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Short communication: Cholesterol oxidation products in traditional buttermilk

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ABSTRACT

The aim of this study was to quantitatively and qualitatively assess the content of cholesterol oxidation products in traditional buttermilk after butter production. Cholesterol oxidation products (COP) exhibit a wide spectrum of biological activity, including cytotoxic, carcinogenic, and pro-oxidative properties. Buttermilk has about 2 mg of COP/kg of fat, including 7β-hydroxycholesterol and 5,6α-epoxycholesterol. Buttermilk immediately after production had a relatively high level of 7β-hydroxycholesterol (1.47 mg/kg), which decreased to 0.61 mg/kg after storage for 10 h at 3°C. During storage, the content of 5,6α-epoxycholesterol increased from 0.50 to 1.40 mg/kg. After 10 h of storage, the antioxidant potential of the buttermilk decreased (expressed as radical scavenging ability; change in 1,1-diphenyl-2-picrylhydrazyl = 32.2%). This study showed the presence of COP in fresh and stored buttermilk and the influence of time on changing the direction of cholesterol oxidation.
Key words: buttermilk, cholesterol, oxysterol, DPPH

Short Communication

Traditional buttermilk (**BM**) is a natural product resulting from the butter-making process during sweet cream or cream churning. It is intended for direct consumption or as an ingredient of cultured buttermilk. The components of BM depends on its acidity, the composition of the cream, and the conditions of churning. In traditional BM obtained in a butter churn machine, the fat content varies from 0.15 to 0.4%, by the continuous cream method, fat content can reach 0.65%, and from sweet cream up to 0.75%. Buttermilk usually has lower acidity than the plasma of churned cream. It resembles skim milk in its composition and appearance but contains most of the milk fat globule membrane (**MFGM**; Fu et al., 2014). During the manufacture of butter, materials derived from the MFGM are recovered in the aqueous phase (BM) when milk fat globules are mechanically disrupted upon churning of cream. The MFGM fragments contain polar lipids (glycerophospholipids and sphingolipids), neutral lipids, proteins, glycoproteins, enzymes, and cholesterol (Fauquant et al., 2014). The major sterol present in milk—cholesterol—accounts for at least 95% of the sterols and is located mainly in the MFGM (Jensen and Newburg, 1995). Cholesterol content in milk ranges from 3.08 to 6.06 g/kg of fat (Mesilati-Stahy and Argov-Argaman, 2014); on average, it is 3.3 g/kg of fat, which equals 135 mg/kg of milk. In the MFGM, on the other hand, cholesterol content is 1.7 mg/m^2 MFGM (Et-Thakafy et al., 2017). For comparison, goat milk contains 1.5 mg/m^2 MFGM and sheep milk 1.8 mg/m^2. The content of cholesterol in MFGM is 40 mg/100 g of fat globules, which is 2 g/100 g of MFGM dry matter or 2% (Dewettinck et al., 2008). The amount of membrane fat globules is 1.9 m^2/g of fat. In MFGM, there is 1.7 mg/m^2 of cholesterol (Et-Thakafy et al., 2017). The estimated mass of the MFGM is 2 to 6% of that of the total fat globules (Lopez, 2011).

Cholesterol [(3β)-cholest-5-en-3-ol; **Ch**] is a 27-carbon animal sterol. Its structure contains a tetracyclic cyclopentanoperhydrophenanthrene, an 8-carbon saturated side chain, and a hydroxyl group in the 3β position. Cholesterol levels in dairy products depend on, among other factors, the fat content, heat treatment, milk homogenization, and the type of lactic acid bacteria present. Milk contains approximately 12 mg of cholesterol per 100 g (Fletouris et al., 1998). According to Kovács et al. (2004), low-fat dairy products have a larger share of the Ch in fat high-fat products, because the small fatty beads have a relatively large surface area, which accumulates Ch.

Cholesterol, due to the presence of Δ^5 unsaturated bond, is subject to numerous chemical reactions, including oxidation. Ex vivo oxidation of Ch occurs primarily through free radicals under the influence of heat, UV, or ionizing radiation in the presence of oxygen. Oxidation of the carbon at the C-7 position is the easiest to accomplish. Through the intermedi-

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ate product, 7-hydroperoxide, isomeric hydroxyl derivatives are formed: 7β-hydroxycholesterol (**7β-OHC**) and 7α-hydroxycholesterol (**7α-OHC**). Another route is the oxidation of Ch on the carbon atoms forming the double bond (C-5 and C-6), where epoxidation occurs easily. As a result, isomeric epoxides are formed, including 5,6α-epoxycholesterol (**α-epoxyC**) and 5,6β-epoxycholesterol (**β-epoxyC**). Many researchers have reported that cholesterol oxidation products (**COP**) in foods can reach 1% of total cholesterol and occasionally 10% or more (Hur et al., 2007). In addition to cholesterol, the formation of COP requires reactive oxygen species and is favored by the presence of UFA or transition metals; in some rare cases, the oxidation is enzymatic (Rose-Sallin et al., 1996; Hur et al., 2007). The probability of COP forming in fresh dairy products is very low because the medium is liquid and the oxygen content is low (Sieber, 2005). Milk has a low level of PUFA and of pro-oxidant trace elements such as iron and copper. However, oxidation in fresh dairy products may be dominated by 7β-OHC and α-epoxyC (Kumar and Singhal, 1992). The formation of COP in milk and dairy products can only occur under extreme conditions, such as high temperatures for a long period or prolonged storage at high temperatures (Sieber, 2005).

In very low concentrations, oxysterols are natural components of the human body and mediate many physiological functions (Kloudova et al., 2017). They participate in the regulation of Ch metabolism. However, oxysterol action is also connected with human pathologies (e.g., atherosclerosis, Alzheimer's disease, Parkinson's disease). Cholesterol oxidation products exhibit a wide spectrum of biological activity, such as cellular cytotoxicity, immunosuppressive effects, mutagenicity, carcinogenicity, and pro-oxidative properties (Jusakul et al., 2013; Kulig et al., 2016). The major nonenzymatically formed oxysterols, 7-ketocholesterol (**7-ketoC**), 7β-OHC and β-epoxyC, have strong cytotoxic properties and are implicated in various pathological states (Lemaire-Ewing et al., 2005; Rimner et al., 2005; Massey, 2006). It has been suggested in the literature that COP produce different effects depending on whether they are applied separately or in mixtures. For example, a mixture of 7β-OHC and 25-hydroxycholesterol (**25-OHC**) had weaker pro-apoptotic effects than 7β-OHC alone (Aupeix et al., 1995; Kloudova et al., 2017). Because of the potential health risk of oxysterols, their formation and presence in foods have been the subject of many studies (Seckin and Metin, 2005). Bierzuńska et al. (2017) examined COP found in cheese from buttermilk and showed a correlation between the amount of oxysterols produced in cheese and the antioxidant potential. In the scientific and industrial literature, however, there is no information about the composition and concentration of oxysterols in traditional buttermilk. In addition, observations on traditional BM production technology have prompted us to consider the effect of storage time on the amount and type of COP. The time that elapses between the end of churning and the further use of BM induces changes that may affect the direction of Ch oxidation, the number and type of COP, and the antioxidant potential. In these experiments, attention has been focused on the most common and abundant COP, including cholesterol 25-OHC, cholestanetriol (**triolC**), 7α-OHC, 7β-OHC, 7-ketoC, and α- and β-epoxyC.

The research material was traditional buttermilk, which was left over after the industrial production of butter from sour cream. The butter production used traditional methods, sometimes on an industrial scale. To cream, comprising 35% fat [9° Soxhlet-Henkel (**SH**) of plasma] after pasteurization (92°C for 30 s), the starter culture Lyofast M030N (Sacco, Cadorago, Italy) was added at 10 units of activity per 1,000 L of cream. The sour cream, comprising 35% fat, pH 5.5 (13.1°SH, 21°SH of plasma), was churned at 10°C for 55 min to achieve a butter grain size of 2 to 4 mm. The BM was taken to the tank by a gravity drain directly after the churning process and immediately cooled to 3 ± 0.5°C (sample **BM-0**). Buttermilk was stored in a covered fermentation tank at 3 ± 0.5°C for 10 h (sample **BM-10**). The tank was manufactured from high-alloy austenitic steel (type 316L-AISI, X2CrNiMo 17 13 2-DIN), a stainless chromium-nickel steel, cold-rolled sheet, annealed, with surface roughness (roughness average, Ra) of 0.6 μm. The current study examined the composition and properties of BM using the methods previously described by Cais-Sokolińska et al. (2016, 2018). The isolation of lactic acid bacteria and determination of the proteolytic and lipolytic activity of the microorganisms was carried out following the method of Bettache et al. (2012).

The main element of the study was to analyze the content of Ch and COP. Fat from BM was extracted according to the Röse-Gottlieb procedure following ISO method 14156 (ISO, 2001). The Ch content was determined using an AOCS Official Method (AOCS, 2009) by gas chromatography, after a prior sample saponification with 1 mol/L KOH in methanol for 18 h at room temperature and triple extraction of the unsaponifiables with hexane:*tert*-butyl methyl ether (**MTBE**) 1:1 (vol/vol). The solvent was evaporated under a stream of nitrogen. The dry residues were dissolved in 0.1 mL of pyridine and silylated with 0.4 mL of Sylon BTZ (Supelco, Bellefonte, PA). Derivatives of the sterols were separated on an HP 6890 gas chromatograph equipped with a DB-35MS capillary column (25 m × 0.20 mm; 0.33 μm; J&W Scientific, Folsom, CA). A sample was