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Antioxidant effect of fruit peel extracts on fish steaks during refrigerated storage

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Abstract
Fish have rich source of essential nutrients required for supplementing both infant and adult diets. Due to high content of polyunsaturated fatty acid fish flesh and fish oil is beneficial in reducing the serum cholesterol. The protein content of fish is also important when considering quality and texture of the fish meat. Fish meat is also a rich source of minerals and the most abundant micro-elements are Zinc (Zn), Iron (Fe) and Copper (Cu). Due to high content of polyunsaturated fatty acid, fish meat is highly prone to oxidative rancidity resulting spoilage, the oxidation of polyunsaturated fatty acids is catalyzed by heat, light, and enzymes, and leads to the formation of peroxides, aldehydes, ketones, and free radicals. Fish lipid oxidation is one of the major problems in fish processing, mainly through the loss of nutritional qualities and the unpleasant odour. To extend the shelf –life of fish meat during storage condition as well as ambient temperature, used commercially some synthetic chemical such as Butylated hydroxyanisole (BHA), Butylated hydroxytoluene (BHT). Although these synthetic chemicals inhibit oxidation of lipid present in fish meat and extend shelf life of product effectively. But many research prove that artificial antioxidants are harmful to the lab animals and they cause disease that lead to liver damage cytotoxicity and carcinogenesis. To overcome this problem natural antioxidants may be used to arrest oxidation without any harmful effects. Fresh fruits and vegetables contain ascorbic acid as an antioxidants components, vegetable oils consists of tocopherols, tocotrienols as antioxidants components. Tea, coffee, soy fruit, olive oil, chocolate, cinnamon, oregano consists of resveratrol, flavonoids and antioxidants components. Fruits, vegetables and eggs consists of lycopenes, carotene. Pomegranate (*Punica granutum*), Banana (*Musa paradisiacal*) and pineapple (*Ananas comosus*) peel, etc., have adequate antioxidative activity. Furthermore, these fruits peels to food product did not show any adverse effect on food quality.

Keywords: synthetic antioxidant, health hazards, natural antioxidants, pomegranate (*Punica granutum*), banana (*Musa paradisiacal*), pineapple (*Ananas comosus*) peel

Introduction
Fish have rich source of essential nutrients required for supplementing both infant and adult diets (Abdullahi *et al*., 2001) [1]. Evidences suggest that due to high content of polyunsaturated fatty acid fish flesh and fish oil is beneficial in reducing the serum cholesterol (Stansby, 1985) [51]. The protein content of fish is also important when considering quality and texture of the fish meat (Majid *et al*., 2011) [34]. Fish meat is also a rich source of minerals and the most abundant micro-elements are Zinc (Zn), Iron (Fe) and Copper (Cu) (Saadettin *et al*., 1999) [45]. Fish foods have recently received more attention from consumers due to their positive benefits on human health and nutrition. Recent studies have clearly shown the importance of n-3 and n-6 fatty acids for human health and nutrition. Fish food is the best source of dietary supply of n-3 fatty acids, including Eicosapentaenoic acid (EPA) and Docosahexaenoic acid (DHA). It has been suggested that consuming EPA and DHA may reduce the risk of mortality from cardiovascular disease in people who have already experienced a cardiac event. Due to its high content of polyunsaturated fatty acids, including EPA and DHA, fish oil is highly susceptible to oxidative spoilage. This causes rancidity of the product, which most often results in the deterioration of color, texture and flavor and even the nutritive value of the food.

Spoilage of fish
Fish freshness is the most important and fundamental single criterion for judging the quality of fish and fishery products. The loss of quality of fish depends on many factors, including the fish species, handling and storage conditions (Venugopal and Shahidi, 1996) [53]. Fresh fish is susceptible to spoilage caused by both microbiological and chemical reactions. Lipid deterioration easily takes place and limits the shelf-life of oily fish during storage (McDonald and H. O. Hultin., 1987) [35].

Both hydrolytic and oxidative rancidities in fish muscle are associated with quality deterioration (Pacheco-Aguilar *et al*., 2000) [39]. Time passed after catch and the temperature ''history" of fish is considered to be the key factor determining the final quality characteristics of a fish product (Olafsdottire *et al*., 2004) [37]. Spoilage is a metabolic process that causes food to be undesirable and unacceptable for human consumption due to changes in sensory and nutritional characteristics (Doyle, 2007) [15]. Fish deteriorate rapidly after death due to the effect of a wide variety of biochemical and microbial degradation mechanisms that can be summarized as endogenous enzyme activity, microbial development, lipid oxidation and browning and non enzymetic browning. These changes directly affect the quality and self-life of fish (Whittle *et al*., 1990; Olafsdottir *et al*., 1997) [54, 36].

Deterioration of fish normally includes four stages: rigor mortis, resolution of rigor, autolysis (loss of freshness) and bacterial spoilage. These stages occur faster or slower depending on the species, the physiological condition of fish, microbial contamination and temperature. Fish decomposition is a progressive proteolysis of the muscle tissue brought about primarily by the action of microorganisms and, to a lesser extent, by autolytic enzymes. Because the changes during the decomposition are known to be very complex, a single chemical index may not be a reliable indicator for a particular sample of fish (Castillo-Yanez *et al*., 2007) [10]. Deterioration of fish, either marine or freshwater, occurs mainly as a result of enzymatic and microbial activities, which lead to loss of quality and spoilage (Arashisar *et al*., 2004) [4]. Spoilage is the result of whole series of complicated deteriorative changes brought about in dead fish tissue by its own enzyme, by bacteria and by chemical action (Shewan, 1976). The early reaction of spoilage is autolytic and bacterial enzymes become progressively the more active in the later stages. After death of fish, the oxygen supply in the tissue ceases due to disruption of the circulatory system. In short time of post-mortem, the mitochondrial system ceases to function. Adenosine triphosphate (ATP) is gradually depleted through the action of various ATPase. After residual supplies of creatine phosphate have been depleted, anaerobic glycolysis continue to regenerate some ATP with the end product, lactate accumulation (Foegeding *et al*., 1996) [18]. Cakli *et al*., (2007) [9] reported that the high levels of moisture, free amino acids and polyunsaturated fatty acids, and nutrient content, in addition to high amount of naturally present autolytic enzymes and high pH render fish an easily perishable product, often going bad within a short period under refrigerated conditions.

Fish meat is probably the most susceptible to oxidation mainly because of its high level of unsaturated fatty acids, and its shelf life is limited by enzymatic and microbial activity (Ucak *et al*., 2011) [12]. The oxidation of polyunsaturated fatty acids is catalyzed by heat, light, and enzymes, and leads to the formation of peroxides, aldehydes, ketones, and free radicals. Fish lipid oxidation is one of the major problems in fish processing, mainly through the loss of nutritional qualities and the unpleasant odour. Live fish is normally considered to be sterile, but microorganisms are found on all the outer surfaces (skin and gills) and in the alimentary tract of live and newly caught fish in varying numbers. When fish dies, its entire body resistance mechanisms breakdown, giving way to microorganisms or the enzymes they secrete to invade or diffuse into the flesh where they react with the complex mixture of natural substances present. During storage a characteristic flora develops, but only a part of this flora, known as the specific spoilage organisms (SSO), contribute to spoilage.

Post-mortem quality management

Flesh quality is usually defined in terms of appearance, taste, smell, firmness, juiciness, freshness and process characteristics. Deterioration of fish flesh results from the complex combination of physical, chemical, biochemical and microbial processes. However, the first changes occurring in post mortem fish muscle are due to endogenous enzymes promoting proteolysis of muscle proteins and connective tissue as well as fat hydrolysis. Loss of freshness is due to a complex combination of biochemical, chemical and physical processes, and is followed by muscle spoilage due to microbiological contamination. Quality attributes of fish flesh, including food safety, organoleptic features, nutritional quality and aptitude to industrial transformation, influence consumption and acceptability of fish as food. Fish sensorial changes and texture properties are closely linked to freshness. Along with ante mortem muscle biochemistry, post mortem biochemical processes are directly linked to final quality attributes. Post mortem degradation of fish muscle proceeds through rigor-mortis, resolution of rigor mortis, autolysis and various other chemical/microbial changes. Most of the past studies on freshness of fish were based on the view that freshness of fish is mainly decreased by bacterial action. Lipid oxidation and microbial growth during storage can be reduced by applying antioxidant and antimicrobial agents to the meat products, leading to a retardation of spoilage, extension of shelf-life, and maintenance of quality and safety (Devatkal and Naveena, 2010) [14].

The shelf life of the fish and fish products can be extended by low temperature preservation like icing, chilling, and frozen storage where the chemical, enzymatic activity and metabolic activity of microorganism are reduced which causes deterioration. The most common method of preservation used for extension of shelf life of fish is freezing and frozen storage at a temperature of -20ºC. Freezing though increases the shelf life of the product, still alters the physical structure of the fish and maintaining of such low temperatures is very expensive and consequently it is not used for routine foods storage (Madigan *et al*., 2009) [33].

To improve the shelf life of the products during refrigerated storage different chemical preservatives are incorporated. But due serious limitations posed by highly persistent chemicals as fish preservatives during storage has elicited interest in seeking alternative methods such as use of natural products derived from plant origin which contain phenolic phytochemicals and flavonoids (Demo *et al*., 2001; Kahkonen *et al*., 1999; Leong and Shui, 2002) [13, 28, 30]. Sarkardei and Howel (2008) [46] reported that natural antioxidants may provide nutritional and therapeutic effects.

Preservation by natural antioxidants

The Natural Products are an important concern for human health and welfare. Natural products are economically beneficial, safe and had promising effect. The natural sources such as plants, fruits and vegetables are rich in bioactive compounds and are valuable products for pharmaceutical industry. Synthetic and natural antioxidants have been successfully used to block or delay the oxidation process in meats. Hirose *et al*. (1998) [24] indicated that use of synthetic antioxidants need to be restricted because of their health risks and toxicity. Juntachote *et al*. (2006) [27] reported that the demand for natural antioxidants has recently increased

because of the toxicity and carcinogenicity of synthetic antioxidants. Herbs of the Lamiaceae family, mainly oregano (*Origanum vulgare L*.), rosemary (*Rosmarinus officinalis L*.) and sage (*Salvia officinalis L*.) have been reported as having significant antioxidant capacity (Shan *et al*., 2005; Wojdylo *et al*., 2007) [48, 55].

Rosemary extracts have a potent antioxidant activity and are widely used in the food industry. The antioxidant activity of rosemary extracts has been associated with the presence of several phenolic diterpenes such as carnosic acid, carnosol, rosmanol, rosmariquinone and rosmaridiphenol, which break free radical chain reactions by hydrogen donation. Natural antioxidants of tea polyphenols, which has protective effect on active oxygen radicals, are extracted by using water, ethanol, methanol and acetone. The spices such as clove, cinnamon, black pepper, ginger, garlic and cardamom extracts were found to contain some active antimicrobial, antioxidant and antimycotic properties (Rajkumar and Berwal, 2003) [40]. Herbs and spices have been used in maintaining and enhancing human beauty since time immemorial. For example - turmeric is used for skin care. The anti-ageing and cosmeceuticals is gaining importance in the beauty, health and wellness sector. Spices like turmeric, cardamom, clove, aniseed, coriander, basil, saffron, garlic and sage are used mainly in beauty and cosmetic industry.

Application of turmeric extract cream (0.5%) regulates sebum in human skin, person with excessive oilskin or suffering from acne will have great benefit from this property. Saffron (*Crocus sativus*) as complexion promoter in skin care and reported that 0.3% of saffron used in cream and lotion will be giving brighter and shiny skin, this effect is mainly due the crocin and cicrocrocin content of saffron, this regulates the melanin biosynthesis in skin. A part from many beneficial effects herbs and spices have been found to reduce inflammation, protect against infection, helps to detoxify the liver and cleanse the lungs and other organs and also protect from cell damage that can lead to rheumatoid arthritis, osteoporosis, heart disease and other degenerative diseases. Some common herbs such as cilantro, basil, thyme, onion, ginger, turmeric, garlic etc., offer great health benefits by virtue of their powerful phytochemical and antioxidant properties. Even though there is limited literature on the health effects of herbs and spices or extracts of these, the number of studies investigating the possible health effects of phytochemicals originating from herbs and spices is at large. Most of the products categorized as herbal and traditional plant medicines are also based on antioxidant-rich dietary plants or isolated phytochemicals.

The natural antioxidants are always good to consume in nature. Fresh fruits and vegetables contain ascorbic acid as an antioxidants components, vegetable oils consists of tocopherols, tocotrienols as antioxidants components. Tea, coffee, soy fruit, olive oil, chocolate, cinnamon, oregano consists of resveratrol, flavonoids and antioxidants components. Fruits, vegetables and eggs consists of lycopenes, carotene. On the other hand, many research prove that artificial antioxidants such as, Butylatedhydroxyanisole (BHA), Butylatedhydroxytoluene (BHT), are harmful to the lab animals and they cause disease that lead to liver damage cytotoxicity and carcinogenesis.

Natural antioxidants from fruits

Natural antioxidants, particularly in fruit and vegetables have gained increasing interest among consumers and researchers because epidemiological studies have indicated that frequent consumption of natural antioxidants is associated with a lower risk of cardiovascular disease and cancer (Renaud *et al*., 1998) [43]. The defensive effects of natural antioxidants in fruit and vegetables are related to three major groups; vitamin, phenolics and carotenoids. Ascorbic acid and phenolics are known as hydrophilic antioxidants, while carotenoids are known as lipophilic antioxidants (Halliwell, 1996) [23]. Fruits and Fruit juices are important dietary components which are rich in antioxidants. Synthetic antioxidants will have potential health risks. Hence an increasing attention should be paid to identify natural and possibly more economic and effective natural antioxidants.

In the recent years, more attention has been paid to the antioxidants contained in fruits. Guo *et al*. (2003) [21] claimed that high fruit intakes were associated with reduced mortality and morbidity of cardiovascular disease and some types of cancer. Fruits are diverse in antioxidant composition and antioxidant activity and those with high antioxidant activity generally contain more antioxidants. Interestingly, the peel fractions of some fruits possess higher antioxidant activity than the pulp fractions. Study by Li *et al*. (2006) [32] reported that pomegranate peel exhibited higher antioxidant activity compared to its pulp. The peel fractions of fruits may potentially contain more antioxidants quantitatively or qualitatively than the pulp fractions.

In fruit processing industry and in fruit shops the fruit peels are thrown or dumped as waste, but the real fact is that the peels are having better biological activities than other fractions. Fruits and vegetables wastes and their by-products formed during industrial processing possess a serious problem as they exert harmful impact on environment. So they need to be managed and utilized (Duda-Chodak and Tarko, 2007) [16]. Sawalha *et al*. (2009) [47] reported that Citrus fruits have peculiar fragrance partly due to flavonoids and limonoids present in the peel and these fruits are good sources of vitamin C and flavonoids. Many studies have reported the antioxidant and antibacterial effect of juice and edible parts of oranges of different origin and from different varieties (Rapisarda *et al*., 1999; Farag *et al*., 2009) [41, 17].

Fruit peel extracts from oranges were found to have a good total radical anti-oxidative potential (Gorinstein *et al*., 2001; Shimon *et al*., 2012) [20]. High content of phenolic compounds and antioxidant activity of apple peels were found to have valuable source of antioxidants and can impart health benefits when consumed (Wolfe *et al*., 2003) [56]. Gorinstein *et al*., (2001) [20] found that the total phenolic compounds in the peels of lemons, oranges, and grapefruits were 15% higher than that of the pulp of these fruits. Peels from apples, peaches, pears as well as yellow and white flesh nectarines were found to contain twice the amount of total phenolic compounds as that contained in fruit pulp. Apple peels were found to contain up to 3300 mg/100 g of dry weight of phenolic compounds. Total phenolic compounds of seeds of several fruits, such as mangos, longans, avocados, and jackfruits, were higher than that of the edible product, Grape seeds and skins, the byproducts of grape juice and white wine production, are also sources of several phenolic compounds, particularly mono, oligo, and polymeric proanthocyanidins.

Bioactive compounds in pomegranate (*Punica granatum*)

Pomegranate is one of the important dietary sources of antioxidant and phenolics (Ozgen *et al*., 2008) [38]. Pomegranate peel is recognized for its many health promoting properties (Chidambara *et al*., 2004) [11] and antimicrobial activity (Braga *et al*., 2005) [6]. Presence of substantial

quantities of phenolic compounds such as ellagictannins, ellagic acids and gallic acids has been attributed to the antioxidant potential of pomegranate peel (Yasoubi *et al*., 2007; Afaf-haniem *et al*., 2010; Ibrahim, 2010) [58, 2, 26]. The peel of the pomegranate, which is about 50% of the total weight, has been used extensively in the folk medicine of many cultures (Reddy *et al*., 2007) [42]. Peels contain 249.4 mg/g of phenolic compounds as compared to only 24.4 mg/g phenolic compounds found in the pulp of pomegranates several studies have shown that this by product is an important source of bioactive compounds such as phenolic compounds, which are secondary plant metabolites and possess anti-inflammatory, anti-atherosclerotic, antitumor, anti-mutagenic, anti-carcinogenic, antibacterial, or antiviral activities, its use remained very limited and traditional (Cai *et al*., 2004; Li *et al*., 2006) [8, 31].

Bioactive compounds in banana (*Musa paradisiacal*)

Banana a tropical plant may protect itself from the oxidative stress caused by strong sunshine and high temperature by producing large amounts of antioxidant. Banana should be considered to be a good source of natural antioxidant for foods and functional food source against cancer and heart disease. Banana (*Musa paradisiacal*) is known to contain various antioxidants compounds such as gallocatechin (Someya *et al*., 2002) [50] and dopamine (Kanazawa and Sakakibara, 2000) [29]. Banana peel is rich in dietary fiber, proteins, essential amino acids, polyunsaturated fatty acids, potassium and B vitamins (Gonzalez-Montelongo *et al*., 2010) [19]. Antifungal and antibiotic principles are found in the peel and pulp of fully ripe bananas (Brooks, 2008) [7].

Ripe bananas fluoresce when exposed to ultraviolet light. The study suggested that this allows animals which can see light in the ultraviolet spectrum (tetrachromats and pentachromats) to more easily detect ripened bananas (Ashraf *et al*., 2010) [5]. This property is attributed to the degradation of chlorophyll leading to the accumulation of a fluorescent product in the skin of the fruit. The chlorophyll breakdown product is stabilized by a propionate ester group (Anhwange *et al*., 2009) [3]. Banana plant leaves also fluoresce in the same way (Ashraf *et al*., 2010) [5]. Green bananas do not fluoresce. Different studies have been done on the various parts of banana plant in which performed the inhibitory effect towards the food borne pathogens, hence banana plant should be considered to be a potential natural source of antimicrobial as well as antioxidant agent. Someya *et al*., (2002) [50] reported that edible pulp of bananas (*Musa paradisiaca*) contains 232 mg/100 g of dry weight. Phenolic compounds, this amount is about 25% of that present in the peel.

Bioactive compounds in pineapple (*Ananas comosus, Bromelaceae*)

Pineapple (*Ananas comosus, Bromelaceae*) is one of the most consumed tropical fruits and its consumption has been related to several beneficial properties such as antioxidant (Hossain and Rahman, 2011) [25], anti-inflammatory (Hale, 2005) [22] and anti-diabetic activities (Xie, 2006; Riya, 2013) [57, 44]. Correia *et al*. (2004) [12] established a relationship between antioxidant activity, beta glucosidase and total phenolic content in pineapple peel/ soy flour extract. Pineapple fruit is considered a highly nutritious fruit because it contains a high level of vitamin C, a natural antioxidant which may inhibit the development of major clinical conditions including heart disease and certain cancers. The fruit also contains phenolic compounds and β-carotene, which constitute natural sources of antioxidants. Pineapple fruits are an excellent source of vitamins and minerals. One healthy ripe pineapple fruit can supply of about 16.2% of daily requirement for vitamin C. Several physiochemical parameters like starch, reducing sugar, non-reducing sugar, total sugar, protein, ascorbic acid are present in juice and waste.

Conclusion

Fish steaks are most used to prepare fish curry on daily feed routine in many states. Fish is an important source of high biological value protein, essential amino acids and highly unsaturated fatty acid and rich in many micro- nutrients. Unsaturated fatty acid are highly susceptible to oxidation. To protect from oxidation fish are stored at low temperature -20°C along with synthetic antioxidants, many changes occurs, responsible for adverse change in nutritional quality of fish. Synthetic antioxidants are found by many researcher with many adverse effects on health. Natural antioxidant are used to overcome these problem of oxidation without any health hazard. Many studies used citrus fruits extract as natural antioxidants and found significant results. Although many study available on natural antioxidants but there is less studies found to use of peel of fruits. Pomegranate (*Punica granutum*), Banana (*Musa paradisiacal*) and pineapple (*Ananas comosus*) peel, etc., have adequate antioxidative activity and antimicrobial activity, these properties of these fruits peel would be definitely arrest antioxidation of fish at refrigerated storage simultaneously microbial contamination. Additionally, these fruits peels to food product did not show any adverse effect on food quality.

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