioxidant solution while still alive. After the fish were killed, the same packing conditions were applied. Both groups showed significant inhibition in TVC growth and small inhibitions in Enterobacteriaceae and psychotropic count (PCT) growth when compared to the control group that was not treated with the antioxidant addition. However, there were few significant differences ($P > 0.05$) in bacterial growth inhibition between both groups with antioxidants, which might suggest that either method would be useful.

Using a combination of two antioxidant extracts, namely, oregano (*Origanum vulgare*) and cranberry (*Vaccinium macrocarpon*), Lin and colleagues[77] showed extensive inhibition of the growth of *Vibrio parahemolyticus*. The addition of 50% oregano and 50% cranberry extract reduced the amount of bacteria by nearly 5 log cfu/g compared to the control group. The mixture was also twice as effective as the addition of oregano or cranberry extract alone. When lactic acid was added to the cranberry and oregano extract mixture, the growth of *V. parahemolyticus* was inhibited completely. The addition of only lactic acid had a small inhibitory effect of its own on growth of this organism.

Chorianopoulos and colleagues[78] performed in vitro studies on the inhibitory effect of antioxidants on the growth of some of the most important foodborne pathogens, including *Escherichia coli*, *Salmonella enteritidis*, *Staphylococcus aureus*, *Listeria monocytogenes*, and *Bacillus cereus*. The study compared

Table 5. Analytical Methods for Determination of Antioxidants in Seafood

antioxidant	instrument	column	mobile phase, amount (%)	product	source of antioxidant	ref
chlorogenic acid caffeic acid	HPLC + photodiode array detector	150 mm × 4.6 mm × 5 μL	water, 90 acetonitrile, 5 glacial acetic acid, 5	minced trout	soybean meal; soy protein isolate	61
genistein, daidzein	RP-HPLC + photodiode array detector	150 mm × 4.5 mm × 5 μL	water, 12 acetonitrile, 8 0.1 M phosphate buffer, pH 3.5, 2 tetrahydrofuran, 0.8	minced trout	soybean meal, soy protein isolate	61
gallic acid catechin epicatechin gallocatechine gallocatechin gallate epigallocatechin epigallocatechin gallate epicatechin gallate	HPLC-DAD-MS + quaternary pump + photodiode array detector + ion trap MS + electrospray source in negative mode	150 mm × 2.1 mm × 3.5 μL	HCOOH, 0.05 acetonitrile, 0.95	minced Atlantic mackerel	witch hazel	74
catechin epigallocatechin epigallocatechin gallate epicatechin epicatechin gallate gallocatechin gallate	HPLC + UV–vis detector	250 mm × 4 mm × 5 μm	acetonitrile, 7 20 mM KH$_2$PO$_4$, 93		green tea extract	100
epigallocatechin epigallocatechin gallate epicatechin gallate epicatechin catechin	RP-HPLC + UV detector	250 mm × 4.6 mm	H$_2$O + 0.06% H$_2$SO$_4$, 86 acetonitrile, 12 ethyl acetate, 2	minced trout	green tea extract	68
epigallocatechin epigallocatechin gallate epicatechin gallate epicatechin catechin	HPLC + UV–visible detector	unknown	2.5% acetic acid, 87, 60 acetonitrile, 13–40	skin and muscle of blue sprat	green tea extract black tea extract oolong tea extract pouchong tea extract	69
thymol carvacrol eugenol carvone γ-terpinene	GC-MS	30 m × 0.25 mm × 0.25 μL	helium	in vitro	essential oils from thyme, savory, and oregano	78

Table 5. continued

antioxidant	instrument	column	mobile phase, amount (%)	product	source of antioxidant	ref
p-cresol eugenol guaiacol syringol	GC-FID coupled with SPME	DB1 30 m × 0.25 mm × 0.25 μm	helium	smoked herring	smoking	81
α-tocopherol	RP-HPLC + absorbance detector	150 mm × 4.6 mm × 5 μL	acetonitrile, 25 methanol, 25 water, 1	rainbow trout muscle	diet supplementation with α-tocopherol	101
BHT ethoxyquin (EQ)	HPLC + fluorescence detector	phenyl-hexyl 150 mm × 3 mm × 3 μL connected in tandem with 125 mm × 3 mm × 3 μL	mobile phase 1: 0.6 mL of acetic acid in 900 mL of water, 20 / 0.1% ascorbic acid in acetonitrile, 80 / mobile phase 2: 0.1% ascorbic acid in acetonitrile	salmon, rainbow trout, and cod muscle	diet suplementation	102
EQ and its oxidation products	HPLC + UV detector	CSC-S ODS-2 300 mm × 3.9 mm × 10 μm	acetonitrile	herring fish meals, salmon fodders	diet supplementation	79
BHA	RP-HPLC + spectrofluorometric detector		mobile phase 1: acetonitrile, 50 methanol, 50 mobile phase 2: water, 95 acetic acid, 5	salmon, rainbow trout, and cod muscle	diet supplementation	102

the effectiveness of essential oils from thyme, oregano, and savory with the pure antioxidants carvacrol and thymol. Savory essential oil was the most effective in inhibiting the growth of *E. coli* (32% of growth inhibition), *L. monocytogenes* (39%), and *S. aureus* (56%), whereas oregano essential oil was the most effective in inhibiting the growth of *S. enteritidis* (33%) and *B. cereus* (34%). All essential oils showed several-fold greater growth inhibition compared to the pure antioxidants, which had only a slight growth inhibitory effect. Those results might suggest that the synergetic effects of various antioxidant compounds present in essential oil are more effective than the addition of just one pure antioxidant.

A combination of two different preservation techniques has also proven to be very effective in reducing the total microbiologically caused rancidity. The combination of MAP and the addition of thyme essential oil showed higher inhibition level of the growth of TVC, *Pseudomonas*, H₂S-producing bacteria, including *S. putrafaciens*, and lactic bacteria when compared to both the control group and the group treated with only thyme essential oil. It did not, however, show any significant effect on inhibiting the growth rate of Enteriobacteriaceae.[71] Also, combination of antioxidant addition in the forms of both ascorbic acid and rosemary extract with storage under low-UV lightning is effective in inhibiting the growth of aerobic psychrotrophic bacteria in sea bream fillets, when compared to the control groups; however, the inhibition effect was not great.[73] Antioxidant effects on bacterial growth in seafood and in vitro are summarized in Table 4.

■ ANALYTICAL METHODS FOR DETERMINATION OF ANTIOXIDANT COMPOUNDS PRESENT IN FOOD PRODUCTS

Up until a few years ago, GC and high-performance liquid chromatography (HPLC) were the most effective and commonly used methods for determining antioxidant residues. GC instruments equipped with a flame ionization detector (FID) had the highest sensitivity; however, in some instances they caused the decomposition of antioxidants (such as EQ) due to the high temperatures they required. On the other hand, HPLC with UV or fluorescence detection were as sensitive as GC but did not cause antioxidants to decompose. This newer instrumentation also reduced the time of analysis for many substances when compared to GC-FID.[79,80]

One of the most important factors in phenolic antioxidants determination is the method of extraction. The most commonly used methods are liquid–liquid extraction or steam distillation followed by either gas or liquid chromatography. However, each of these methods is time-consuming. Moreover, they require the use of organic solvents, which are hazardous to both the environment and human health. Because of those disadvantages, Serrot and Lafficher[81] proposed the use of SPME coupled with GC-FID. This method, however, possesses several important disadvantages of its own. The estimated content of a particular substance may be low, especially when the content of that substance is very high. Moreover, obtaining accurate results requires the optimization of the time and temperature of the analysis, which often varies significantly from one type of sample to the next, thereby making standardization difficult if not impossible. Because of the above problems, scientists prefer to combine SPME with HPLC when determining the quantity of phenolic compounds and other natural antioxidants in foods or extracts of plants. It does not require a derivatization process and can be combined with electrochemical detector, which allows detection at low concentrations, or UV detection, which requires

higher levels of phenols but is more sensitive to some compounds (e.g., nitrophenols).[82]

Because the analysis of antioxidants and other compounds, such as sweeteners or preservatives, is difficult to determine simultaneously using conventional HPLC due to large differences in the polarity of these compounds, two alternative methods have been proposed. One uses micellar electrokinetic capillary chromatography (MECC)[83] and a Waters quanta 400 system equipped with a 60 × 75 μm i.d. fused silica capillary column. Additives in cola beverages and low-calorie jam were successfully determined using the method of mixed micellar systems. The method successfully determined the quantity of PG, octyl gallate (OG), dodecyl gallate (DG), BHT, and BHA.

The other method uses flow injection analysis (FIA) coupled with monolithic column chromatography. This method has proven to be effective in the determination of various additives in which the synthetic antioxidants BHA, BHT, and PG were present in in vitro models, dehydrated soups, and cola beverages. All additives were separated with an efficiency equal to that of HPLC; however, the resolution of the substances of interest was poorer, but still sufficient for analysis. The method has many advantages over HPLC: the FIA is much faster, and the apparatus used is less expensive and simpler to use.[84]

All of the above-mentioned methods have their advantages and disadvantages. The most commonly used methods for determining antioxidants remain HPLC and RP-HPLC. This is probably due to the great variety of analyses that are possible with these instruments and the lack of need for derivatization in some analyses when compared to GC. In addition, many other instruments can be easily connected to the main apparatus. HPLC-based methods also have the advantages of high sensitivity and relatively low analysis times per sample. Moreover, because HPLC is still the most popular method, new applications and upgrades are constantly becoming available. Furthermore, one can readily find inexpensive instruments. Table 5 summarizes the different methods for identifying and quantifying antioxidants present in food samples.

The use of antioxidants in the seafood industry is growing rapidly. The beneficial impact of these compounds on lipid oxidation and their antibacterial properties cannot be overstated. However, because of the health risks associated with the use of synthetic antioxidants, there is increased interest in natural antioxidants originating from plants, such as spice extracts, essential oils from spices, or just pure antioxidants, extracted from plants, such as catechin, gallic acid, or caffeic acid. Numerous studies have shown that antioxidants reduce the levels of TVB-N, TMA-N, TBARS, and PV. They also decrease the growth of a wide range of bacteria from simple TVC and psychrotrophic bacteria to pathogens such as *Pseudomonas* spp., *S. aureus*, *S. enteritidis*, *E. coli*, and *L. monocytogenes*.

Moreover, antioxidants can also be used to improve the taste, reduce unpleasant odors, and enhance the color of the final product, thus taking the role of both preservative and colorant and taste amplifier. Natural antioxidants also enjoy the higher trust of consumers, which can be a big economic benefit for seafood producers.

Recent studies have shown that the combination of antioxidant application with different preservation techniques, such as MAP or UV lighting, should yield even more promising results. Consequently, future research should focus on these kinds of synergic effects, so that the industry can prolong the shelf life of their products with minimum use of antioxidants. This is very important because some researchers fear that we

cannot be sure if the natural antioxidants will not cause the same health risks as their artificial equivalents. Furthermore, the improper handling and application of antioxidants can reduce rather than increase the quality of seafood; for example, the addition of excessive rosemary extract can produce a bitter unpleasant taste and off-odors in fish.

In conclusion, the use of antioxidants is a very efficient way of prolonging the shelf life of products and decreasing the major health risks from oxidation, but as always we should not get carried away and use them carelessly or indiscriminately.

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Notes
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