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Review Article
Use of Natural Antioxidants in Muscle Foods and their Benefits in Human Health: An Overview

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Abstract
Meat and meat products, especially fish and poultry is more prone to oxidation. Lipid oxidation in meat and fish-products leads to rancidity and off-flavor and development of many harmful substances. Antioxidants provide protection by neutralizing free radicals, which are toxic by-products of natural cell metabolism. Synthetic antioxidants have been successfully used to block or delay the oxidation process in meat and fish. To prevent or delay oxidation reactions, several endogenous antioxidant systems are found in muscle tissue. Amines, peptides and amino acids are known to have significant antioxidant properties. Due to the potential health hazards of synthetic antioxidants, natural products, especially natural antioxidants have been intensively examined. Use of natural antioxidants is emerging as an effective methodology for controlling rancidity and limiting its deleterious consequences. Thus, most of the recent study has been directed towards identification of novel antioxidants from natural sources, particularly of plant origin. This study suggested about the endogenous antioxidants in fish meat, the potential of natural antioxidants and their beneficial role for human health.

Key words: Muscle foods, poultry, fish, lipid oxidation, rancidity, synthetic antioxidant, natural antioxidants, health benefits

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INTRODUCTION

Presently, consumers are very concerned about their diet and the food they eat and attention has been diverted toward processed meat products that are lean, low fat and high in protein content[1]. Different reformulation strategies are being carried out to confer meat as functional food by modifying its lipid and fatty acid content and/or by incorporating a series of functional ingredients like fiber, vegetable proteins, phytochemicals, prebiotics and other natural antioxidants[2]. Lipid oxidation is a highly complex process in which polyunsaturated fatty acids after reacting with molecular oxygen via a free radical chain mechanism form fatty acyl hydroperoxides, which are referred to as either simply peroxides or primary products of oxidation[3]. This primary auto-oxidation is followed by a series of secondary reactions thereby causing degradation of lipids and development of oxidative rancidity[4]. Phytochemicals from plants in meat based products will be an interesting area for the meat industry to explore as these functional extracts with antioxidative and antimicrobial properties are able to replace the current usage of synthetic food additives in the development and improvement of healthy food products[5].

All meat and fish products are prone to oxidation. Amongst meat products, poultry meat is more prone to the development of oxidative rancidity compared to red meat. This is due to the higher content of phospholipids in poultry meat. During lipid oxidation poly-unsaturated fatty acids are degraded to volatile short-chain oxidation products, which is lead to off-odour and off-flavour formation[6]. Lipid oxidation in meat and fish-products leads to rancidity and off-flavor and development of many different substances some of which have even adverse effects to human health[7]. The addition of antioxidants is therefore necessary to increase storage stability, sensorial quality and nutritional value of muscle food products. Beside the traditionally used antioxidants in meat and fish a wide variety of herbs, spices and fruits with antioxidative capacity are more and more used as additives. In addition to their antioxidative capacity, many of these natural substances have positive effects in the human body and health benefits are therefore, highly appreciated as food additives.

OXIDATION IN MUSCLE FOODS

Oxidation of muscle lipids, specifically unsaturated fatty acids in triacyl-glycerols, phospholipids and cholesterol is a critical concern. The generation of peroxides, Reactive Oxygen Species (ROS) and secondary oxidation products has implications for flavour, colour and loss of myofibrillar protein functionality. All oxidative reactions generally result in compromised quality and an undesirable sensory experience for the consumer[6]. In general, oxidative susceptibility is directly proportional to the degree of unsaturation in the constituentfatty acids.

Lipid oxidation is described as an oxygen-dependent, oxidative deterioration of saturated and unsaturated fatty acids. This modification of fatty acids is principally carried out by an autocatalytic mechanism of free radicals, called auto-oxidation and consisting of 3 phases: Initiation, propagation and termination. Lipids and their derivative fatty acids are present in muscles as structural components of muscle membranes, as storage droplets of tri-acylglycerol between muscle fibers and as adipose tissue. The form and nature of these fatty acids decide color stability, drip loss and the development of oxidative rancidity, which ultimately decide the sensory and nutritional quality of meat products[7].

Lipid oxidation is one of the most important changes that can affect the fish muscle, which has high proportion of unsaturated lipids in the tissues and large amounts of heme pigments and metallic traces[8]. Oxidation of fish lipid leads to the formation of volatile compounds associated with rancidity, which significantly reduces the shelf-life of fish products, most especially during storage period[9]. Furthermore, the reaction between lipid oxidation products and proteins, amino acids or vitamins can alter the texture of the fish and reduce its nutritional value through the subsequent loss of essential amino acids. The high proportion of long chain n-3 poly-unsaturated fatty acids (PUFA) found in fish is a crucial factor in the muscle's high vulnerability to oxidation. Fish contain significantly higher levels of poly-unsaturated fattyacids, particularly eicosa-pentaenoic (20:5 n-3) and docosa-hexaenoic (22:6 n-3), than terrestrial animals[10]. Heme proteins, transition metals and lipoxygenases are the primary endogenous components that have the ability to promote radical oxidative chain reactions in fish. The use of antioxidants is an effective way to minimize or prevent lipid oxidation in food products, retarding the formation of toxic oxidation products, maintaining nutritional quality and prolonging the shelf life of foods[11].

ENDOGENOUS FISH ANTIOXIDANTS

The antioxidant defense system of fish muscle is able to control oxidation under normal *in vivo* conditions in such a complicated matrix rich in PUFA, which is a substrate prone to develop oxidative degradations and loaded with pro-oxidant substances. There are several natural compounds that

participate in the antioxidative defense mechanism of fish. These include enzymes (catalase, peroxidase and glutathione and superoxide dismutase), carotenoids, peptides, amino acids and phenolic compounds (tocopherols, ubiquinones). These compounds are found in the cell plasma, mitochondria of cell membranes[12].

Amines, peptides and amino acids are known to have significant antioxidant properties. In general, they function as synergists or primary antioxidants. Amino acids, peptides and nucleotides are believed to be important metal chelators present in fish. Amino acids are also suggested to have antioxidant properties as reaction products with carbonyls from oxidizing lipids. Their antioxidant properties are attributed to the formation of reductone (enaminone) structures that have both reducing and metal complexing properties. Furthermore, the basic amino acids (histidine, lysine and arginine) are known to produce the most effective antioxidant products with sugars[13]. Antioxidants may directly or indirectly inhibit the initiation and propagation steps of lipid oxidation. They sometimes have multiple effects and their mechanisms of action are therefore often difficult to interpret. According to their mode of action, antioxidants can be categorized into preventive inhibitors and true antioxidants.

ENDOGENOUS MEAT ANTIOXIDANTS

Skeletal muscle is susceptible to oxidative deterioration due to a combination of lipid oxidation catalysts and membrane lipid systems that are high in unsaturated fatty acids. Biological tissues from which meat and fish is derived are under constant oxidative stress from free radicals, reactive oxygen species and pro-oxidants, hence, they developed antioxidant systems to protect from oxidative damage[14]. To prevent or delay oxidation reactions, several endogenous antioxidant systems are found in muscle tissue. These include α-tocopherol, histidine containing dipeptides and antioxidant enzymes, such as glutathione peroxidase, superoxide dismutase and catalase.

The oxidative stability of skeletal muscle is also influenced by the histidine containing di-peptides, carnosine and anserine. In pigs, beef and turkey muscle, carnosine concentrations are greater than anserine, while the opposite is true in rabbit and chicken muscle. Anserine and carnosine are found in greater concentrations in muscle high in white fibers with chicken white muscle containing over 5-fold more anserine and carnosine than red muscle. The antioxidant mechanism of carnosine and anserine involves both metal chelation and free radical scavenging. Carnosine is capable of chelating metals, yet its chelating activity is dependent on metal ion type. Studies on the oxidation of bio-molecules and the NMR spectra of carnosine indicate that carnosine chelates copper much more strongly than iron[15].

USE OF SYNTHETIC ANTIOXIDANTS

Antioxidants can prevent lipid peroxidation using the following mechanisms: Preventing chain inhibition by scavenging initiating radicals, breaking chain reaction, decomposing peroxides, decreasing localized oxygen concentrations and binding chain initiating catalysts, such as metal ions[16]. The antioxidants can be of synthetic or natural origin. In industrial processing, mainly synthetic antioxidants, such as butylated-hydroxy-anisole, butylated-hydroxy-toluene and propyl-gallate are used in order to prolong the storage stability of food. However, the demand for natural antioxidants has recently increased because of the toxicity and carcinogenicity of synthetic antioxidants[17].

Synthetic antioxidants have been successfully used to block or delay the oxidation process in meat and fish. In addition to their ability to increase lipid stability, antioxidants added to foods may have ability to reduce the risk of various diseases related to the production of free radicals. Many researchers have indicated that lipid oxidation in meat products can be controlled or minimized by the addition of commercial synthetic antioxidants[18]. Although, synthetic additives have been widely used in the meat and fish industry to inhibit lipid oxidation and microbial growth, the recent trend is to decrease their use because of the growing concern among consumers about such chemical additives.

USE OF NATURAL ANTIOXIDANTS

The use of natural antioxidants is emerging as an effective methodology for controlling rancidity and limiting its deleterious consequences. Natural phenolic compounds with antioxidant activity, such as rosemary extract, tea catechin, tannins, etc. have been gaining attention due to their safety and efficacy[19]. Due to the potential health hazards of synthetic antioxidants, natural products, especially natural antioxidants have been intensively examined as safe alternatives to synthetic compounds. Polyphenols (PP) are the natural antioxidants prevalent in fruits, vegetables, beverages (tea, wine, juices), plants, seaweeds and some herbs and show antioxidative and antimicrobial activities in different fish and fish products. The major active nutraceutical ingredients in plants are flavonoids. As is typical for phenolic compounds, they can act as potent antioxidants and metal chelators. They

also have long been recognized to possess anti-inflammatory, antiallergic, hepatoprotective, antithrombotic, antiviral and anticarcinogenic activities[19].

Grape seed extracts contain a number of polyphenols including procyanidins and proanthocyanidins are powerful free radical scavengers. It has been investigated *in vitro*, such as cooked beef[20], pork patties[21] and chicken meat patties[22]. Tea catechins are polyphenolic antioxidants which possess a range of health promoting properties. Tea catechins have demonstrated significant antioxidant, anti-carcinogenic, anti-mutagenic properties in several *in vitro* studies related to human and animals. Tea polyphenols are metal chelating agents and also act on free radicals, since their benzene rings, inhibit chain reactions during lipid oxidation[21].

Seaweeds are considered to be a rich source of antioxidants. Recently, the potential antioxidant compounds were identified as some pigments (fucoxanthin, astaxanthin, carotenoid etc.) and polyphenols (phenolic acid, flavonoid, tannins etc.). Those compounds are widely distributed in plants or seaweeds and they exhibit higher antioxidant activities. Seaweeds are noted to contain not only labile antioxidants (i.e., ascorbate, glutathione) when fresh but also, more stable molecules, such as carotenoids, mycosporine like amino acids and a variety of polyphenols like catechins and phlorotannins[22]. Further, the evidence available in the literature suggests the potential protective effects of seaweeds against oxidative stress in target tissues and lipid oxidation in foods[23].

ANTIOXIDANTS AND HUMAN HEALTH

Oxygen has double-edged properties, being essential for life, it can also aggravate the damage within the cell by oxidative events[24]. A free radical can be defined as any molecular species capable of independent existence that contains an unpaired electron in an atomic orbital. Free radicals and its adverse effects were discovered in the last decade. These harmful substances are produced during the normal metabolic processes in the body along with toxins and wastes. Free radicals are generated during oxidation of carbohydrates, fats and proteins through both aerobic and anaerobic process. The studies to understand the role of free-radical reactions in human disease, biology, toxicology and in the deterioration of food has become an area of intense interest. Unsaturated lipid molecules of cell membranes and other biological molecules including RNA, DNA and protein enzymes are susceptible to oxidative damage.

The term Reactive Oxygen Species (ROS) is often used to include not only the radicals OH^{-}, RO_2^{-}, NO^{-} and O_2 but also the non-radicals HOCl, O_2, $ONOO^{-}$, O_3 and H_2O_2. "Oxidative stress" is the term that refers to the imbalance between generation of ROS and the activity of the antioxidant defenses. Severe oxidative stress can cause cell damage and death[25]. A role of oxidative stress has been postulated in many conditions, including atherosclerosis, inflammatory condition, certain cancers and the process of aging. Oxidative stress is now thought to make a significant contribution to all inflammatory diseases (arthritis, vasculitis, glomerulonephritis, lupus erythematous, adult respiratory diseases syndrome), ischemic diseases (heart diseases, stroke, intestinal ischema), hemochromatosis, acquired immunodeficiency syndrome, emphysema, organ transplantation, gastric ulcers, hypertension and pre-eclampsia, neurological disorder and many others[26].

An antioxidant is a molecule stable enough to donate an electron to a rampaging free radical and neutralize it, thus reducing its capacity to damage. Antioxidants provide protection by neutralizing free radicals, which are toxic by-products of natural cell metabolism. Some of such antioxidants, including glutathione, ubiquinol and uric acid are produced during normal metabolism in the body. Other lighter antioxidants are found in the diet. The body cannot manufacture the micronutrients, so they must be supplied in the diet. Increased intake of antioxidants can prevent diseases and lower the health problems. Foods may possibly enhance antioxidant levels because they contain a lot of antioxidant substances[27]. Antioxidants study to protect lipids from per-oxidation by free radicals and this can be defined as a molecule stable enough to donate an electron to a free radical and neutralize it, thus reducing its capacity to damage[28].

CONCLUSION AND FUTURE RECOMMENDATION

The damage by free radicals contributes to the etiology of many chronic health problems such as cardiovascular and inflammatory diseases, cataract and different types of cancers. Antioxidants prevent free radical induced tissue damage by preventing the formation of radicals, scavenging them or by promoting their decomposition. Synthetic antioxidants are recently reported to be dangerous to human health. Thus, the search for effective, non-toxic natural compounds with antioxidative activity has been intensified in recent years. There has been a huge demand for natural antioxidants mainly because of adverse toxicological reports on many synthetic compounds. Thus, most of the recent study has been directed towards identification of novel antioxidants from natural sources, particularly of plant origin. Although these

extracts are derived from plant generally regarded as safe but further study is needed to determine their safe limits and toxicological effects in meat and fish products as the extraction or processing conditions may alter their properties.

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