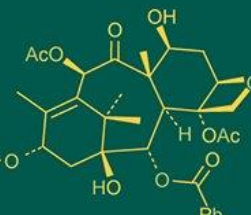
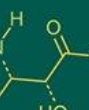
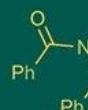


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Jungivala Mansi Dipakbhai
M. F. Sc. (Fish Processing Technology), Dept. of Fish Processing Technology, College of Fisheries Science, Kamdhenu University, Veraval, Gujarat, India

Dr. BG Chudasama
Professor & Head, Dept. of Fish Processing Technology, College of Fisheries Science, Kamdhenu University, Himmatnagar, Gujarat, India

Dr. DV Bhola
Associate Professor, Dept. of Fish Processing Technology, College of Fisheries Science, Kamdhenu University, Veraval, Gujarat, India

Rushirajsinh J Rana
M. F. Sc. (Aquaculture), Dept. of Aquaculture, College of Fisheries Science, Kamdhenu University, Veraval, Gujarat, India

Corresponding Author:
Jungivala Mansi Dipakbhai
M. F. Sc. (Fish Processing Technology), Dept. of Fish Processing Technology, College of Fisheries Science, Kamdhenu University, Veraval, Gujarat, India

Reformulation of preserved fish products: A review

Jungivala Mansi Dipakbhai, BG Chudasama, DV Bhola and Rushirajsinh J Rana

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Abstract

Fish is a highly demanded and nutritious food product, yet the perishability remains the biggest challenge for its preservation. So that, fish and fish products must need to preservation to retard the deteriorative changes. Therefore, fish products can be reformulated by the incorporation of active ingredients, as additives, coatings or as part of the packaging of the fish product. Natural ingredients are a rich source of active components and have been studied in order to compensate for salt reduction, reduce lipid oxidation, and inhibit bacterial growth. A large concentration of a certain ingredients can produce changes in the color, texture, taste, and flavor of the fish, affecting the quality of the final product.

Keywords: Reformulation, salt, natural, antioxidant, antimicrobial

1. Introduction

Many food products are perishable by nature and require protection from spoilage during their preparation, storage and distribution to reach final consumers as safe and high-quality products. Because many food products are sold in areas of the world that are far from their production sites, the need for extended shelf life for these products has also expanded (Holley *et al*, 2005) [46]. Fish is a highly demanded and nutritious food product, yet perishability remains the biggest challenge for its preservation. This food must be stored refrigerated or frozen, and, even under those conditions, it has a very short shelf-life, particularly for refrigeration (5–7 days and 9–12 months under refrigeration and frozen conditions, respectively). The deterioration of fresh fish during storage is attributed to different damage mechanisms, like microbiological spoilage, autolytic degradation, and lipid oxidation (Tavares *et al*, 2021) [19]. Different types of preservation methods such as drying, smoking, freezing, chilling, brining, fermentation and canning are reported to extend the self-life of sea foods and meat products. However, low temperature storage and chemical techniques for controlling water activity, enzymatic, oxidative and microbial spoilage are the most common in the industry today (Ghaly *et al*, 2010) [37]. As an alternative or in addition to those techniques, fish products can be reformulated by the incorporation of active ingredients, as additives, coatings or as part of the packaging of the fish product (Valenzuela *et al*, 2017) [113].

2. Reduce salt content products and associated issues

Salt (NaCl) is essential for life. Total body sodium in an average 70 kg person is of about 4,200 mmol, of which 40% is found in bone and 60% in the fluid inside and outside of cells. Na⁺ and Cl⁻ are the principal ions in the extracellular compartment, which includes blood plasma, interstitial fluid, and transcellular fluid. As such, they play critical roles in a number of life-sustaining processes. Salt is one of the most important determinants of high blood pressure and increased cardiovascular risk worldwide. However, a high salt intake has other adverse effects beyond those involving the cardiovascular system (Cappuccio, 2013) [14]. The World Health Organization (WHO) recommended that adults consume no more than 5 gms of salt per day (WHO, 2020) [124].

Available evidence suggests a direct relationship between sodium intake and blood pressure (BP) values (Intersalt, 1988; Chaudhary & Wainford, 2021; He *et al*, 2002; Denton *et al*, 1995) [50, 15, 45, 21].

Excessive sodium consumption has been shown to produce significant increase in BP and has been linked with one set of hypertension and its cardiovascular complications (Weinberger, 1996; Strazzullo *et al*, 2009) [123, 106]. The changes in salt intake present a major challenge to the kidney to excrete these large amounts of salt. The consequence is that the high salt intake causes a rise in BP (Intersalt, 1988; Chaudhary and Wainford, 2021; He *et al*, 2002), thereby increasing the risk of CVD and renal disease (He *et al*, 2002) [50, 15, 45]. In several countries in Europe, over 40% of the population has hypertension, mostly in countries that present high salt consumption (Brown *et al*, 2009) [10].

Sodium chloride (NaCl) is an essential food additive used for preservation / flavor enhancement. Food processing with NaCl has higher sodium contents, raising the concern between the excess dietary sodium as a risk factor for several health problems (Gomes *et al*, 2021) [39].

Although natural food products contain salt, more than 75% of it is received from processed food products (Dötsch-Klerk *et al*, 2015) [26]. Salt is one of the most widely used food additives as a preservative, enhancing flavor (Silva *et al*, 2003), and to improve water adsorption (Lawrence *et al*, 2004) [102, 61]. A high salt concentration generates changes in cellular metabolism because of its osmotic effect, which, influences microorganisms in different concentrations, but can reduce the nutritional value of preserved foods because water soluble components such as vitamins and minerals can be eliminated (Luck & Jager, 1995) [68]. Salt effects on different properties of the product such as, taste perception, water holding capacity, behavior of proteins and on lipid oxidation (Albarraçin *et al*, 2011) [1].

Fish products containing salt include dry-cured fish such as cod, bottarga (dry cured roe), or mojama (dry cured tuna loin) (Andrés *et al*, 2007), sea bream (Chouliara *et al*, 2004; Goulas & Kontominas, 2005), chub mackerel (Goulas & Kontominas, 2005), smoked salmon (Gallart-Jornet *et al*, 2007; Sigurgisladottir *et al*, 2000) and canned, chilled & frozen fish (Webster *et al*, 2010) [4, 16, 40, 35, 101, 122]. Sodium content in different seafood is shown in Table-1.

Table 1: Sodium content in different seafood

Food Group	Sodium Content (In mg/100gm)
Anchovy	3,668
Sardine	307
Haddock	261
Canned tuna	377
Mackerel	83
Catfish	119
Cod	78
Salmon	61
Tilapia	56
Tuna	54
Shrimp	947
Octopus	460
Crab	395
Oyster	212
Squid	44

*Source: dePorter. (2017) [22]

Strategies to reduce salt consumption include nutritional education; salt reduction at industrial level; price discount, tax reduction policies in healthy products; use of salt substitute inorganic salts; use of aromatic herbs and spices; use of derivatives of amino acids and ribonucleotides; use of

sea plants and the application of emerging technologies, such as high pressure processing, PEFs & high intensity ultrasound (Parniakov *et al*, 2020) [82].

In meat products, NaCl is a multifunctional and indispensable component. In addition to enhancing the taste, it supports the shelf life of meat products, texture and color, reduces water activity and regulates the solubility of proteins (Rysová & Šmídová, 2021) [98].

Nguyen *et al*, (2011) [114] showed that only reducing NaCl concentration, conformational changes of cod muscle proteins during brine salting occur, due to the protein aggregation. Therefore, when reducing salt content in fish, other ingredients must be used as substitutes to maintain palatability, texture, processing yield and shelf life (Kilcast & Angus, 2007) [58].

The effects of partial substitution of NaCl with KCl, MgCl₂ & CaCl₂ on gelation properties of myofibrillar protein mediated by microbial transglutaminase during the heating process were studied and result showed the ranking of chlorinated salt efficiency was NaCl > KCl > MgCl₂ > CaCl₂. The gel strength was all decreased in the myofibrillar protein gels containing KCl, MgCl₂ & CaCl₂ compared to the NaCl group, while KCl group reduced the gel strength to a less extent (Feng *et al*, 2018). Mackerel (*Scomber japonicas*) fillets were salted with NaCl and/or KCl to determine the most acceptable level by sensory evaluation; effects of ascorbic acid, vacuum packaging and cold stores on lipid oxidation. 50% replacement of NaCl by KCl reduced NaCl level with minimal impact on sensory quality, the higher level of ascorbic acid (0.05% weight basis), the higher the antioxidant effect observed with thiobarbituric acid value and peroxide value (Park *et al*, 2009) [81]. “The partial replacement of sodium in meat and fish products by using magnesium salts” study result show that magnesium has more difficulty to penetrate inside the muscle and slightly modifies the WHC of proteins, their solubility and enzymatic activity. Salty taste, bitterness and off-flavor are the most affected. The study of “salt substitution in surimi seafood and its effects on instrumental quality attributes” compared effects of salt substitute and salt on physicochemical properties of heat set surimi gels. This study demonstrates that salt substitute and salt resulted in similar physicochemical properties of surimi gels, but gels with salt substitute had lower sodium content. Therefore, salt substitute can replace NaCl in low sodium surimi seafood products (Tahergorabi *et al*, 2012) [107]. The study of “salt microspheres and potassium chloride usage for sodium reduction: case study with sushi” was to estimate possibilities of salt substitutes usage in the preparation of two sushi types (nigiri & maki) prepared with different seafood. Potassium chloride and regular salt were used in the lowering sodium content in sushi meal with the usage of salt substitute (Đorđević *et al*, 2018) [25]. The study of “The effect of partial sodium replacement on physicochemical parameters of smoked sea bass during storage” was to study the effect of partial sodium replacement by potassium and packaging conditions on the physicochemical properties of smoked sea bass during cold storage. As the result, smoked fish exhibited significant differences in color and texture as compared to fresh fish; the type of packaging had an effect on the pH, WHC and texture; textural parameters decreased during storage; the pH of Na sample was initially higher than in Na: K samples, so that the type of salt did not affect the texture or other physicochemical parameters (Fuentes *et al*, 2012) [34].

Osheba, (2013) [77] study three technological attempts were applied to produce low sodium smoked herring fish with high quality. The first attempt was reducing time of salting (from 48 to 24hrs). The second attempt was using wet salting method with different concentration of brine solutions (8, 18 and 26% NaCl) instead of dry salting method. The third attempt was replacement of NaCl with some salt replacers at different level (20, 40 and 60%). Results suggested that salt replacers should be used until a level of 40% and salt replacers should be used at 15% NaCl and dry method for 24hr instead of 48hr to obtain low sodium smoked herring fish with high eating quality. The study of “effect of partial substitutes of NaCl on the cold-set gelation of grass carp myofibrillar protein mediated by microbial transglutaminase” were to investigate the changes and the relationship between structure and physiochemical properties of low sodium salt substitutes (KCl, $MgCl_2$ & $CaCl_2$) on grass carp myofibrillar protein gels mediated by microbial transglutaminase during cold-set gels. The results showed the gel properties of cold-set gels varied both with the low sodium salt types and incubation time and myofibrillar protein gels added with NaCl and KCl had significantly higher WHC than $MgCl_2$, $CaCl_2$ and control groups. The most recommended salt to replace NaCl is KCl (Aliño *et al*, 2010; Greiff *et al*, 2015) [2, 42]. It is being shown that both salts have equivalent antimicrobial properties against different pathogenic bacteria (Bidlas & Lambert, 2008; Pelroy *et al*, 1982) [7, 85].

3. Natural antioxidant from plant as a replacers for conventional antioxidants

Lipids are compounds of vital importance for proper human nutrition. In addition to providing energy to the biological process of body, lipids contain a large number of substances such as essential fatty acids or fat-soluble vitamins that can only provide by diet (Purriños *et al*, 2011) [89]. They influence the flavor and contribute to improving the tenderness and juiciness of meats (Amaral *et al*, 2018) [3]. Lipids are susceptible to degradation. Lipid oxidation is the main non-microbial cause of quality deterioration in meat and meat products (Min & Ahn, 2005; Lorenzo & Gómez, 2012) [71, 67]. Oxidation reaction reduces the nutritional value of meats due to the loss of essential fatty acids and vitamins. The first change observed results in a gradual reduction of sensory quality. These include changes in color, texture and appearance of rancid odor and flavor (Purriños *et al*, 2011) [89]. In addition, multiple toxic compounds are produced during lipid oxidation (Domínguez *et al*, 2019) [23]. The lipid oxidation is a very complex process, which includes multiple mechanisms that interact with each other. To explain it simply unsaturated fatty acids, react with

molecular oxygen via a free radical mechanism. As a result of these reactions, hydroperoxides are produced which is considered as the first oxidation products. Hydroperoxides are highly unstable, so they decompose rapidly resulting in a large of secondary compounds that include hydrocarbons, aldehydes, ketones, alcohols, esters and acids (Gallart-Jornet *et al*, 2007) [35]. Now a days, consumers demand more natural products, which limits the industry in their use of currently allowed synthetic antioxidants in foods, leaving manufacturers with few options (Gomes *et al*, 2021) [39].

Sea food is prone to lipid oxidation because of the high concentration in polyunsaturated fatty acids (PUFAs), in particular eicosapentaenoic acid (C20:5, n-3) and docosahexaenoic acid (C22:6, n-3). Moreover, the presence of heme pigments (myoglobin & hemoglobin) and trace amounts of metallic ions like iron and copper makes the fish more susceptible to lipid oxidation (Hsieh & Kinsella, 1989) [48]. Some technologies have been developed seeking to reduce lipid oxidation and to increase shelf life of these products, such as vacuum packaging, modified atmosphere, and use of antioxidants. Natural products of animal origin are currently receiving more attention, since the addition of substances such as butylated hydroxy toluene (BHT), butylated hydroxy anisole (BHA), and tert-butyl hydroxy quinone (TBHQ) in food products is strictly limited (Lima *et al*, 2013) [20]. The basic definition of antioxidant is that it is simply a chemical compound or a substance that inhibited oxidation (Ryan *et al*, 2021) [97]. A broader definition was introduced in 1995 by Halliwell & Gutteridge as, “Any substance that when present at low concentration compared to that of an oxidized substrate significantly delays or prevents oxidation of that substance. In other words, an antioxidant is a substance that slows the rate of an oxidation reduction (Ryan *et al*, 2021) [97].

Antioxidants act by inhibiting formation of free radicals or by interrupting propagation of the free radicals by one of several mechanisms (Valenzuela *et al*, 2017) [113]:

- Scavenging spp. that initiates peroxidation. Chelating metal ions such that they are unable to generate reactive spp. or decompose lipid peroxides.
- Quenching $\cdot O_2^{\cdot -}$, preventing the formation of peroxides.
- Breaking the auto oxidative chain reaction
- Reducing localized O_2 concentration.

Therefore, antioxidants through molecular mechanisms promote preservation of sensory and nutritional properties in food. Antioxidants can be synthetic or natural and can originate from different sources (Valenzuela *et al*, 2017) [113]. The most important types of antioxidants are summarized in Table 2:

Table 2: Types of antioxidants.

Group of compounds	Mechanisms of action	Example of Inhibitor
Antioxidants	Reaction with free radicals	Propyl galate, Tocopherol
Hydroperoxides deactivators	Reaction with hydroperoxides	Cysteine, Selenometathione
Synergists	Regeneration of an antioxidant	Ascorbyl palmitate
Singlet oxygen quenchers	Transformation of singlet oxygen into triplex oxygen	Carotenes
Chelating agent	Binding heavy metals into inactive complexes	Polyphosphates, citric acid.

*Source: Valenzuela *et al*, 2017 [113]

Some important antioxidants (Pokorny, 2007) [87]:

- Synthetic antioxidants: Butylated Hydroxy Toluene (BHT), Butylated Hydroxy Anisole (BHA), Di-tert-butyl hydroxy quinone (DBHQ), Propyl galate (PG), Octyl galate (OG), tert-dodecyl galate (DG).
- Natural antioxidants: Tocopherol, antioxidants from oil seeds (olive oil, sunflower seed oil, cotton seed oil, rape seed oil), antioxidants from cereals & grain legumes, compound from fruit & vegetables (ascorbic acid), extracts from herbs and spices.

- Other antioxidants: substance decomposing hydroperoxides, synergists, singlet oxygen quenchers, metal chelating agents.

The use of synthetic antioxidants (particularly BHT & BHA) has been linked to increased tumoral activity (Kahl & Kappus, 1993) ^[54], resulting in strict regulation of their use in foods; thus, research interest for new sources of natural antioxidants grew (Valenzuela *et al*, 2017) ^[113]. The antioxidants of natural origin are a solution to this problem (Valenzuela *et al*, 2016) ^[112]. Antioxidants mostly used in the industry are ascorbic acid and derivatives (vit.C), α -tocopherol and derivatives (vit.E), polyphenols and carotenoids (Valenzuela *et al*, 2017) ^[113].

In the fish processing industry, the extract is applied by dip treatment for chilled and frozen stored products. Combination of plant extract treatment with vacuum packaging or MAP is also proven to be an effective preservation method for the extension of the shelf life of fresh fish and fishery products.

4. Antioxidant from plant

Natural antioxidants occur in all higher plants and in all parts of the plant – wood, bark, pods, leaves, fruit, roots, flowers, pollen and seeds. These are usually phenolic or polyphenolic compounds. Typical compounds that possess antioxidant activity include tocopherol, flavonoids, cinnamic acid derivatives, phosphatides and polyfunctional organic acids (Pratt, 1992) ^[88]. 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.

Natural antioxidants in food may be from (Pratt, 1992) ^[88]:

- Endogenous compounds in one or more components of the food.
- Substances formed from natural sources.
- Food additives isolated from natural sources.

Some sources of natural antioxidants and component from plants are listed in Table 3 and Table 4:

Table 3: Some sources of natural antioxidants from plants

Algae	Oil seed
Amla	Olives
Citrus poly & peel	Osage orange
Cocoa powder or shell	Plant (extracts)
Heated products	Protein hydrolysate
Herbs & spices	Resin
Microbial products	Soy products
Oat flour	Tempeh

*Source: Pratt, 1992 ^[88]

Table 4: Some components of natural antioxidants from plants

Amino acid	Other organic acid
Ascorbic acid	Reductions
Carotenoids	Peptides
Flavonoids	Tannins
Melanoidin	Tocopherols

*Source: Pratt, 1992 ^[88]

Addition of plant extract to ice had enhanced the quality of fishes during storage (Quitral *et al*, 2009; Özyurt *et al*, 2012) ^[90, 79]. Polyphenols are the secondary plant metabolites containing one or more aromatic rings with several hydroxyl groups (Boudet, 2007) ^[9]. Polyphenolic compounds present in plants extract demonstrate potential

antioxidant properties due to their redox potential; that enable them to act as hydrogen donors, reducing agents, nascent oxygen quenchers & chelating metal ions in numerous food applications (Gramza *et al*, 2006) ^[41]. The active hydroxyl groups present in the molecular structure of polyphenols are active components that can interact with the free radicals to inhibit lipid oxidation (Mitsumoto *et al*, 2005) ^[72].

Polyphenols can be broadly divided into 4 categories: flavonoids, phenolic acids, hydroxy cinnamic acids & lignans (Maqsood *et al*, 2013) ^[70]. Flavonoids are further classified as Flavonol, flavonones, isoflavones, flavones, chalcones, and anthocyanins. Most common flavonoids include quercetin. Many of flavonoids & related compounds have strong antioxidant characteristics in lipid aqueous & lipid food system (Pratt, 1992) ^[88]. Use of natural preservation to increase the shelf life of chilled fish products is a promising technology since many herbs, plants, vegetables & fruits extract or powder have antioxidant & antimicrobial properties.

5. Antioxidant from seaweed

Sea weed is a term encompassing for macroscopic, multicellular or benthic marine algae. They attached to the bottom in relatively shallow coastal waters. They grow in deep sea areas of dept up to 180 m & also in estuaries. They are abundantly found in solid substrates & commonly presenting onto dept 30-40 m. In India about 620spp have been reported (Pal *et al*, 2014) ^[80]. Seaweed may be categorized into one of multi cell algae; they may be chlorophyceae (green algae), pheophyceae (brown algae) and rhodophyceae (red algae). Sea weeds are the source of producing chemical compounds like agar-agar, carrageenan and alginates. These chemical compounds also known as phytochemical and are mainly use for human consumption, animal feed, manure, *etc*. Bioactive compounds of seaweeds are fatty acids (PUFA), steroids (cholesterol), carotenoids, polysaccharide, dietary fibers, agar, carrageenan, alginate and phycocolloids (Pal *et al*, 2014) ^[80].

6. Fucoxanthin

Fucoxanthin, a specific carotenoid found in brown seaweed, has a unique structure including an allenic bond and a 5, 6-monoepoxide in the molecule. It is one of the most abundant carotenoids accounting for > 10% of estimated total natural production of carotenoids (Maeda *et al*, 2008). It is largely found in kelp (brown algae seaweeds) and microalgae (diatoms) and play a key role in algae photosynthesis (D'Orazio *et al*, 2012) ^[24]. Fucoxanthin isolated from different seaweed species such as *Myagropsis myagroides*, *Underia pinnatifida* & *Sargassum fulvellum* exhibited antioxidant effects (Woo *et al*, 2010) ^[125]. Sachindra *et al*. (2007) ^[99] assessed the antioxidant activities of fucoxanthin *in vitro*, where fucoxanthin exhibited antioxidant activities higher or similar to that of α -tocopherol.

7. Phlorotannins

Phlorotannins (1,3,5-trihydroxy benzene), is a group of polyphenolic compounds which are commonly present in the brown algae accounting for about 5-12% of dry mass (Venkatesan *et al*, 2019) ^[115]. The development of phlorotannin can occur through polymerization of phloroglucinol oligomers via acetate-malonate pathway (Li *et al*, 2011) ^[64]. A variety of low-, intermediate- and high-

molecular weight phlorotannins (molecular size up to 650kDa) have been reported from marine algae (Singh & Sidana, 2013) ^[103]. In algae, phlorotannins are known to exist in soluble or cell-wall bound forms. The soluble phlorotannins are stored in membrane bound vesicles called physodes. When physodes fuse with the cell walls, phlorotannins form a complex with alginic acid present in cell wall (Singh & Sidana, 2013) ^[103]. The pharmacological activities of phlorotannins include antioxidant, anti-HIV, anti-diabetic, anticancer, anti-inflammatory and enzyme inhibitory activities. Eckol, a phlorotannin first isolated as fibrinolytic component from *Ecklonia kurome*, is a classic example of pharmacological exploitation of a known compound (Singh & Sidana, 2013) ^[103].

8. Sulfated polysaccharide

Sulfated polysaccharide comprises a complex group of macromolecules with a wide range of important biological properties (Mourão, 2007) ^[73]. Marine algae are the most important source of non-animal sulfated polysaccharides (Li *et al*, 2008) ^[62]. Sulfated polysaccharides are found in varying amounts in three major divisions of marine algal groups, rhodophyta, phacophyta and chlorophyta (Fonseca *et al*, 2008) ^[33]. The general sulfated polysaccharides of phaeophyta are called fucans, which comprise families of polydisperse molecules based on sulfated-L-fucose. Heterofucans are also called fucoidans (Costa *et al*, 2010) ^[18].

9. Application of natural antioxidants to preserve fish products

9.1 Polyphenols

Tea polyphenols are gaining importance among the 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 one week during ice storage (Fan *et al*, 2008) ^[30]. Their study proved that dip treatment in 0.2% tea polyphenols has effectively inhibited adenosine triphosphate (ATP) degradation as well as lipid oxidation (Fan *et al*, 2008) ^[30]. Yi *et al*, (2011) ^[127] 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 Crocker as it has retained the myofibrillar functional properties significantly higher than that of control (Zhao *et al*, 2013) ^[6]. Rosemary (*Rosmarinus officinalis*) is a plant species 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 system (Zhang *et al*, 2010; Li *et al*, 2006) ^[129, 65]. 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) ^[111].

Kenar *et al*, (2010) ^[57] reported that dipping in ethanolic extracts of rosemary and sugar tea (*Salvia officinalis*) has extended the shelf life of vacuum-packed sardine fillets by 7days when stored at 3±1°C. Gao *et al*. (2014) ^[36] 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 (1.55 each, v/v) were found to retain quality characteristics of vacuum-packed rainbow trout (*Oncorhynchus mykiss*) during a refrigerated storage of over a period of 20 days (Pezeshk *et al*, 2011). Viji *et al*, (2015) ^[86, 118] 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. Zarei *et al*, (2015) ^[128] applied a combination of citrus and pomegranate peel extract and chitosan nanoparticles for shelf life extension of silver carp fillets.

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, and 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. Grape polyphenols/ extracts are known for inhibition of oxidation in several food systems. Pazos *et al*. (2005) ^[84] demonstrated that flavonols fractionated from grape pomace had potent inhibition against oxidation in frozen white muscle of Atlantic mackerel.

Studies on the use of plant extracts in smoked fish are limited compared to chilled and frozen fish products. Fasakin & Aberejo (2002) ^[31] demonstrated that pulverized plant materials obtained from *Piper guineense* & *Aframomum melegueta* could be used to deter egg hatchability and adult emergence of fish beetle, *Dermestes maculatus* in smoked catfish during storage. Application of plant extract 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. The results from all these studies confirm their potential to reduce synthetic additives to prevent oxidation and quality deterioration in food systems, especially fish and fish products.

9.2 Algae and seaweed

Several studies evaluated the effect of sea weeds in corporation on nutritional, sensorial and textural properties of seafood products (Roohinejad *et al*, 2017) ^[96]. The ability of different *in vitro* antioxidant assays to predict the efficiency of cod protein hydrolysate and brown seaweed (*F.vesiculosus*) ethyl acetate extract on lipid oxidation in hemoglobin fortified washed cod mince and iron containing cod liver oil emulsion was investigated (Jónsdóttir *et al*, 2016) ^[53]. Ortiz *et al*, (2014) ^[76] studied the effect of different seaweed extracts incorporation, as covering lipids, on lipid and sensory quality of canned Atlantic salmon (*Salmo salar*). The feasibility of using Nori and Hijiki seaweeds as natural additives, instead of synthetic preservatives to improve the shelf life and quality of minced tilapia has evaluated (Ribeiro *et al*, 2014) ^[95]. Wang *et al*. (2010) investigated the effects of seaweed (*F.vesiculosus*) extract and fractions towards haemoglobin catalyzed lipid oxidation in washed cod protein isolates during ice storage. The effect of using sea weed (*Euchema*) powder on the quality of fish cutlet was studied (Senthil *et al*, 2005) ^[100].

9.3 Antimicrobial preservatives from microorganisms and plants

Fish and fishery products are frequently contaminated with bacterial pathogen. Fishery products have been recognized as a major carrier of food-borne pathogens like *Salmonella* spp., *Staphylococcus aureus*, *Vibrio parahaemolyticus*, *Listeria monocytogenes* & *Escherichia coli* (Venugopal *et al*, 1999) ^[116]. The consumption of these infected fish and their products can result in mild to chronic illness (Ramachandran & Raymond, 2019) ^[92]. Among all, *Salmonella* is the main cause of seafood borne bacterial illness, followed by *Listeria monocytogenes*, which is commonly observed in seafood due to its presence as an environmental inhabitant in processing facilities (Norhana *et al*, 2010) ^[75]. Besides, *Vibrio* species are present in oysters and the most virulent species are *Vibrio vulnificans*. Moreover, the toxin produced by *C. botulinum*, a spore forming anaerobe, is a big concern because it is heat stable and highly toxic and can grow at temperature exceeding 3.3 °C. finally, *A. hydrophila* is commonly found in seafood and in fresh flowing stagnant & brackish water (Singh *et al*, 2016) ^[104].

Antimicrobial agents are compounds that, at low concentrations, suppress or inhibit the growth of microorganisms. Antimicrobial agents have been used widely as feed additives (Cromwell, 1991) ^[19]. An antimicrobial agent is a chemical preservative that can be incorporated into the food or in the packaging material to induce antimicrobial activity. Natural antimicrobials can be derived from plants, animals, algae and bacteria. They can be applied in different ways, such as dipping and spraying solutions, or they can also be incorporated into edible films and coatings, as carriers (Erkan *et al*, 2015) ^[29].

9.4 Essential oils

Essential oils (also known as volatile or ethereal oils) are complex mixtures of volatile compounds produced by living organisms or from plant materials like flowers, bud, seeds, leaves, wood, fruits, roots, twinges and barks (Vergis *et al*, 2015) ^[117]. The essential oils are proven to have anti-viral, anti-myotic, anti-parasitic, antioxidant and insecticidal properties. In addition to the antibacterial action, the phenolic components are responsible for the antibacterial properties of essential oils (Cosentino *et al*, 1999) ^[17]. The hydrophobicity of essential oils enables them to purate the lipid layer of bacterial cell membrane and mitochondrion, making the structures more permeable. This leads to leakage of ions and other cell contents (Lambert *et al*, 2001) ^[60]. The direct addition of essential oils to food leads to reduction in bacterial population immediately but may also alter sensory characters. Thus, the use of essential oils in film production may be an alternative method (Vergis *et al*, 2015) ^[117]. The antimicrobial activity of essential oils depends on the oxygen availability. The use of vacuum packaging with oregano oil has synergistic effect on the inhibition of *Listeria monocytogenes* and spoilage microorganisms (Tsigarida *et al*, 2000) ^[110].

Various essential oils provide safety from the most important food borne pathogenic bacteria like *Listeria*, *Salmonella*, *Aeromonas*, *Clostridium botulinum*, *Enterobacter*, *Staphylococci* and their toxins due to their lipophilic action (Vergis *et al*, 2015) ^[117]. It should be stressed that phenolic compounds such as thymol, carvacrol and eugenol are main group responsible for the preservative

effects of EOs (Burt, 2004; Jayasena & Jo, 2014) ^[11, 52]. Eos that have been commonly studied to extend shelf life of fish products are derived from thyme, rosemary, bay, marjoram, oregano, basil, garlic, sage, green tea, nettle, flaxseed, black cumin, clove, cinnamon, grape fruit, lemon and lemon grass (Kostaki *et al*, 2009; Sterniša *et al*, 2016) ^[59, 105]. It was reported that oregano EO is the most frequently used for applications as fish preservatives, followed by rosemary and thyme EOs (Patel, 2015) ^[83].

The study of “Efficiency of rosemary and basil essential oils on the shelf life extension of Atlantic mackerel (*Scomber scombtus*) fillets stored at 2 °C”, and the result indicated that rosemary and basil treatments were effective for inhibiting the formation of total volatile basic nitrogen (TVB-N) and lipid oxidation products during storage (Karoui & Hassoun, 2017) ^[56]. The study of “Evaluation of effects of nanoemulsion based on herb essential oils (rosemary, laurel, thyme and sage) on sensory, chemical and microbiological quality of rainbow trout (*Oncorhynchus mykiss*) fillets during ice storage”, and the result showed that the lowest bacterial numbers were obtained from rosemary followed by thyme group. Among the essential oils, it can be concluded that rosemary and thyme can be recommended for nanoemulsion as a preservative for fish fillets (Ozogul *et al*, 2017) ^[78].

9.5 Plant extracts

Although the polyphenols present in plant extracts are mainly known by their antioxidant activity, some of them also present antimicrobial properties and have been used in fish preservation. The antimicrobial activity of phenolic extracts has been attributed to cell membrane disruption (Kanatt *et al*, 2010) ^[55]. The effects of a tea polyphenols dip treatment on quality changes of silver carp (*Hypophthalmichthys molitrix*) during iced storage; the results indicated that the effect of tea polyphenol dip treatment on the fish sample was to enable the good quality characteristics to be retained for longer and to extend the shelf life during the iced storage (Fan *et al*, 2008) ^[30]. The effect of edible coating containing pomegranate peel extract (PPE) on quality and shelf life of silver carp fillet during refrigerated storage; the result showed that addition of PPE considerably delayed lipid oxidation in silver carp fish fillet compared to control samples (Tarkhasi, 2016) ^[108]. The effects of turmeric, shallot extracts, and their combination on quality characteristics of vacuum-packed rainbow trout stored at 4±1°C; the results indicated that the effect of the T, Sh and T+Sh on the fish samples were to enable the good quality characteristics to extend the shelf life during the refrigerated storage (Pezeshk *et al*, 2011) ^[86].

9.6 Chitosan

Chitosan is the principle derivative of chitin. Chitosan is a linear polymer of $\alpha(1\rightarrow4)$ -linked 2 amino-2-deoxy- β -D-glucopyranose and easily derived by *N*-deacetylation. The production of chitosan from crustacean shells obtained as a food industry waste is economically feasible, especially if it includes the recovery of carotenoids. The shells contain considerable quantities of asthaxanthin (a carotenoids that has so far not been synthesized, and which is marketed as a fish food additive in aquaculture, especially for salmon) (Dutta *et al*, 2004) ^[27]. The properties of chitosan are listed in Table 6:

Table 5: Studies of effect of essential oils on different fish and fishery products.

Fish & fishery products	Preservations	Mode of applications (concentration)	Additional preservation	Effects	References
Atlantic mackerel (<i>Scomber scombrus</i>) fillet	Rosemary and basil	Immersion (1%)	Stored at 2 °C	pH, TVB-N, PV, TBA ↓ shelf life (2-5 days) ↑	(Karoui & Hassoun, 2017) [56]
Grass carp (<i>Ctenopharyngodon idellus</i>) fillet	Oregano, thyme and star anise	Immersion (0.1%)	Stored at 4 °C	TVB-N, TBA, K value, Hx, Putrescine, TVC, HSPB, Pseudomonas, Aeromonas ↓ Sensory score ↑ Shelf-life (2 days) ↑	(Huang <i>et al</i> , 2018) [49]
Rainbow trout fillet	Mentha piperita, Artemisia dracunculus and Zataria multiflora	Coating (0.2%)	Sodium alginate + chitosan + stored at 4 °C	TVB-N, TBA, TVC, PTBC, LAB ↓	(Raeisi <i>et al</i> , 2020) [91]
Bream (<i>Megalobrama ambycephala</i>) fillet	Clove	Coating (1%, 1.5%)	Pectin + stored at 4 °C	pH, TVB-N, PV, TBA, TVC, PBC, HSPB, LAB, Pseudomonas, Enterobacteriaceae ↓ Sensory score ↑ Shelf-life (at least 6 days) ↑	(Nisar <i>et al</i> , 2019) [74]
Bluefin tuna (<i>Thunnus thynnus</i>) fillet	Clove	Film wrapping	Soy protein + stored at 2 °C	TBV-N, TBA, TVC, MBC, HSPB, Pseudomonas spp., Enterobacteriaceae ↓ pH ↑	(Echeverría <i>et al</i> , 2019)
Cobia (<i>Rachycentron canadum</i>) fish steak	Ginger	Film wrapping (0.3%)	Chitosan + stored at 2 °C	pH, TVB-N, TBA, MBC, PTBC, HSPB, Pseudomonas, Brochothrix thermosphacta ↓ Sensory score ↑ Shelf-life (15 days) ↑	(Remya i, 2017) [93]

Table 6: Properties of chitosan

Chemical Properties	Biological Properties
Linear polymine	Biocompatible
Reactive amino groups	Binds to mammalian and microbial cells aggressively
Reactive hydroxyl groups available	Regenerative effect on connective gum tissue
Chelates many transitional metal ions	Accelerated the formation of osteoblast responsible for bone formation
	Hemostatic
	Fungistatic
	Spermicidal
	Antitumor
	Anticholesteremic
	Accelerates bone formation
	Central nervous system depressant
	Immunoadjuvant

*Source: Dutta *et al*, 2004 [27]

Chitosan has been used as part of films and coatings, with or without other active ingredients, to control bacterial growth and extend the shelf life of fish and seafood products. For example in Catfish (*Ictalurus punctatus*) fillet (Bonilla *et al*, 2018); grass carp fillets; pacific white shrimp (*Litopenaeus vannamei*) (Wang *et al*, 2015); rainbow trout (Rezaeifar *et al*, 2020); large yellow croaker (Wu *et al*, 2018); snakehead fish fillet (Cao *et al*, 2020); turbot (*Scophthalmus maximus*) fillets (Li *et al*, 2020); Antarctic krill (*Euphausia superba*) (Liu *et al*, 2020) [8, 121, 94, 13, 63, 66].

9.7 Probiotics

Probiotics are living microbial food ingredients which have a beneficial effect on human health. Probiotics were originally used to influence both animal and human health through intestinal micro biota alternations (Isolauri *et al*, 2004) [51]. Lactic Acid Bacteria (LAB) are among the most important probiotic microorganisms typically associated with the human gastrointestinal tract. LABs are gram +ve, nonsporing, catalase- negative organisms that are aero tolerant, fastidious, acid-tolerant, and strictly fermentative (Holzapfel *et al*, 2001) [47]. Traditional fermented fish

products could be an alternative and readily available source for LAB starter inoculums with significant functional properties including improved technological and probiotic characteristics (Gupta *et al*, 2021) ^[43].

LABs include lactobacilli, bifidobacteria, non-pathogenic *E. coli*, bacilli and yeasts such as *Saccharomyces boulardii*. LABs produce bacteriocins, peptides, and proteins with antibacterial activity (Valenzuela *et al*, 2017) ^[113]. The production of bacteriocins by LAB is very significant for applications in food systems. Among numerous bacteriocins so far characterized, nisin is best defined, and only purified

bacteriocin preparation approved for use in food products (Ghanbari *et al*, 2013) ^[38].

Particularly, the presence and growth of *L. monocytogenes* is of major concern in case of seafood. *L. monocytogenes* is a frequent contaminant in the raw material: it survives the salting and cold-smoking (< 30 °C) process and it able to grow in the final product at chilling temperature. In this regard, the use of lactic acid bacteria isolated from seafood and/or their bacteriocins have proved successful in preventing to delaying the growth of this pathogen under conditions mimicking those existing in lightly preserved fish products (Ghanbari *et al*, 2013) ^[38].

10. Summary

Ways of reformulation	Application in fish and fish products	Benefits
Reduce salt content	By substitute of partial substitute of NaCl	Reduce Na content and also increase the shelf life of the fish and fish products
Use of natural antioxidant	By the use of plant and seaweed antioxidant	Prevents or delays the oxidation of the substance and increase the shelf life of fish and fish products
Use of antimicrobial agents	By the use of essential oils, plant extracts, chitosan and probiotics	Suppress or inhibit the growth of microorganisms and induce antimicrobial activity of the fish & fish products.

11. Conclusion

Natural ingredients are a rich source of active components and have been studied in order to compensate for salt reduction, reduce lipid oxidation, and inhibit bacterial growth, at least in laboratory and small industrial scale. A large concentration of a certain ingredients can produce changes in the color, texture, taste, and flavor of the fish, affecting the quality of the final product. Besides, the majority of these studies focus on only one of this phenomenon, missing the fact that many of the components may have multiple activities. In addition, only a few studies study the synergies or antagonistic effects among different ingredient and with the food matrix. Industrial-scale validation must be performing that includes consumer validation in terms of food safety, quality perception, and organoleptic approval. All final ingredients must fulfill specific regulation in terms of safety, stability, labeling, and information to the agencies and to the consumers.

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