

Cholesterol Oxidation Products Analysis in Meat and Poultry

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Abstract: The purpose of this research is to assess the formation and determination of cholesterol oxidation products (COPs) in meat and poultry products and its effects on human health. Cholesterol is a molecule with an unsaturated or double bond which is prone towards oxidation and formation of COPs. The impact of COPs towards human health depend on the type of COPs formed. Extraction, purification and detection methods in quantifying COPs play an important role in the analysis as COPs occur mostly at low levels. Direct cold saponification was the most suggested method due to high efficiency, good method precision, minimal artifact formation and ease of handling to recover COPs. High performance liquid chromatography (HPLC) and gas chromatography (GC) couple with mass spectrometry (MS) detector have become more important in providing better quantitative information in COPs analysis. Determination of cholesterol using gas chromatography usually need to go through derivatisation process to improve volatility and thermo stability, to optimize peak shape, to decrease retention time and to increase sensitivity. However, it makes no difference between GC analysis of free cholesterol and those of trimethylsilyl (TMS) or acetate derivatives. The gas chromatography mass spectrometry (GCMS) is the most commonly used method to determine COPs for many food samples including meats and poultry.

Key words: Cholesterol Oxidation Products • Cholesterol • Gas Chromatography • Meat • Poultry

INTRODUCTION

Cholesterol is a monounsaturated lipid with a double bond on carbon-5. Cholesterol oxidation products (COPs) oxidation products (COPs) are similar to cholesterol, which contain an additional functional group, such as a hydroxyl, ketone or an epoxide group in the sterol nucleus and/or on the side chain of the molecule [1-3]. COPs have received considerable attention in recent years because of their biological activities associated with human diseases [4]. Cholesterol-containing foods, when consumed fresh, contain low levels of COPs and the levels go up during processing, storage and cooking [5].

Thus for health reasons, it is important that COPs in food are identified and quantified accurately [6]. The validation and re-validation of the analytical methods

are necessary to obtain reliable data. Several methods were developed along the years to determine cholesterol in foods in order to obtain accurate results. Therefore, the purpose of this paper is to assess the formation and determination of COPs in meat and poultry products and its effects on human health.

The Relation of Lipids, Fats and Oils: The fats and oils in foods belong to a group called lipids. Fats and oils are differentiated in two ways: (1) fats are solid at room temperature, whereas oils are liquid and (2) fats are usually derived from animal sources, whereas oils are derived predominantly from plants [7]. Lipids are classified into three major groups which are particularly concerned in food and nutrition studies: neutral fats known as triacylglycerols or tryglycerides, phospholipids and sterols [8].

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Triacylglycerols: Triacylglycerols are made up of three fatty acids combined with one molecule of an alcohol called glycerol (Figure 1). Glycerol has three carbon atoms and three hydroxyl groups (-OH). Fatty acids are commonly composed of linked chain of carbon atoms with a carboxyl group (-COOH) on the end of the chain. The fatty acids are joined to the glycerol molecule by an ester linkage [8].

Phospholipids: Structurally, phospholipids are similar to triglycerides. They contain glycerol attached through an ester linkage to two fatty acids. The difference is there is phosphorus compound to replace the carbon in one of the fatty acids which makes the phospholipids soluble in water, whereas its fatty acid components are soluble in fat. Phospholipids are very important in the body as a component of cell membranes by assisting the fat soluble vitamins and hormones to move in and out of the cells [7].

Sterols: Sterols are important compounds in maintaining the human body such as cholesterol, hormones and vitamin D. The sterol of most significance in food is cholesterol [7]. Cholesterol is only found in foods of animal origin such as meat, egg yolks, fish, poultry and milk fat [8]. Plants do not contain cholesterol, but they may contain other types of sterol called phytosterols [7].

Cholesterol Deposition, Biological Functions and Oxidation: Cholesterol is a eukaryotic sterol that in higher animals is the precursor of bile acids, vitamin D3 and steroid hormones and a key constituent of cell membranes, mediating their fluidity and permeability. Most is synthesized by the liver and other tissues, but some is absorbed from dietary sources [9]. Dietary cholesterol enters the body through the chylomicron pathway and is removed from the plasma by the liver as a component of chylomicron remnants. Cholesterol is a molecule with an unsaturated or double bond; therefore, it is prone to oxidation. It is sensitive to free radical oxidation by diatomic molecular oxygen (O₂) in the air. Cholesterol molecules are arranged in double layers with the 3-hydroxyl groups in juxtaposition and side chains exposed [10].

Structure of Cholesterol: Cholesterol is a structure containing 27 carbons (Figure 2), commonly found as the component in cell membrane. Biologically it is an important precursor for bile acid, pro-vitamin D3 and several steroidal hormones [11].

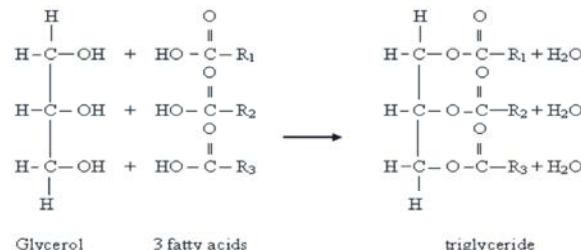


Fig. 1: Chemical reaction on formation of triglyceride

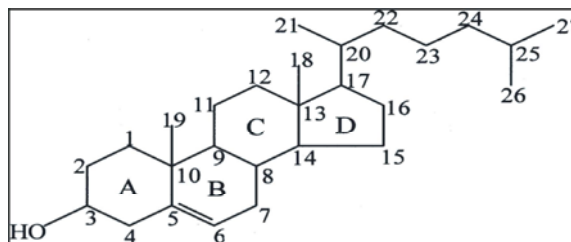


Fig. 2: The chemical structure of cholesterol with positional numbering system [12].

Oxidation of Cholesterol: Oxidation is defined as the addition of oxygen or removal of hydrogen from a molecule. Oxidation affects most food components, including the micronutrients-colors, flavor compounds, vitamins and minerals. The macro constituents, carbohydrate, lipids and proteins are also susceptible to oxidation [13]. Due to the presence of one double bond at position Δ5-6 of the nucleus, cholesterol is susceptible to oxidation when exposed to light and molecular oxygen [14-16]. The molecule undergoes autooxidation and photo-oxidation by a free-radical mechanism leading to the formation of hydroperoxides and then to a number of oxidation products, the so-called oxysterols [17]. Oxysterols have various structures depending on the type of oxidation and the physical state of the substrate [16]. These oxidation products are similar in structure to cholesterol, but contain an additional oxygen function such as hydroxyl group, ketone group, or an epoxide group at the sterol nucleus or at the side chain of the molecule [18]. Oxidation occurs through a free radical reaction, giving rise to more than 60 autooxidation products [19], however up to 70 oxysterols have been identified [4]. Oxysterols that tend to be more polar to cholesterol may enter the blood circulation with dietary and biliary cholesterol as part of chylomicron structure [20].

In the process of oxidation of cholesterol in foods, presence of unsaturated fatty acids, cholesterol level, heat, oxygen, light, UV light, gamma-radiation, water activity and technologically related events and influence the formation of cholesterol oxidation products (COPs) in

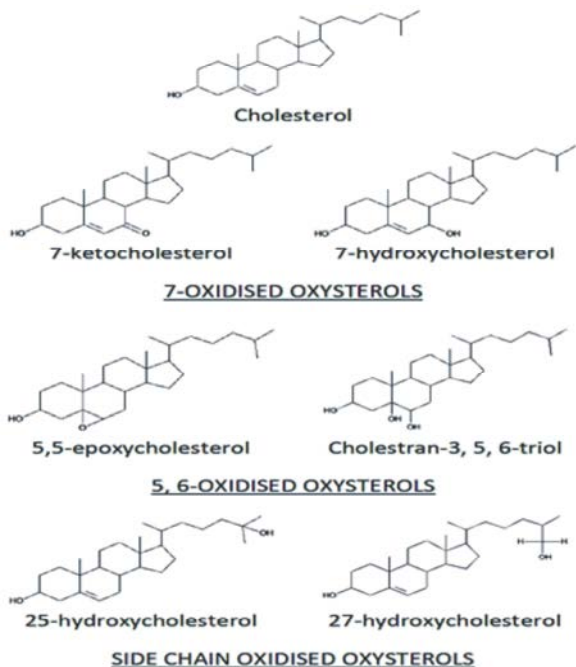


Fig. 3: Structure of Cholesterol Oxides (Oxysterols) [25].

food [1,3,5,21]. The hydroperoxides derive from oxidation of unsaturated fatty acids play a significant role to facilitate cholesterol oxidation at $\Delta 5-6$ double bond, which is more sensitive to oxidation [22]. Interaction of triglycerides with cholesterol may accelerate the oxidation of the sterol and cholesterol may also influence triglyceride oxidation [23]. Free radicals that formed on unsaturated fatty acids during lipid oxidation by removing hydrogen from $\Delta 5-6$ double bond, can be migrated to the position 4 or 7 of rings respectively allowing the reaction of molecular oxygen at the position 4, 5, 6 or 7 [3]. However position 7 is the most common position and stable that may form either a hydroxyl derivative by decomposition (7α -hydroxycholesterol or 7β -hydroxycholesterol) or a keto derivative by dehydration (7-ketocholesterol) [24].

The attack rarely occurs at C-4 because of the possible shielding effect provided by the neighbouring hydroxyl group at C-3 and the trialkyl substituted C-5 [10]. Another possible reaction is a direct reaction with the double bond forming 5, 6-epoxycholesterol then by further dehydration it can be transformed into a triol (cholestane- 3β , 5α , 6β -triol) [18]. Figure 3 shows the main structure of cholesterol oxides of pathophysiological interest recently.

Meanwhile, both the C-20 and C-25 are at the tertiary positions at the lateral chain more susceptible to oxidative attack than other carbons producing the 20-hydroperoxide

and 25-hydroperoxide respectively [16,18]. These molecules can be further reduced into 20α -hydroxycholesterol and 25-hydroxycholesterol and 27-hydroxycholesterol [26].

Cholesterol can also be oxidized by enzymic oxidation. Monooxygenase, dioxygenase, dehydrogenase and oxidases are the main enzymes that can oxidize cholesterol. The COP, 7α -hydroxycholesterol, 25-hydroxycholesterol, 20α -hydroxycholesterol, 25R-26-hydroxycholesterol, 22R-hydroxycholesterol are produced by enzymic oxidation of cholesterol [26].

Cholesterol Oxidation Products (COPs): COPs formed from cholesterol during food processing or storage, are widely distributed in cholesterol-rich products such as eggs and [27,28].

The most frequent COPs found in foods are 7-ketocholesterol (7-Keto), 20-hydroxycholesterol (20-OH), 25-hydroxycholesterol (25-OH), 7α -hydroxycholesterol (7α -OH), 7β -hydroxycholesterol (7β -OH), 5,6 α -epoxide, 5,6 β -epoxide and Triol. 7-Keto is one of the major oxygenated products found in oxidized low-density lipoproteins (LDL) and in atherosclerotic plaque, where it is believed to play a role in arterial pathology [29]. 7-keto occurs in relatively high concentrations in many foods [30,31,32,33,34], with the content varying, depending on the type of matrix [34] and it has been proposed as an indicator of cholesterol oxidation [35,36,37]. By comparison, 25-OH occurs in smaller concentrations [30], however, 25-OH and Triol are considered to be the most atherogenic of these products [38,39].

Issues on COPs and the Effects to Human Health: Over the past few years, considerable attention has been devoted to the study of cholesterol oxidation due to the biological activities of its oxidation products (COPs) associated with human diseases. Research provides evidence that some cholesterol oxides are toxic and may facilitate development of coronary artery disease and certain cancers [16,24,40-45].

Oxysterols that tend to be more polar than cholesterol may also be absorbed in the intestinal track by a mechanism similar to that of cholesterol absorption [40]. Oxysterols are able to pass through lipophilic membranes much more quickly than cholesterol does [46]. Oxysterols may enter the blood circulation with dietary cholesterol and biliary cholesterol (endogenous cholesterol) as part of chylomicron structure [20].

Table 1: Studies on the effect of oxysterols on vascular and nonvascular cells.

Studies have been done on effect of oxysterols on vascular and nonvascular cells.	Researcher
25-OH triggered the apoptotic in murine lympho cells and in normal murine thymocytes.	50
Apoptosis occurred shortly after treatment of human monocytic cell lines with 20-30 μM (8.053-12.080ppm) of 25-OH and 7 β -OH.	51
Cells become necrogenic towards cultivated macrophages after treated with 50 μM (20.132ppm) of 7 α -OH, 7 β -OH and 27-OH.	52
30 μM of 7-keto(12.020ppm) and 25-OH (12.080ppm) can induce apoptosis in rabbit cultured smooth muscle cells (SMCs)	53
20-25% of SMCs were apoptotic after 48 hours incubation after treated with 20-30 μM (8.053-12.080ppm)	
of 7 β -OH and 20-25% of SMCs were apoptotic after 48 hours incubation after treated with 40-60 μM (24.039ppm) of 7-keto.	54
Concentration of oxysterols 20-50 μM exerting proapoptotic effect on the other cell types present in the arterial wall.	54
30 μM (12.080ppm)7 β -OH or 25-OH induced cell death trough a necrogenic mechanism.	55
Treated murine macrophagic cells in culture with 7-keto lead vascular cells to apoptosis and higher amount of oxysterols induce straight necrosis.	56

In both in vivo and in vitro studies the COPs have been shown to have a variety of potentially atherogenic, cytotoxic, mutagenic and possibly carcinogenic effects [4]. In the extreme situation, accumulation of oxysterols in the liver and possibly in other organs may occur through phagocytosis by local macrophages [25]. COPs also may contribute to the progression of chronic liver disease through their proapoptotic and pronecrogenic effects. Oxidation of cholesterol could contribute to brain tissue damage under a condition of oxidative stress [18].

Atherosclerotic lesions not only contain cholesterol, but also a series of oxysterols [47]. The impact of oxidatively modified low-density lipoprotein (LDL) particles is very important and widely accepted model for the pathogenesis of atherosclerosis [48]. Oxidized LDL is potentially more atherogenic than native LDL in several ways. It can stimulate release of a chemotactic factor from endothelial cells [49]. But the contribution of dietary oxysterols to the total amount of oxysterol measurable in plasma and lipoproteins of human is still unclear [18].

Table 1 shows several studies that have been done on the effect of oxysterols on vascular and nonvascular cell. There are many research done to investigate the harmful effects of oxysterols towards human cells. However, practically all toxicologic studies have been carried out on the major oxysterols taken individually, whereas in biology they always occur in mixtures [25]. Naturally, oxysterols are always present as a mixture, whether in foods, oxidized LDL, or in the core region of atherosclerotic plaque and molecular interactions often occur among mixed compounds. The oxysterol mixture was markedly profibrogenic but an equimolar amount of 7-keto was not [56]. However further research need to be done on the threshold amount of oxysterols which can affect the cells and the effect of mixture and single oxysterols to the cell.

Food Processing and COPs: Foods that are naturally characterized by high cholesterol content are major sources of oxysterols when processed. Processed animal foods are the main sources of COPs in human diet [3].

Unlike fresh or raw foods, prepared food and storage procedures favor the auto oxidation of cholesterol, resulted in high levels of cholesterol oxides preparation or storage procedures favor the autoxidation of cholesterol and showed high levels of cholesterol oxides [25]. Cholesterol-containing foods, when consumed fresh, contain low levels of COPs and the levels go up during processing, storage and cooking. The consumption of pre-cooked foods of animal origin is becoming more popular in the world due to the low cooking time involved in preparing them at home. Therefore, the intake of foods containing COPs has increased [5]. Cholesterol rich foods having the same cholesterol content may yield significantly different amount of cholesterol oxides [57].

For example, in eggs, despite the high cholesterol content, they are well protected from oxidation; the lipids are located in the yolk, rich in natural antioxidants and surrounded by the albumin and shell which provide further protection against atmospheric oxygen. cholesterol content, but their well protected from oxidation because the lipid are located in the yolk, which is rich natural antioxidants and surrounded by the albumin and shell which provide further protection against atmospheric oxygen.

Cooking of food under standard domestic conditions increased production of COPs [58]. Thermal degradation of cholesterol causes all the major COPs of pathophysiologically interest to form [26]. Presence of several reactive oxygen species, unsaturated fatty acid, cholesterol, transition metals and in rare cases, enzyme are needed for COPs to form [59].

The highest 7-ketocholesterol production was found with combined roasting and microwave heating, followed by microwave alone, barbecuing, frying and roasting [22]. Meanwhile boiling was relatively the safest procedure. Furthermore frying oil repeatedly exposed to high temperature for a large number of cycles could enhance the amount of COPs present in food; therefore cholesterol free food can also be supplemented with considerable amount of COPs [60,61,62]. The level of 7-ketocholesterol increased linearly with heating time but not with

temperature [62]. Studies on the effects of heating were mostly conducted in frying oil such as tallow and lard. The formation of 7-ketocholesterol was nearly linear with heating time, reaching about 10% of the initial cholesterol content at 376 h heating 155°C.

The oxidation rate may also be facilitated in the presence of light [16]. Photo-oxidation of cholesterol mainly involves singlet oxygen and is catalysed by natural pigment such as chlorophylls, flavin and myoglobin [26]. Prolonging cooking time also can increase the COPs content of fried and boiled eggs [63].

An interesting case of the effect of these conditions on cholesterol oxidation has been observed in fresh meat. This product is generally packaged with transparent films that allow a complete exposure to light and oxygen, thus favoring lipid oxidation and, in particular, cholesterol oxidation [34]; this effect is mainly due to the presence of photocatalysers, such as hemoglobin and hematoporphyrines. An 8h exposure to neon light has proved to increase 10 times the initial COPs content present in the raw meat [34]. However, the intensity of oxidation directly depends on the radiation spectrum; in fact, the level of oxysterols in meat exposed to red light for 8h is only twice as much as the initial one [34]. It is, therefore, important to control the quality of the raw materials, in order to evaluate the actual effect of processing, handling and storage on the cholesterol oxidation of the final product. The rate of cholesterol oxidation in pork was greatly accelerated during storage following cooking and appeared to follow the same trend as lipid oxidation in general [64].

Food storage and packaging represents the crucial step in foodstuff preparation. It also plays a significant role in producing the COPs. The concentration of COPs in egg powders increases with time and temperature storage - the longer the time and the higher the temperature of storage - the bigger the amounts of oxysterols produced [65]. Aluminium foil as a light and gas barrier is important for preventing light-induced cholesterol oxidation in food which paper-based packaging materials cannot sufficiently prevent [66]. The presence of oxygen and light during storage also promote the oxysterol formation. The efficiency of vacuum conditions in reducing cholesterol oxidation during frozen storage was higher for cooked than for raw samples. Cooked samples stored aerobically showed the highest COPs amounts, especially roasted samples. This result indicates that cooking enhances cholesterol oxidation during storage [67].

Therefore, in order to avoid the formation of COPs, thermal treatments exposure to light and oxygen should be reduced to a minimum during food processing, handling and storage [26].

Overview of Cholesterol Analysis: Food analysis is expensive and complex and the methods vary in terms of cost, accuracy and complexity. The validation and re-validation of the analytical methods are necessary to obtain reliable data. Several methods were developed along the years to determine cholesterol in foods in order to obtain accurate results. Earlier data on cholesterol analysis before and during the early 1980s and even recently were produced by colorimetric, gravimetric and enzymatic methods. Colorimetric and gravimetric method lack specificity, requires a strict control of the analytic conditions in order to ensure accurate results [68-70].

Chromatography is preferred for cholesterol analysis in foods because of its specificity to separate cholesterol from other unsaponifiable compounds based on their differences in physical and chemical properties and interaction with stationary and mobile phases [71-73]. Meat products, especially processed meats with non-meat ingredients, contain not only cholesterol but also plant sterols, tocopherol, tocotrienol, saturated hydrocarbons, squalene, aliphatic alcohols, terpene alcohols, triterpene alcohols and steryl esters, all of which are unsaponifiable [73]. HPLC has been employed more because it is thought to decrease cholesterol oxidation at a lower operational temperature and allows for a non-destructive separation. However, the GC is still preferred for its high sensitivity compared with HPLC [74].

Analysis of COPs in Meat and Poultry: Determination of COPs involved very tedious and long steps. Most common analytical protocol used in COPs analysis, has more or less the same route: (i) extraction of COPs from food matrix; (ii) purification of the sample extract; (iii) derivatization to suitable compounds and (iv) quantification with a suitable chromatographic method [75,76].

Since studying on COPs in meat and poultry become more important nowadays, the quantification of COPs should be design in a way to guarantee efficient recovery and minimized the artifacts during sample clean-up and work-up steps. The quantification of COPs in food is difficult because there are interruptions by large amounts of interfering cholesterol, triglycerides, phospholipids and other lipids [75]. Technical difficulties with cholesterol oxide analysis may be due to similar chemical structures and presence of cholesterol oxides at trace levels (parts

per million). with cholesterol oxide analysis and become a major part due to similar chemical structures and presence of cholesterol oxides at trace levels (parts per million). Since COPs occur mostly at low levels, they need extensive workup and cleaning procedure before final quantification. Therefore, the extraction, purification and detection methods in quantifying COPs play a major role.

Purification, Saponification and Enrichment in COPs

Analysis: There are two methods of enrichment; saponification and transesterification. Saponification has two common procedures; cold and hot saponification. Cold saponification at 25°C for 18-22 hours has shown high recovery and low artefact formation [61]. Meanwhile, using hot saponification at 60°C for 45-60 minutes has reduced the saponification time. However, hot saponification leads to artifact formation by degrading 7-ketocholesterol and isomeric epoxides. The saponified triglycerides form a soap solution giving bad separation of the evolved emulsions and micelle formation, which leads toward loss of compounds of interest [77]. There are two more methods identified by previous studies in improving COPs analysis; has been identified by previous studies to overcome the previous problem on COPs analysis; direct saponification and transesterification. Direct saponification is chosen to simplify sample preparation procedure [78-81] and most studies showed that direct saponification has superior recovery and accuracy compared with conventional lipid extraction and saponification [79,82-86].

Direct saponification was more accurate in recovering total cholesterol content of standard reference materials (e.g. egg yolk and whole egg) from the National Institute of Standards and Technology [82]. Higher recoveries (99.8%), excellent precision [coefficient of variation (CV) of 1.74%] and slightly greater cholesterol concentrations in meat matrices was reported when compared with the traditional procedure [83]. Moreover, a comparison made on direct saponification of dairy fat, against conventional dairy fat extraction in dairy products yielded higher recoveries from samples made through the direct saponification method [85]. with a conventional dairy fat extraction technique in dairy products, similarly direct saponification delivered higher recoveries [85]. In the same study, direct saponification gave an improved precision for low-fat dairy products and cheese (CV of 1.49 against 3.16% of conventional fat extraction).

Another method used to free sterol and its oxide from the bulk of the accompanying lipids is transesterification. Transesterification is an alternative to saponification of extracted lipids of total lipids and subsequent enrichment of COPs [87]. Transesterification convert esterified

oxysterols and tryglicerides into fatty acid methyl esters in mild condition. After esterification, the lipid primarily consists of acid methyl ester, free cholesterol and its oxidation products and some minor apolar and polar compounds.

Derivatization of COPs: Determination of cholesterol using gas chromatography usually needs to go through the derivatization process to improve volatility and thermo stability, to optimize peak shape, to decrease retention time and to increase sensitivity [72,73,88]. Trimethylsilyl (TMS) ether was the most commonly used derivative for many years until now [73,83,85,88-91]. Compared with acetate derivatives, TMS derivatives are more suitable for GC analysis with mass spectroscopic (MS) quantitation because they allow more informative fragment ions upon ionization [93,94]. The TMS reagents and derivatives are easily hydrolyzed; therefore, moisture-free environment to handle derivatized samples and moisture trap for the GC system are usually required [73]. However, cholesterol can be analyzed without derivatization with the peak possibly being subjected to distortion or tailing because of its interaction with silanol groups on the column surface [73,95,96]. On the other hand derivatization can create artifacts that contaminate the detectors, especially FID [11,73]. As part of a collaborative study of several sample treatments for cholesterol quantitation in foods, pretreatments such as preparative chromatography or derivatization were not required because pretreatment could lead to loss of analyte [81,96-99]. There was no difference between results obtained by GC analysis of free cholesterol and those of TMS or acetate derivatives [99].

Quantification of COPs: Another important step in COPs analysis is quantification. COPs are present in small amount in food. Detection and quantification are very challenging in order to give a good result. COPs have different characteristics such as diverse polarity and chemical properties due to the different functional groups in their chemical structure. Isomers of some COPs show similar chemical, spectrometric and fragmentation characteristics. Therefore determination of COPs required sensitive methods and systems. Many research has been carried out on the analysis system for the determination of COPs in various type of samples. Thin layer chromatography (TLC), High Performance Liquid Chromatography (HPLC) and GC are the most common method used to detect COPs in previous studies [100].

It has been reported in a HPLC analysis with a spectrometric detection, that the inability in detection of some human harmful COPs is due to poor UV absorption

[101] Therefore, HPLC coupled to Mass Spectroscopy (MS) detectors has become a very powerful tool over the past years due to the increment of the sensitivity [22].

Another instrument is GC. There are few factors affecting COPs response in GC such as injection technique and conditions, reagents and conditions use to get derivatives [102]. Cholesterol derivatives can be detected and quantified accurately by either Flame Ionisation Detector (FID) or MS detector in GC [73,93,95]. The FID gives good sensitivity and a wide range of linearity; and it is widely used for cholesterol determination in foods including meat and poultry products [73,85,91,96,103,104].

In recent years, the MS detector has become more has become increasingly important, coupling with both GC and HPLC to provide better quantitative information if cholesterol co-elutes with other unsaponifiable compounds [73,93,95,105]. The best combination of techniques to identify COPs rapidly and sensitively is GC-MS with a selected ion monitoring mode (SIM) and a capillary column [61,102]. The outcome of this, in the form of mass spectrum, can be compared with the chemical library to identify COPs with high accuracy.

The success of GC-MS technique requires efficiency of derivatization procedures, especially on steroids containing hydroxyl groups because the conversion of hydroxyl groups to TMS ether groups makes the compounds more responsive to the ionization process [105].

CONCLUSION

Cholesterol is a molecule with an unsaturated or double bond; therefore, therefore making it prone towards oxidation and COPs formation COPs. COPs have various structures depending on the type of oxidation and the physical state of the substrate. The impact of COPs towards human health depends on the type of COPs formed. Extraction, purification and detection methods in quantifying COPs play an important role in the analysis as COPs occur mostly at low levels. Direct cold saponification was the most suggested method due to high efficiency, good method precision, minimal artifact formation and ease of handling to recover COPs. HPLC and GC are the most common methods used to detect COPs in previous studies. The MS detector has become more and more important, coupling with both GC and HPLC to provide better quantitative information if cholesterol co-elutes with other unsaponifiable compounds. In some of the researches, COPs need to some of the research COPs need to go through the derivatization process prior to GC analysis to improve

volatility and thermo stability. However, it makes no difference between GC analysis of free COPs and those of TMS or acetate derivatives. The GCMS is the most commonly used method to determine COPs for many food samples including meats and poultry. Since COPs are dangerous to human health and occur at low levels, method development on extraction, purification and quantification of COPs at trace levels may become more important for further research.

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