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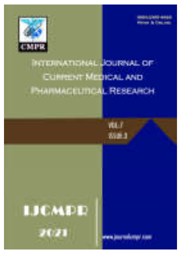

Research Article

POTENTIAL MEANS TO LOWER CHOLESTEROL OXIDATION PRODUCTS (COPs) IN THE DIET: A REVIEW

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ABSTRACT

Cholesterol is a monosaturated sterol, present in the foods of animal origin. It has highest odds to get oxidized into a series of oxidized products called as cholesterol oxidation products (COPs) or oxysterols during processing and storage. Most of them are produced during thermal processing of animal origin foods. These oxysterols are well known for their diverse negative biological effects. These zenobiotic effects have been researched a lot and many studies exhibit domino effect of natural and synthetic antioxidants and fermented dairy products on lowering the concentration of oxysterols in foods. The present review focuses on certain potentially significant and evidenced means of lowering these exogenous and endogenous COPs. This review will positively prove noteworthy to every segment of community as well as food industry so as to lower the concentration of oxysterols in food production, processing and storage.

INTRODUCTION

Cholesterol oxidation has long been maintained in focus by the research faculty all over the globe. Degraded cholesterol which is well branded as cholesterol oxidation products (COPs) or oxysterols have been extensively studied for their zenobiotic and deleterious biological effects on human body.

There are more than 70 COPs have been identified as of now and some may still remain to be unidentified. The most researched and evident hazardous effect of COPs among all others is considered to be on cardiac system by atherogenesis mechanism. They are more reactive than non-oxidized cholesterol and lead to a dysfunction of endothelial cells and even to the fibrotic degeneration of the arterial wall and rupture of the plates (Poli *et al* 2009; Vicente *et al* 2012).

Cholesterol oxidation proceeds in three different ways such as autoxidation, photo-oxidation and enzymatic oxidation. Oxidation can occur endogenously as well as exogenously. In exogenous case, it can happen during cooking, processing, storage etc by exposure to various variants like light, heat, air, water, other chemical reactants etc. Although cholesterol oxidation cannot be ceased absolutely but it can undeniably be reduced and controlled while cooking and processing it. Although as of now there are no specific, precise and experimented means to prohibit the production of endogenous and exogenous COPs, certain natural and synthetic antioxidants and few other ways can potentially be used to lower their production exogenously as well as endogenously.

This review's major aim is to recognize these potential means of lowering the production of COPs endogenously and exogenously. And hence following article briefs the probable and apparent approaches to control the production of oxysterols.

Cholesterol and Its Oxidation

Cholesterol is an animal sterol found in the body tissues of vertebrates. It is found in large concentration in brain, spinal cord and liver. It is a significant and vital constituent of membrane cells in order to provide them stability. It is a key precursor in the synthesis of vitamin D, of the bile acid, of the various steroid hormones such as cortisol, cortisone, and aldosterone in the adrenal glands, and of the sex hormones progesterone, estrogen, and testosterone. Cholesterol also has an important role for the brain synapses as well as in the immune system. Myelin contains 70% of brain cholesterol. Brain is the most cholesterol rich organ of the human body. Hepatic cells synthesize the greatest amount of cholesterol in vertebrates. Its biosynthesis is a highly regulated process.

Cholesterol ($C_{27}H_{46}O$) is one of the most studied lipid moiety found in animal cells. It has 27 carbons in its structure and a

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single double bond at 5, 6-position at the sterol nucleus. (Please see the figure 1)

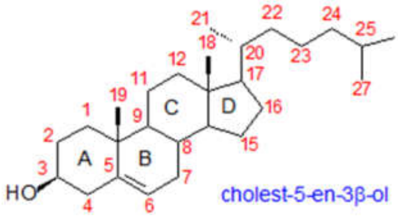

Figure 1 Structure of Cholesterol

On account of this single double bond, this lipid moiety is highly susceptible to oxidation. Cholesterol undergoes auto-oxidation, photo-oxidation and enzymatic oxidation, producing relevant hydroperoxides (Ubhayasekera *et al*., 2004). It undergoes autoxidation by a free radical mechanism leading to formation of hydroxyperoxides and then to a number of cholesterol oxidation products (COPs), well known as oxysterols. These are a group of sterols similar to cholesterol in structure but exhibit an additional oxygen functional group like hydroxyl group, ketone group or an epoxide group at the sterol nucleus or at the side chain of the molecule. Many numbers of oxysterols (equal to 70) have been identified and are under study (Schroepfer, 2000) and many probably remain to be identified (Valenzuela *et al*, 2003).

Formation of COPs

Oxidation of cholesterol follows the oxidation pattern such as autoxidation, photo-oxidation and enzymatic oxidation, producing relevant hydro peroxides.

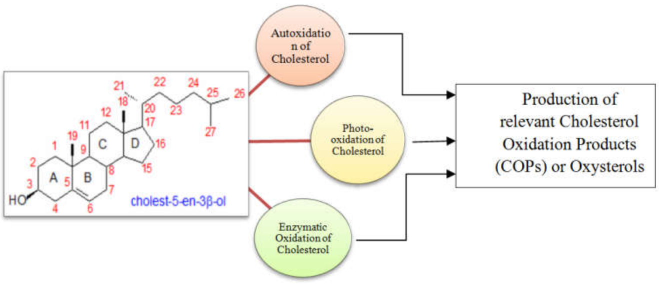

Figure 2 Cholesterol oxidation patterns

Autoxidation of cholesterol

It is a self catalytic reaction with molecular oxygen. The oxidation of cholesterol starts at C-7 by removal of hydrogen and addition of oxygen, forming the primary COP, isomers of 7-hydroperoxycholesterol. The 7-dehydroperoxycholesterol further gets converted to 7α-hydroxycholesterol and 7β-hydroxycholesterol, which are commonly found in food (Lercker *et al*. 2002). 7-ketocholesterol is formed by dehydration of isomeric 7-hydroxycholesterol in the presence of radicals. It is a major COP in the food matrix (Shingla and Mehta. 2018).

Photo-oxidation of cholesterol

During photo-oxidation of cholesterol, singlet oxygen is formed from triplet oxygen by light in the presence of an active sensitizer (natural pigment or synthetic colorant). Cholesterol can react with singlet oxygen in the presence of photo-sensitizer, forming dominant hydroperoxide at C-5. (Lercker. 2002).

Enzymatic-oxidation of cholesterol

Some enzymes in food oxidize cholesterol. The main enzymes involved in the enzymatic oxidation of cholesterol are monooxygenase, dioxygenase, dehydrogenase, and oxidases. The COPs like 7α-hydroxycholesterol, 25-hydroxycholesterol, 20α-hydroxycholesterol, (25R)-26-hydroxycholesterol, 22R-hydroxycholesterol are produced by enzymatic oxidation of cholesterol. (Lercker *et al*. 2002).

These are the obvious mechanisms of cholesterol oxidation. As discussed earlier, the influence of cholesterol oxides on human health deserves some more emphasis, because these oxides have a potential to cause gradual but harsh physiological changes (Valenzuela and others 2004). COPs contribute significantly to the complex cellular and molecular events leading to the formation of fibrous and atherosclerotic plaques, once thought to be involved in several key steps, such as the induction of proinflammatory, proapoptotic, and profibrogenic effects, in the development of certain diseases (Dantas *et al*, 2015). A large body of evidence supports the hypothesis that oxidized lipoproteins particularly low density lipoproteins (LDL) play a major role in atherogenesis (Staprans I *et al*, 2003).

Possible Means to Lower Cholesterol Oxidation Products (COPs) in the Diet

In the view of potentially hazardous effects of COPs on human health, efforts are being diverted towards prevention or reduction in the generation of exogenous COPs. There are some possible dietary ways which can lower the production of exogenous COPs.

Eggs and egg-derived products are the main dietary sources of oxysterols. Thermally processed milk and milk-derived products are also another source of COPs in the diet. Fried meats, and other miscellaneous foods, such as French fried potatoes, when prepared using vegetable/animal frying oil, are another important source of oxysterols in the western diet. To sum up, dietary sources of COPs are cholesterol-rich foods, such as dairy, processed eggs, and meat products etc. (Valenzuela A *et al* 2003).

Antioxidants and COPs

Many studies have indicated that natural and synthetic antioxidants exhibit positive results on the reduction of COPs. Scientists have always been conducting experiments regarding involvement of high cholesterol and oxidative stress in cardiovascular diseases; most of the results indicated that the high cholesterol diet modulates proteins related to lipid metabolism, which might result in the malfunction of the heart and α-tocopherol (vitamin E) shows its beneficial effects.

Antioxidants, both of synthetic or natural origin, are widely applied to prevent lipid oxidation in processed foods, raw materials, or fats and oils used in manufacturing (Valenzuela & Nieto, 1996). Antioxidants are organic lipid- or water-soluble substances that can scavenge the active forms of oxygen involved in the initial steps of oxidation, or break the oxidative chain reaction. Antioxidants may react with the fatty acid peroxy radicals to form stable antioxidant radicals, which are either too non reactive for further reactions or form non-radical products (Valenzuela & Nieto, 1996).

Synthetic Antioxidants: Most widely used synthetic antioxidants in human foods are 2,6-di- tertiarybutyl-4-methylphenol (BHT), tertiarybutyl- 4-hydroxyanisole (BHA), tertiary butylhydroquinone (TBHQ), and the n-propyl ester of 3,4,5- trihydroxybenzoic acid (propyl gallate, PG) (Valenzuela *et al*., 2003). Natural antioxidants, which are present in variable amounts in vegetables such as fruits, leafy greens, flowers, roots, grains, and seeds, have gained prominence as alternatives to synthetic antioxidants (Aruoma, 1997).

***Natural Antioxidants**:* Research interest in the properties and occurrence of natural antioxidants center upon vitamin E (mainly the alpha, beta, and gamma tocopherol homologous), some herbs extracts, such as rosemary extract, carotenoids, and various flavonoids (Valenzuela, 2002; Valenzuela *et al*., 2003). Since cholesterol oxidation proceeds via a free radical mechanism, similar to polyunsaturated fatty acid oxidation (Kubow, 1993), antioxidants used to inhibit general fat and oil oxidation are also able to prevent or retard cholesterol oxidation. α-tocopherol has been identified as the most effective antioxidant, with better activity than other antioxidants, such as BHA or BHT. Csallany *et al*. (2002) have recently demonstrated that α-tocopherol, BHA, and BHT can inhibit the oxidation of cholesterol in the presence of superoxide anion, water, and hydrogen peroxide.

In an *in vivo* model, Galobart *et al*. (2002) demonstrated that feeding laying hens with linseed oil or sunflower oil added with α tocopheryl acetate may reduce cholesterol oxidation of spray-dried omega-6/ omega-3 fatty acid-enriched eggs during storage. The effect of α-, β-, γ-, and δ-tocopherol on cholesterol-containing liposomes, when fatty acid and cholesterol oxidation is induced by Fe^{2+}, was recently communicated (Valenzuela *et al*., 2002).

Rosemary extract is a food grade natural antioxidant oleoresin obtained from the leaves of the evergreen shrub rosemary (Rosmarinus officinalis. L) (Okamura *et al*., 1994). The extract contains four effective antioxidants: carnosol, rosmanol, isorosmanol, and rosemarydiphenol (Wu *et al*., 1982). These four compounds are odorless and tasteless diterpenolactones when processed, and among these lactones, rosmary diphenol and rosemanol, show antioxidant activity stronger than BHA and BHT. The effect of rosemary extract on cholesterol oxidation was recently tested using highly purified soybean oil as the substrate for oxidation. In these experimental conditions, rosemary extract exerts a strong antioxidant effect by preventing both soybean oil oxidation and cholesterol oxidation (Valenzuela *et al*., 2002).

***Flavonoids**:* Flavonoids comprise a well-known family of natural substances that exhibit antioxidant properties (Cook & Samman, 1996). These structures are ubiquitous in photosynthesizing cells, seeds, fruits, pollen, and flowers (Havsteen, 1983). Flavonoids have been defined as "high level" antioxidants (Robak & Gruyglewski, 1988). That is, they act by scavenging those free radicals or exited forms of oxygen involved in the first stages of lipid oxidation, such as the singlet oxygen, the superoxide free radical or the hydroxyl free radical (Fraga *et al*., 1987). The effectiveness of some flavonoids as antioxidants for fats and oils has been previously demonstrated (Das & Pereira, 1990; Nieto *et al*., 1993). The effect of four flavonoids (quercetin, catechin, morin, and rutin) was recently assayed against cholesterol oxidation when oxidation is induced by temperature, and using soybean oil as substrate for oxidation (Valenzuela *et al*., 2002). In these conditions, quercetin was highly effective to prevent cholesterol oxidation.

Janoszka B conducted a study on COPs in 2010. The effect of onion and garlic on the formation of two cholesterol oxidation products (COPs): 7-ketocholesterol and 7-hydroxycholesterol was evaluated by comparing their concentrations in meat and gravy samples obtained from three pork dishes prepared in the presence and absence of these flavorings. The addition of onion (30 g/100g of meat) caused a decrease in 7-ketocholesterol and 7-hydroxycholesterol concentrations in all of the investigated pork dishes by 79%, whilst the addition of 15 g of garlic to 100g of meat lowered the concentration by 88%.

Another study indicated that sage was effective in controlling lipid and cholesterol oxidation, minimizing the pro-oxidant effects of salt, cooking, and storage (Mariutti *et al*, 2011).

Fruit Phytonutrients and Their Effects on COPs

Certain fruits have exhibited the potential to lower the risk of endogenous COP production.

***Citrus Fruits**:* The different radicals responsible for the cell oxidation process are the following: singlet oxygen ($1O_2$); superoxide anion (O_2), hydroxyl radical (OH•) and peroxyl radical R-O-O• (Rio D *et al* 1997, Saez *et al* 1994, Okwu D, 2007). The hydroxyl radical is the most cytotoxic of all these radicals. Also, polyunsaturated fatty acids present in cell membranes are easily oxidized by both; enzymatic and oxidative preoxidation through free radical chain reaction (Rio D *et al* 1997, Okwu D, 2007). Initiation of lipid per oxidation can be induced by free radicals (superoxide, hydroxyl and singlet oxygen) produced in biological systems (Rio D *et al* 1997, Okwu D, 2007). These electrically inert species have the ability to interact and alter genetic constitution. They exhibit catatonic, mutagenic and carcinogenic actions. It has been reported that lipid peroxidation can be inhibited by flavonoids acting as strong radical scavengers and singlet oxygen quenchers (Rio D *et al* 1997, Okwu D, 2007). It has also been proposed that citrus flavonoids react with peroxyl radicals; thus, bringing about the termination of the radicals reaction (Rio *et al* 1997).

Citrus fruits such as Indian gooseberry (*aamla*), guava, lemon, orange, sweetlime, grapefruit etc exhibit exceptional antioxidant properties owing to the presence of vitamin C (ascorbate), citrate and certain phytonutrients like polyphenols, terpenes (limonene), limonoids, glucarates, flavonoids like quercitin, naringin, hesperidin, rutin, citrus carotenoids like β-carotene, zeaxanthin, retinal, leutine, lycopene etc (Okwu D. 2008).

Citrus phenolic compounds particularly flavonoids have been reported to possess an important antioxidant activity toward radicals. The citrus flavonoids have the ability to capture electrons, block and/or scavenge the radicals. The citrus flavonoids form a tautomeric dislocation, which prevents the propagating chain reactions of these oxygen free radicals. (Rio *et al* 1997, Okwu D, 2007).

Dried figs: Dried figs have also been reported to possess antioxidant properties for they are the potent source of flavonoids and polyphenols and various other compounds like arabinose, b-amyrins, b- carotines, glycosides, b-setosterols and xanthotoxol (Soni *et al* 2014).

Dried figs are well known for numerous therapeutic benefits and are used in traditional medicines to treat different disorders like gastrointestinal disorders (colic, indigestion, loss of appetite and diarrhoea), respiratory disorder (sore throats, coughs and bronchial problems), and cardiovascular disorders and also used as anti-inflammatory and antispasmodic remedy (Duke *et al* 2002, Werbach M, 1993) . Ficus carica has also been found to have antidiabetic, hypolipidemic [Canal *et al*, 2000], hepatoprotective (Gond and Khadabadi, 2008), antispasmodic (Gilani *et al*, 208), antipyretic (Patil V *et al* 2008), antibacterial (Mi-Ran *et al*, 2009), antifungal (Houda *et al*, 2010), scavenging activity and immune response (Xiao-Ming Y, 2009).

Pomegranate: Punicalagin is the main ingredient present in pomegranate peel as evaluated by ferric reducing antioxidant power assay and was found to be the richest source of antioxidants. Ismail *et al*., 2012, reported that punicalagin composes 85% of the total pomegranate tannins and accounts for more than 50% of the antioxidant activity of pomegranate juice. Xu *et al*., 2014, reported that punicalagin shows an antioxidant activity by increasing Nrf2-mediated HO-1 expression and also induces the PI3K/Akt-mediated HO-1 expression. Punicalagin enhances the SOD1 mRNA expression and thereby inhibits reactive oxygen species generation and NO overproduction by macrophages. The antioxidant activity of punicalagin acts by electron donation to free radicals that convert them to relatively more stable compounds. Gil *et al*., 2000, reported that punicalagin exhibits 3-fold antioxidant effect than that of green tea and red wine.

Cherry: Strong evidence indicates that natural products derived from plants, such as anthocyanins, poses antioxidant properties and may inhibit lipid oxidation and its metabolites. Montmorency cherries (*Prunus cerasus*) contain polyphenols in amounts of 3000 mg/100g dry weight, because of this they could act as a natural source of strong antioxidants. Results demonstrated that anthocyanins may have a dose-dependent antioxidant activity, specifically targeting CO. Montmorency tart cherries have the ability of to penetrate lipid membrane and scavenge ROS (Meza *et al*, 2018).

Fermentation and COPs

Recently in 2013, Machorro-Mendez *et al* assessed the ability of *Lactobacillus casei* ATCC 334 to remove COPs in aqueous solution. Results showed the ability of both growing and resting cells to remove COPs. All COPs-bacterium interactions were specific, being resting cells the most efficient for COPs removal. Binding to the cell wall and/or cell membrane incorporation appeared to be the most likely mechanisms involved on COPs removal by *L. casei* ATCC 334.

The most common application of *L. casei* is industrial, specifically for dairy production. It is a dominant species in naturally fermented green olives, cheddar cheese etc.

Among the best-documented, probiotic *L. casei*, *L. casei* DN-114001, and *L. casei* Shirota have been extensively studied and are widely available as functional foods (examples are Actimel, Yakult). Another commercially available form of *L. casei* can be found in Danactive made by Dannon. They registered trademarked *L. casei* as *L. casei* Immunita.

Concluding Remarks

From the above discussion, it is apparent that cholesterol oxidation products' production can be reduced or minimized by taking into thought their production means and factors responsible for the same.

Several measures can be adopted to lower the production of COPs in foods. As cholesterol oxidation in maximum in cholesterol rich foods, minimizing or avoiding foods with high cholesterol content or avoiding cooking food in cholesterol containing fat; this may help in achieving the goal. Consciously using foods with high antioxidant, phenolic content as discussed in the above review. Use of synthetic antioxidants wherever possible during processing can also be a potential alternative to lower dietary oxysterols. Processing foods at low temperatures is an exceptional option for achieving this aim; this also has many positive effects on retaining overall food quality. Using oxygen excluding packaging system can also aid in avoidance of oxidation of cholesterol. Incorporating fermented dairy products in major meals of the day can also be useful. And finally storing food in the dark and away from harsh lights can also pave way for avoiding oxysterol formation in foods.

Thus to sum up, it is utterly clear that reducing the total cholesterol content of the foods would have a significant effect on the amount of cholesterol oxides in foods.

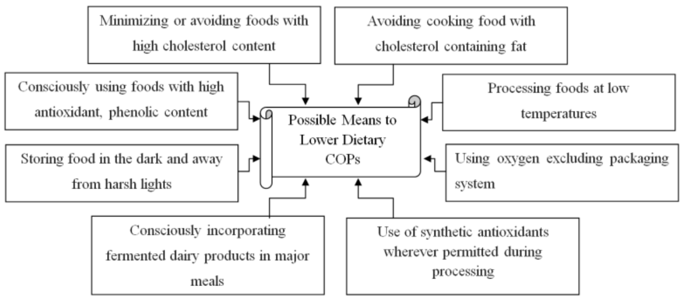

Figure 3 Possible Ways to Minimize Dietary COPs

Although these means have exhibited the efficacy in lowering or inhibiting the oxysterols production during processing of foods as well as in raw materials, more advanced researches should be carried out to demonstrate the best possible way of application of antioxidants or any other method to prevent cholesterol oxidation.

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