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Review

7-Ketocholesterol as marker of cholesterol oxidation in model and food systems: When and how

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ABSTRACT

Cholesterol can undergo oxidation through enzymatic or chemical mechanisms, generating a wide range of oxidation products (COPs) with adverse biological effects. COPs are characterized by different functional groups and are produced in different ratios or amounts, depending on the treatment and storage conditions. To follow the cholesterol oxidation process, 7-ketocholesterol (7-KC) has been often used as an oxidation marker in both model and food systems, since it is easily formed and is one of the most representative ring COPs. However, 7-KC does not always rise with increasing time/temperature conditions, especially in complex systems and high-protein or extensively processed foods. The following review provides a critical picture of the utilization of 7-KC as a cholesterol oxidation marker in model and food systems, focusing on the possible causes and effects of the different behaviours and trends, as well as on the advantages and disadvantages of using 7-KC when the extent of cholesterol oxidation is to be assessed.

Contents

1. Introduction

Cholesterol is a monounsaturated constituent of cell membranes and is involved in their permeability and fluidity. Due to the presence of a double bond (carbon 5), a wide range of cholesterol oxidation products (COPs) can be produced endogenously or exogenously through different reaction mechanisms and pathways (chemical, photosensitized and enzymatic oxidation). Metabolic dysfunctions or the frequent consumption of COP-containing foods can be potentially harmful to health, since COPs can have negative biological actions (atherogenic, cytotoxic, mutagenic, apoptotic and carcinogenic effects), and are likely to be involved in several chronic and degenerative diseases, as well as in disturbances of cell functionality and lipid metabolism [1–3]. Several reviews have

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discussed the routes of oxysterol formation and their major biological effects [1–6].

The first products of cholesterol oxidation are hydroperoxides (ROOH, mainly in positions 5 and 7), which can undergo dismutation that generates 7α-hydroxycholesterol (7α-HC) and 7β-hydroxycholesterol (7β-HC), together with 7-ketocholesterol (7-KC). The formation of epoxy derivatives (5α,6α-epoxycholesterol (α-EC) and 5β,6β-epoxycholesterol (β-EC)) occurs via a bimolecular reaction mechanism through the interaction of a hydroperoxy radical and cholesterol [4]. In the presence of water and acidic conditions, epoxy derivatives in turn can experience oxirane-ring opening and thus produce cholestanetriol (CT). Generally, ring COPs tend to be formed non-enzymatically, whereas side-chain oxysterols usually have an enzymatic origin, except for 25-hydroxycholesterol (25-HC) and 7α-HC, which can be produced by both routes [2].

7-KC has been often used as a marker of cholesterol oxidation in both model and food systems, since it is easily formed and is one of the most representative oxysterols (>30% of total COPs) [4,7]. Several kinetic models have been proposed for cholesterol oxidation in food and model systems [6]. However, 7-KC does not always rise with increasing time/temperature conditions, especially in complex systems and high-protein or highly processed foods. The stability of cholesterol in complex mixtures is influenced by interactions among these components and/or their decomposition products. Moreover, the large number of molecules generated through oxidation, together with the presence or absence of antioxidants and proxidants and reactions with other macromolecules (proteins, carbohydrates, lipids) cannot only shift the cholesterol oxidation rate, but also modify the shape of the oxidation curve itself and the relative oxysterol distribution.

This review aims to provide a critical view of the utilization of 7-KC as a marker of cholesterol oxidation in model and food systems, focusing on the possible causes and effects of the different behaviours and trends, as well as on the advantages and disadvantages of this marker choice.

2. Model systems

In most model system studies, 7-KC has been reported as the most abundant COP (30–70% of total COPs). Cholesterol degradation follows a first order kinetic model if cholesterol is present as a solid or in solution and is subjected to either thermoxidation or photoxidation [6]. A first order kinetic model has also been suggested for 7-KC formation when cholesterol was thermoxidized in the solid state at 150 °C [8]. Cholesterol has proven to be virtually stable during heating at 100 °C for 24 h, but is unstable at temperatures above 120 °C [9]. When cholesterol was heated at 140 °C, only 7-hydroperoxycholesterol was formed until 213 s, whereas 7-KC, 7α-HC and 7β-HC were generated between 213 and 593 s; this was subsequently followed by the formation of α-EC and β-EC [10]. Cholesterol degradation at 140 °C occurs slowly, as this temperature is below its melting point [11]. However, this behaviour was not observed at higher temperatures (180 and 220 °C), where all these five COPs were already present at the first sampling time point [10]. At these high temperatures, cholesterol not only generates the most common COPs, but is also involved in the formation of dehydration compounds, oligomers and volatile compounds [10,12]. Moreover, under these conditions, 7-KC can also dehydrate and give rise to cholesta-3,5-dien-7-one. The latter can also be generated during photoxidation at room temperature (RT), probably due to 7-KC dehydration through energy released during light exposure [13].

The physical state of the model system (liquid or powder) can also affect the rate of 7-KC formation. In model food powders [14], surface composition and structure greatly influenced the cholesterol oxidation rate. Although α-EC was always the most abundant COP at the beginning of degradation (regardless of model food powder composition), 7-KC and 7β-HC became the predominant oxysterols after 6-month storage in darkness at RT [14]. In aqueous model systems, the pH value can also modify the trend of cholesterol oxidation, since CT formation is greatly favoured under acidic conditions. The pH conditions can also affect the activity of proxidant or antioxidant compounds, since they can modify the molecule chemical properties (protonated or reduced forms). Ionizing radiation can also generate COPs in aqueous systems; although the products are similar to those formed by autoxidation, they present different relative amounts [15]. When exposed to gamma radiation, the main COPs are 7-KC and the 5,6-epoxy derivatives.

On evaluating the influence of the degree of unsaturation of different triacylglycerols (TAG) upon cholesterol thermoxidation at 180 °C, 7-KC was found to be the most abundant COP, except in the presence of trilinolenin where 7β-HC was predominant [16]. Furthermore, when cholesterol was heated alone or with tristearin, a decrease in 7-KC was noted after 20 and 120 min, respectively, yielding an overall decrease in total COPs. Other authors have noted similar 7-KC behaviour under analogous thermoxidation conditions, but the decline in 7-KC was faster probably due to the different heating transfer modalities and sample amount:vial volume ratios involved [17]. Cholesterol degraded more rapidly when it was heated alone than in the presence of TAG, which could be ascribed to a dilution effect, TAG physical protection against oxygen contact and/or TAG competition for oxidation. TAG physical arrangement and molecular hindrance, as well as chemical group interaction and viscosity increase due to polymerization, might also have influenced these trends. Another research group [18] incubated cholesterol with fish oil TAGs (with different degrees of unsaturation) at RT; although 7-KC rose continuously during the whole 39-day storage period, both 7β-HC and β-EC became the most abundant COPs after 27 days. In an analogous study [19], 7-KC was found to be a reliable marker in all cholesterol-TAGs model systems oxidized at 100 °C for 24 h, but dropped after 12 h in the most unsaturated TAG systems (linseed and sardine oils).

When cholesterol was oxidized in the presence of superoxide anion, water and hydrogen peroxide, α-tocopherol, butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) markedly retarded the formation of 7-KC [20]. A protective role of carotenoids against 7-KC formation in solution has been also observed [21]. When evaluating the combined effect of riboflavin and fatty acid methyl esters (FAME) upon cholesterol photoxidation (25 °C for 28 days), the most relevant COPs were either 7-KC or β-EC, depending on the amount of antioxidant and the type of FAME involved [13]. Moreover, 7-KC tended to decrease after 14 days of storage in all the trials, except when cholesterol alone was photoxidized. In another study [22], cholesterol was thermally oxidized at 140 °C in the presence of stearylamine, yielding epoxy and triol derivatives as the main oxysterols. Stearylamine was able to reduce both the oxidation and degradation rates of cholesterol, which could be due to the formation of antioxidant compounds through the reaction between amines (primary and secondary) and alkenals or epoxyalkenals [23].

3. Food

The behaviour of COPs and 7-KC in food greatly depends on the type of food matrix involved (physicochemical and enzymatic characteristics), as well as on the processing and storage conditions to which they are subjected. Therefore, the suitability and reliability of 7-KC as a marker of cholesterol oxidation should be assessed according to the food matrix involved.

3.1. Egg products and egg-based products

The great consumption of industrialized egg-containing foods (such as bakery products, salad dressings and pasta) has led to