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# Role of Plant Extracts as Natural Additives in Fish and Fish Products - A Review

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## Abstract

Quality and shelf life of fish and fish products are often enhanced by using various food additives during handling, processing and storage. Due to potential health hazards, synthetic additives are being widely replaced by their natural counter parts. Extracts containing bioactive compounds isolated from various plant sources *viz.*, spices and herbs, fruit and vegetables, seaweed etc. have shown remarkable *in-vitro* antioxidant and antimicrobial activities. Based on this, successful application of plant extract treatments have been carried out on chilled, frozen and dried whole fish, fillet as well as fish mince as antioxidant or antibacterial agent. Use of plant extracts also demonstrated to have potential for replacing sulphating agents in crustaceans. In this article, use of plant extracts as natural additives in fish and fish products is reviewed.

**Keywords:** Plant extracts, preservative, fish products, antioxidant activity

## Introduction

Seafood, unlike other muscle foods, is highly susceptible to both microbiological and chemical deterioration. High levels of moisture, nutrient content and pH render fish an easily perishable product, often going bad within a short period of

time post mortem (Li et al., 2012). Several antimicrobial compounds *viz.*, nitrites, sulphites and organic acids and antioxidants *viz.*, butylated hydroxy anisole (BHT), butylated hydroxy toluene (BHT) and tertiary butyl hydroxy quinone (TBHQ) have been used since long to delay microbial proliferation and oxidative changes in fish (Rajesh et al., 2002; Manju et al., 2007). Even though highly active, the metabolites of these synthetic preservatives have been reported to have potential toxicological effects (Juntachote et al., 2006; Naveena et al., 2008). Hence, in the past decade, researchers have been focusing on the exploration of safe, effective and acceptable natural preservatives for controlling the microbial and chemical mechanisms responsible for spoilage in fish.

Natural products, such as plant extract, either as pure compounds or as standardized extracts, provide unlimited opportunities for control of microbial growth owing to their chemical diversity (Negi, 2012). Plant extracts rich in polyphenols and flavonoids are easily obtained from natural sources like spices and herbs, fruits and vegetables by extraction with organic solvents. Constituents/extracts from plant sources are generally regarded as safe (GRAS) either because of their traditional use without any documented detrimental impact or the evidences generated from numerous dedicated toxicological studies (Smid & Gorris, 1999). Natural antioxidant and antimicrobial activity of plant extracts have been recognized for many years and applied in several food systems as antioxidant or antibacterial agents (Ahn et al., 2007; Fernandez-Lopez et al., 2005; Kanatt et al., 2007).

In the fish processing industry too, replacement of synthetic antioxidants by plant extracts is being widely encouraged by researchers and processors.

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Generally, the extract is applied by dip treatment for chilled and frozen stored products. Combination of plant extract treatment with vacuum packaging or modified atmospheric packaging is also proven to be an effective preservation method for the extension of shelf life of fresh fish and fishery products (Ucak et al., 2011; Houchier et al., 2013). Addition of plant extracts to ice had enhanced the quality of fishes during storage (Quitral et al., 2009; Ozyurt et al., 2012). The interactions between phenolic compounds and proteins also play a role in the processing and quality enhancement of certain food products. Various plant extracts have recently been used as gel enhancers in surimi produced from dark fleshed and lean fish (Balange et al., 2009). In addition, application of plant extracts to protein based films has been reported to enhance its mechanical properties (Gomez-Guillen et al., 2007; Rattaya et al., 2009). The objective of this paper is to review the latest literature on plant extracts that are being used as natural additive/preservative in fish and fish products.

### **Active compounds in plant extracts and mechanism of action**

Phenolics are generally extracted from fresh or dried samples from different plant parts like leaves, roots, stems, fruit peel, seeds and bark. Solvent extractions are the most commonly used procedures to prepare extracts from plant materials due to their ease of use, efficiency, and wide applicability. A variety of solvents including methanol, ethanol, acetone, ethyl acetate, and their combinations have been used for the extraction of phenolics from plant materials (Dai & Mumper, 2010). After extraction, the solvent is removed and the extract is dried by traditional oven drying method or spray drying. The bioactivities of plant extracts are due to the presence of phytochemicals especially polyphenolics which vary in their diversity and concentration depending on the type of plant material and extraction procedure followed.

Polyphenols are the secondary plant metabolites containing one or more aromatic rings with several hydroxyl groups (Boudet, 2007). Polyphenols can be broadly divided into four categories—flavonoids, phenolic acids, hydroxycinnamic acids and lignans (Maqsood et al., 2013). According to the degree of oxidation of the heterocyclic ring, flavonoids are further classified as flavanol, flavanones, isoflavones, flavones, chalcones, and anthocyanins. Some of the

most common flavonoids include quercetin, a flavonol abundant in onion, broccoli, and apple; catechin, a flavanol found in tea and several fruits; naringenin, the main flavanone in grapefruit; cyanidin-glycoside, an anthocyanin abundant in berry fruits (black currant, raspberry and blackberry); and daidzein, genistein and glycitein, the main isoflavones in soybean (D'Archivio et al., 2007). Phenolic acids can be divided into two classes: derivatives of benzoic acid such as gallic acid, and derivatives of cinnamic acid such as coumaric, caffeic and ferulic acid. Caffeic acid is the most abundant phenolic acid in many fruits and vegetables, most often esterified with quinic acid as in chlorogenic acid, which is the major phenolic compound in coffee. Another common phenolic acid is ferulic acid, which is present in cereals and coumaric acid is abundant in citrus fruits.

Polyphenolic compounds present in plant extracts demonstrate potential antioxidant properties due to their redox potential; that enable them to act as hydrogen donors, reducing agents, nascent oxygen quenchers, and chelating metal ions in numerous food applications (Gramza et al., 2006). The active hydroxyl groups present in the molecular structure of polyphenols are the active components that can interact with the free radicals to inhibit lipid oxidation (Mitsumoto et al., 2005). In addition, polyphenols can exhibit scavenging activity against free radicals (Rice-Evans et al., 1996), superoxide radicals, peroxy nitrite, chelate copper and iron, preventing metal catalysed free radical formation (Lin & Liang, 2000). The presence of the functional group “-OH” in the structure and its position on the ring of the phenolic or flavonoid molecule determine the antioxidant capacity.

Plant phenolics and its constituents may be lethal to microbial cells or they might inhibit the production of secondary metabolites. Major site of interaction with a bacterium is the outer cell membrane or cytoplasmic membrane; damage to this vital membrane can result in death of the bacterium which can occur in the following ways: (i) physical disruption of the membrane (Shimamura et al., 2007); further results in loss of macromolecules from the interior (ii) interaction with protein, causing deformation in the structure and functionality (Rico-Munoz et al., 1987) and (iii) interfering with membrane-associated functions like electron transport, nutrient uptake, protein and nucleic acid synthesis and enzyme activity (Bajpai et al., 2008).

The effect of phenolic compounds can be concentration dependent. At low concentration, phenols affect enzyme activity, particularly those associated with energy production, while at high concentrations; they cause protein denaturation (Brijesh et al., 2009). A major constraint associated with the use of natural antimicrobials in foods is the lack of reproducibility of their activity; despite the great diversity of compounds they contain (Negi, 2012). Qualitative and quantitative variations in the content of bioactive phytochemicals in plant extracts result in their variable effectiveness.

### Application of plant extracts in fish and fish products

Use of natural preservatives to increase the shelf life of chilled fish product is a promising technology since many herbs, plants, vegetables and fruits extract or powders have antioxidant and antimicrobial properties. Tea polyphenols are gaining importance among researchers as an effective natural food preservative. Dipping in 0.2% tea polyphenol for 90 minutes has extended the shelf life of silver carp by 1 week during iced storage (Fan et al., 2008). Their study proved that dip treatment in 0.2% tea polyphenol has effectively inhibited adenosine triphosphate (ATP) degradation as well as lipid oxidation. In a similar study, Li et al. (2012) applied 0.2% tea polyphenol for treating whole ungutted crucian carp which could effectively retard microbial growth, delayed chemical deterioration and extended the shelf life by 6-8 days compared to control during refrigerated storage. Yi et al. (2011) demonstrated that shelf life of vacuum-packed *Collichthys* fish balls could be prolonged for an additional 6 days during 0°C storage by adding tea polyphenols. Apart from this, tea polyphenols also displayed effective impact on muscle protein integrity post-mortem in large yellow croaker as it has retained the myofibrillar functional properties significantly higher than that of control (Zhao et al., 2013).

Rosemary (*Rosmarinus officinalis*) is a plant species of the Labiatae family, and its major and most active extract components like carnosic acid, carnosol, rosmarinic acid etc. are reported to inhibit lipid oxidation and microbial growth in several food systems (Zhang et al., 2010; Li et al., 2006). Rosemary extract displayed superior antioxidant activity and slowed down lipid oxidation in brined anchovies stored at 4°C for 28 days (Turhan et al., 2009). Kenar et al. (2010) reported that dipping in ethanolic

extracts of rosemary and sage tea (*Salvia officinalis*) has extended the shelf life of vacuum packed sardine fillets by 7 days when stored at 3±1°C. Rosemary extract at 0.4 and 0.8% was found to be effective in controlling bacterial growth and biochemical indices in vacuum packed Atlantic mackerel burgers during chilled storage (Ucak et al., 2011). Ozogul et al. (2011) has observed a positive effect of rosemary and sage tea extracts in lowering ammonia and biogenic amine formation in vacuum packed sardine fillets stored at 3±1°C. Recently, a study carried out by Gao et al. (2014) displayed the synergistic effect of rosemary extract along with nisin in inhibiting protein decomposition, lipid oxidation, nucleotide breakdown and microbial growth in pompano fillet (*Trachinotus ovatus*) throughout the storage at 4°C.

Extracts from turmeric (*Curcuma longa*), a rich source of antibacterial agent alone or in combination with shallot (*Allium cepa*) extract (1.5% each, v/v) were found to retain quality characteristics of vacuum-packaged rainbow trout (*Oncorhynchus mykiss*) during a refrigerated storage of over a period of 20 days (Pezeshk et al., 2011). Few attempts have also been done on investigating the efficacy of fruits and vegetable peel as preservative agent in fish. Farvin et al. (2012) has applied potato (*Solanum tuberosum*) peel in refrigerated horse mackerel mince and the extract has revealed protective effect on lipid and protein oxidation. In their study, ethanolic extracts of potato peel was found to be very effective in retarding lipid oxidation. Viji et al. (2015) demonstrated that citrus (*Citrus aurantium*) peel extract has good antioxidant and antimicrobial activities and the shelf life of Indian mackerel by 2 days compared to control by delaying the spoilage mechanisms when stored at -2°C. Zaeri et al. (2015) applied a combination of citrus and pomegranate peel extract and chitosan nanoparticles for shelf life extension of silver carp fillets. Pomegranate peel extract was significantly stronger than orange extract in delaying lipid oxidation.

Selmi & Sadok (2008) evaluated the combined effects of powdered thyme (*Thymus vulgaris*) sprinkling (0.1% w/w) and vacuum packaging on the quality of tuna (*Thunnus thynnus*) during storage at 0°C and found that thyme treatment minimized auto-oxidation of lipids as evident from lower TBARS and higher PUFA values when compared to control. The antimicrobial effect of *Majorana syriaca* plant extract (0–3900 ppm), obtained by ethyl acetate

has been demonstrated in minced yellowfin tuna stored at 0°C (Al-Bandak et al., 2009). Winarni et al. (2012) assessed the efficacy of *Aloe vera* concentrate and crown of god fruit (*Phaleria macrocarpa*) powder on the quality attributes of Indian mackerel during storage at 4°C and they reported that 20% *A. vera* and 1.5% crown of god fruits treatments were found to be the best treatments to reduce changes in sensory and microbial quality. The effect of kiam wood (*Cotyleobium lanceotatum*) extract on the postponement of haemoglobin-mediated lipid oxidation of washed Asian sea bass mince has been reported by Maqsood & Benjakul (2013). Shi et al. (2014) demonstrated that clove (*Syzygium aromaticum*) bud extracts and grape (*Vitis vinifera*) seed extracts have efficiently inhibited lipid and protein oxidation in silver carp fillets during chilled storage. Recently, Viji et al. (2015) reported the positive role of mint and citrus extracts in controlling biochemical and microbiological changes in chill stored Indian mackerel.

Incorporation of plant extracts into ice was also found useful for inhibiting the microbial and biochemical spoilage of fresh fish compared to conventional icing. This novel icing strategy is found to be particularly useful for improving the quality of fishes stored on board and during transportation to interior places. Oral et al. (2008) investigated the preservative effects of ice incorporated with wild thyme hydrosol against the spoilage of a freshwater barb and found that this modification in icing system extended its shelf life from 15 to 20 days. In a similar way, Quitral et al. (2009) investigated the effects of ice prepared from rosemary and oregano (*Origanum vulgare*) extracts on the chemical changes in Chilean jack mackerel during storage and found that the plant extract icing system has delayed the chemical changes compared to traditional ice. In another study, icing with 0.05-0.1% rosemary extract improved the sensory and chemical quality parameters and extended the shelf life of sardine by 3 days than that stored in traditional ice (Ozyurt et al., 2012). Further, icing with this extract reduced the formation of biogenic amines especially histamine and putracine in their study. Recently, Bensid et al. (2014) evaluated the effects of ice containing thyme (0.04% w/v), oregano (0.03% w/v) and clove (0.02% w/v) extracts on the quality parameters of anchovy (*Engraulis encrasicolus*) by chemical, sensory and microbiological methods. The employment of these icing systems led to a marked antioxidant effect along

with a significantly lower reduction of aerobic mesophiles and psychrotrophic bacteria in anchovy muscle as compared with the traditional ice batch.

Oxidation is an important cause of quality deterioration defect in seafood during frozen storage. Glazing with antioxidant chemicals after freezing and dip treatment in chemical solutions prior to freezing has been followed to retard oxidation reactions in frozen stored fish and fish products. Dark fleshed species like mackerel, tuna, bonito are highly susceptible to oxidation and discoloration during frozen storage. Successful application of plant extract treatments to prevent oxidative rancidity has been carried out on frozen minced, whole fish as well as fish fillet from these species.

Rosemary extract has been applied in filleted and minced frozen horse mackerel where it delayed lipid oxidation with significantly lower amount of malonaldehyde compared to control during storage (Vareltzis et al., 1997). A commercial rosemary extract, rosmol P has turned the muscle of horse mackerel fillet to be less prone to oxidation during frozen storage (Aubourg et al., 2004). The effects rosemary extract (200 and 500 mg kg<sup>-1</sup>) on lipid and protein alterations in sea salmon mince during frozen storage at -11±2°C were evaluated by Tironi et al. (2010) and they could observe higher concentration of extract delaying the onset of oxidation along with 50% reduction in oxidation and also a reduction in loss of red color of the muscle.

Grape polyphenols/extracts are known for inhibition of oxidation in several food systems. Pazos et al. (2005) demonstrated that flavonols fractionated from grape pomace had potent inhibition against oxidation in frozen white muscle of Atlantic mackerel. Grape antioxidant dietary fibre that combines a high amount of dietary fibre and polyphenolic antioxidants such as phenolic acids, anthocyanins, catechins and other flavonoids, when added to minced horse mackerel muscle considerably inhibited lipid oxidation during storage at -20°C (Sanchez-Alonso et al., 2007). Yerlikaya & Gokoglu (2010) investigated the positive effects of green tea, grape seed and pomegranate peel extract dip treatment (1% w/v) on the sensory and physical properties of frozen bonito fillets and reported a marked reduction in muscle structure deformation during frozen storage. Lin & Lin (2005) have assessed the effect of glazing with 3 types of tea extracts on the storage quality of frozen Bonito fillet

over a period of 16 weeks. The result revealed that green tea and pouchong tea afforded better protection against protein and lipid oxidation of frozen bonito flesh.

Addition of commercial pomegranate seed extract at 2% concentration in chub mackerel mince has significantly inhibited the formation of lipid hydroperoxides and TBARS during storage at -18°C (Ozgen et al., 2011). Effect of aqueous flax seed extract in lipid oxidation of whole Atlantic mackerel during frozen storage was evaluated by Studolink et al. (2005). The results demonstrated that dipping in 1% flax seed extract prior to freezing has led to lower free fatty acid and secondary oxidation product formation during the frozen storage compared to control. Ibrahim & Sherif (2008) demonstrated the positive influence of rosemary, thyme, black cumin extract and their combinations (0.3%) in controlling the values of TVBN, TMA, TBARS and moisture loss during the storage of frozen tilapia fillet.

Drying and smoking are the oldest methods of fish preservation. The most common method of smoking is cold smoking by using temperatures of 25-30°C which is not enough to kill the microorganisms. Although smoke components can act as antioxidants, the problem of lipid oxidation still persists in mildly processed fish. In addition, a high proportion of smoked or dried fish are destroyed by the attack of maggots and dermestids, thereby reducing their market value. Studies on the use of plant extracts in smoked fish are limited compared to chilled and frozen fish products. Fasakin & Aberejo (2002) demonstrated that pulverised plant materials obtained from *Piper guineense* and *Aframomum melegueta* could be used to deter egg hatchability and adult emergence of fish beetle, *Dermestes maculatus* in smoked catfish during storage. In the study of Gomez-Estaca et al. (2007), coating cold smoked sardine with edible film enriched with oregano or rosemary extracts has been found to increase the phenol content and antioxidant power of muscle, particularly when used in association with high pressure, due to migration of antioxidant substances from the film. The added plant extracts lowered lipid oxidation levels and also reduced microbial growth. Effect of aqueous extracts of pepper and nutmeg was investigated by Klin-Kabari et al. (2011) in smoked cat fish. Pepper extract was more active in terms of reducing lipid oxidation as measured by peroxide value and

TBARS values of smoke-dried catfish stored at room temperature for 6 weeks.

Melanosis, the blackening in crustaceans is induced by a biochemical process, in which phenols are oxidised to quinones by polyphenoloxidase (PPO). Polymerisation of the colorless quinones gives rise to black high molecular weight pigments. Sulphite-based formulations are currently used to prevent or delay melanosis; however, high concentrations of additives are required to effectively arrest melanosis (Gomez-Guillen et al., 2005). Moreover, increasing regulatory attention associated with these additives has led to the interest in natural additives to prevent melanosis in shrimp. In the last few years, extensive work has been done on the application of plant extracts to control melanosis development. Plant phenolic compounds, including catechin (Nirmal & Benjakul, 2009a) and ferulic acid (Nirmal & Benjakul, 2009b), could delay the melanosis formation and extend the shelf life of Pacific white shrimp when stored in ice. Inhibition of melanosis in shrimp has also been achieved by using enokitake (*Flammulina velutipes*) extract, grape seed extract, green tea extract, lead seed (*Leucaena leucocephala*) extract and pomegranate peel extract. Shrimp (*Trachypenaeus curvirostris*) immersed in 2.5 g of wet enokitake extract/mL for 10 min had no melanosis up to 20 h at 24°C (Jang et al., 2003). Shrimp (*Parapenaeus longirostris*) treated with 1.5% grape seed extract had the lowest melanosis as compared to other treatments when stored at 4°C (Gokoglu & Yerlikaya, 2008). Melanosis development was retarded in pacific white shrimp treated with methanolic pomegranate extract (1.25 g 100 ml<sup>-1</sup>) for 15 min followed by chilled storage. Pomegranate extract treatment was equally effective to 1.25% sodium metabisulphite treatment up to 6 days of storage (Basiri et al., 2015).

A dose dependent inhibition of melanosis was observed in pacific white shrimp by lead seed extract where mimosine, an analogue to tyrosine played a major role with phenolic compounds (Nirmal & Benjakul, 2011). Phenolic compounds in plant extracts can retard the melanosis formation by a combined mechanism such as PPO inhibitor Chang (2009), interacting with the active site of the enzyme and chelation of copper at the active site of PPO (Kubo & Kinoshita, 1998) and reduction of quinone to hydroquinone, etc. Phenolic compounds also have the ability to quench O<sub>2</sub> involved in the hydroxylation process, thus preventing the forma-

tion of *o*-quinone (Edenharder & Grunhage, 2003). Polyphenols in plant extracts can function as potential inhibitors of melanosis in crustaceans on account of their multiple functional properties.

Gel strength of fish protein plays an important role in determining the texture and physical properties of surimi seafood. Textural properties of fish protein can be improved by exogenous agents of chemical and biological origin (Benjakul & Visessanguan, 2003; Chiou et al., 2006). Naturally derived plant phenolic compounds have also been shown to serve as potential gel enhancers through their protein cross-linking ability (Rawel et al., 2002). Interaction of phenolic compounds with proteins brings changes in protein secondary structure resulting in higher thermal stability (Ali, 2002). Balange et al. (2009) demonstrated superior breaking force and deformation of mackerel surimi gel with 0.15% oxidized ethanolic kiam wood extract containing tannic acid. Furthermore, aqueous kiam wood extract and ethanolic kiam wood extract containing 251.90 and 456.30 mg of tannin g<sup>-1</sup> of dry extract, respectively, was used as an effective gel strengthening agent in the surimi gels prepared from mackerel (Balange & Benjakul, 2009). Shitole et al. (2013) has applied seaweed (*Sargassum tenerrimum*) extract at different levels (0.5 to 2.5% of surimi) to improve the properties of gels from lesser sardine (*Sardinella brachiosoma*). Gels added with 2% extract had increases in gel strength by 76.27% compared with the control. Addition of seaweed extract had no detrimental effect on the sensory properties of surimi gel.

In the recent years, biodegradable protein based films finds a vital role in food packaging by serving as selective barriers to moisture transfer, oxygen uptake, lipid oxidation and loss of aroma volatiles (Maqsood et al., 2013). Most protein films have good barrier characteristics against gas, organic vapours and oils as compared to synthetic films (Jiang et al., 2007). However, poor mechanical characteristics and high water permeability of protein films limit their application as packaging materials. This can be overcome by the use of chemical cross linking agents and enzymes which can modify the polymer network of protein. Nevertheless, some cross-linking agents are toxic and few others are of high cost (Cao et al., 2007). Therefore, non-toxic natural compounds having protein-cross linking ability are of much interest to the researchers.

Cross-linking effects of plant extracts in gelatin-based films is well demonstrated. Aqueous extracts of murta (*Ugni molinae* Turcz) leaves added to tuna-fish (*Thunnus tynnus*) gelatin-based edible films improved their viscoelastic properties, increased transparency, and provided protection against UV light and enhanced antioxidative capacity, which is attributed to the interference of polyphenolic compounds in the arrangement of gelatin molecules (Gomez-Guillen et al., 2007). Fish skin gelatin film incorporated with phenolic compounds like oregano and rosemary extracts also displayed enhanced antioxidative activity when tested by ABTS radical scavenging activity and FRAP assays (Gomez-Estaca et al., 2009). Fish skin gelatin films containing 6% oxygenated seaweed (*Turbinaria ornata*) extract exhibited a higher elongation at break (EAB) than the control film (Rattaya et al., 2009). These studies suggest that plant extracts could effectively modify the functional properties of the film which could be attributed to the interaction between hydrophobic groups of polyphenols and proteins. Furthermore, hydroxyl group of polyphenols enter into hydrogen bonding with protein, making more protein cross links. Hence, polyphenolic compounds could serve as a novel cross-linker in gelatin films, which could impart different properties to the resulting films, in comparison with toxic cross-linking compounds.

Many of the plant extracts which are being used for food applications has been used by human for thousands of years. Although international guidelines exist for the safety evaluation of food additives, due to problems in standardisation of extracts, it is difficult to assign Acceptable Daily Intake (ADI) or no observable adverse effect level (NOEL) for the plant extracts (Negi, 2012). The safety assessment of plant extracts is complicated by many factors such as compositional diversity, lack of identity of the active ingredients etc. However, most plant derived extracts are generally regarded as safe by USFDA (21 Code of Federal Regulations CFR 182, 184).

Application of plant extracts has proven to extend the shelf life of food products including fish and fish products. Potential effects of various extracts have delayed lipid oxidation, inhibited microbial growth and improved textural properties. There are also reports pertaining to protein cross linking and enzyme inhibiting activities of a wide source of extracts. The results from all these studies confirm their potential to replace synthetic additives to prevent oxidation and quality deterioration in food

systems, especially fish and fish products. Hence, plant extracts extend the scope for multifunctional natural food ingredients serving many useful functions to fish processing sector.

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