Evaluation of antioxidant activity of whey protein to improve cholesterol oxidation stability in fresh white cheese from buttermilk

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Summary

The health-promoting value of fresh white cheese made from buttermilk can be increased by adding to it a whey protein concentrate (WPC). At the same time, the antioxidant potential of whey protein can be used to stabilize the lipid fraction. WPC-enriched cheese had higher ferric reducing antioxidant power (*FRAP*) values than the control buttermilk cheese. The 1,1-diphenyl-2-picrylhydrazyl radical-scavenging activity (*DPPH*) was also higher in WPC-enriched cheese, but not as noticeable as in the case of the *FRAP* assay results. A strong correlation was established between *FRAP* and *DPPH* antioxidant activity values, and the amount of cholesterol oxidation products in WPC-enriched cheese. The longer the WPC-enriched buttermilk cheese was stored, the lower the number of cholesterol oxidation products (mostly 7β-hydroxycholesterol and 5,6-β-epoxycholesterol) was determined. After 30 days, the content of total cholesterol oxidation products in WPC-enriched cheese was 4.7-fold lower than in the control cheese.

Keywords

cheese; buttermilk; antioxidant activity; whey protein

Dairy industry is one of the most innovative food sectors. Consumers seek out those dairy products that provide for the proper physiological functioning of the body and that contain bioactive components [1, 2]. Products with new or improved ingredients and health-promoting properties are appearing on the market, including acid whey cheeses. The most important cheese in this assortment group is fresh white cheese (tvorog, quark, twaróg, biały ser, farmer cheese, Weißkäse, beli sir, tvaroh). The raw substance used in the production of traditional fresh white cheese is milk subjected to acid or acid–rennet coagulation. Milk can also be replaced by buttermilk or a mixture of non-fermented milk and BM. The use of buttermilk in cheese was mentioned by FERREIRO et al. [3]. Buttermilk resembles skim milk in composition and appearance, but contains most of the milk fat globule membrane (MFGM), which is composed of phospholipids, including phosphatidylcholine (lecithin), phosphatidylethanolamine and sphingomyelin. Due to high concentration of polyunsaturated fatty acids, buttermilk is susceptible to oxidative deterioration [4]. MFGM contains a mixture of proteins, glycoproteins and phospholipids, all of which can act as emulsifiers and help the fat globule remain suspended in milk. Since buttermilk contains MFGM (along with casein), it is an interesting source of ingredients with the potential to impart certain specific characteristics to cheese made from it [5]. Using buttermilk in cheese production is also justified economically.

In traditional cheese-making, casein is almost the exclusive curd structure. Casein contains four fractions: αS1-casein, αs2-casein, β-casein, and κ-casein. Almost the entire whey protein fraction, β-lactoglobulin (β-LG) and α-lactoalbumin (α-LA), upon precipitating at isoelectric point and curd processing, remains in solution and is lost in whey. This represents a very significant huge loss, because whey proteins have antioxidant properties [6]. These properties depend on, among others, the presence of sulfuric amino acids in the structure. More precisely, β-LG is the

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source of γ-glutamylcysteine dipeptide, the precursor of glutathione that possesses strong antioxidant properties [7]. The main components of antioxidant peptides are the amino acid residues of histidine, tyrosine, methionine, lysine, and tryptophan, which are also antioxidants in their free form. It is not only the amino acid sequence, but also the spatial configuration of the peptide, that give it its antioxidant properties [8, 9]. The ability to neutralize free radicals and inhibit enzymatic and non-enzymatic oxidation of lipids is exhibited by a fragment of β-LG, a peptide with the sequence WYSLAMAASDI. This peptide, obtained through proteolysis by corolase PP, demonstrated a greater ability to neutralize free radicals than did butylhydroxyanisole. β-LG and α-LA may also release peptides of sequences MHIRL and YVEEL, both exhibiting antioxidant properties [10].

In dairy products, the initiators of free-radical processes are the cholesterol oxidation products (COPs), which are highly reactive components. The most bioactive and cytotoxic COPs include cholesterol 25-hydroxylase (25-OHC), cholestanetriol (triolC), 7α- and 7β-hydroxycholesterol (7α-OHC and 7β-OHC), 7-ketocholesterol (7-ketoC) and epoxy derivatives of cholesterol, 5.6β- and 5.6α-epoxycholesterol (β-epoxyC and α-epoxyC) [11]. Their presence in milk depends on the initial content of cholesterol, temperature and time of storage, as well as on availability of light and oxygen [11, 12]. Milk fat in cheeses is considered oxidatively more stable than, for example, milk powder. Only limited data are available in scientific literature on analysis of oxysterols in fresh cheese, and even fewer studies regarded oxysterols in buttermilk.

The objective of this study was to utilize whey proteins and employ their antioxidant potential to stabilize the lipid fraction of buttermilk cheese. The evaluation of stability was based on the determination of cholesterol oxidation products in cheeses made from buttermilk.

MATERIALS AND METHODS

Collection, packaging, storage and sampling of cheeses

The research material was fresh white cheese made from buttermilk, a left-over after the industrial production of butter from cream (Great Poland). Model cheese parts ($n = 7$) were made on a pilot scale (Poznań/Września, Poland). The cheese was prepared from buttermilk, with or without the addition of whey protein concentrate (WPC). For this purpose, 2.2 kg of a commercial powder product, sweet WPC80 (Spomlek Dairy Cooperative, Radzyń Podlaski, Poland), was dissolved in 5 l of whey after the cheese had been made from buttermilk. Then, a solution of 4 cm^3 of 60% $CaCl_2$ was added to a 50-litre mixture of buttermilk and WPC to integrate the buttermilk-based casein and WPC-enriched whey proteins introduced to it. From that point on, the cheese-making process proceeded unchanged. The technical and technological conditions of the buttermilk processing, with and without the addition of WPC, were chosen so that coagulation occurred after 2 h at 50–52 °C. The curd had titratable acidity of 0.7 % (expressed as percentage of lactic acid) and pH of 4.6. It was sliced into cubes 10 cm long, and then 4 cm long, which were later stirred and heated to 34 °C. The heating rate was 1 °C per 10 min. The titratable acidity of the whey after heating did not exceed 0.6 % of lactic acid. Draining was performed in a layer ($h = 10$ cm) on a special curd-finishing table. Ironing took 3.5 h at the initial and final temperatures of 20 °C and 10 °C, respectively, and at a maximum pressing force of 3 kg per 1 kg of cheese. Fresh white cheese made from buttermilk and buttermilk with WPC was then shaped into cubes weighing 170 g each and packaged in paper foil. The cheese was stored at 4 ± 0.5 °C and examined within the first 24 h (Day 0) and then after 10, 20 and 30 days.

Compositional analysis

Moisture in the cheese was determined by standard methods AOAC 926.08 [13]. The content of total nitrogen (*TN*) was determined by the Kjeldahl method [14] with the assistance of the distillation unit of the Kjeltec System 1026 apparatus (Tecator, Örebro, Sweden). Content of casein nitrogen (*CN*), non-casein nitrogen (*NCN*) and non-protein nitrogen (*NPN*) were determined according to Svanborg et al. [15]. Content of total protein (*TP*) and whey protein (*WP*) were calculated according to the following equations:

$$TP = (TN - NPN) \times 6.38 \quad (1)$$

$$WP = (NCN - NPN) \times 6.38 \quad (2)$$

where number 6.38 represents the factor indicated for protein derived from milk.

Fat content and titratable acidity (expressed as percentage of lactic acid) in the cheese were determined by standard methods ISO 1735 and AOAC 920.124, respectively [16, 17]. The pH value of the examined cheeses was determined by standard methods using a pH-meter CP-315 (Elmetron, Zabrze, Poland), which was equipped with a combined ESAgP-301W electrode (Eurosensor, Gli-

wice, Poland), consisting of a glass half-cell and a saturated chloro-silver half-cell [18].

Fat from milk and cheese was extracted according to Röse–Gottlieb procedure following the method ISO 14156 [19].

Determination of cholesterol

The cholesterol content was determined using AOCS Official Methods Ch. 6–91 [20] by gas chromatography, after saponification with 1 mol·l^{-1} KOH in methanol for 18 h at room temperature and triple extraction of the unsaponifiables with hexane:*tert*-butyl methyl ether (MTBE) 1:1, v/v. The solvent was evaporated under a stream of nitrogen. Dry residues were dissolved in 0.1 ml pyridine and silylated with 0.4 ml of Sylon BTZ (Supelco, Bellefonte, Pennsylvania, USA). Derivatives of the sterols were separated on a gas chromatograph HP 6890 equipped with a DB-35MS capillary column (25 m × 0.20 mm; with a stationary phase film of 0.33 μm; J&W Scientific, Folsom, California, USA). A sample was injected in splitless mode. Column temperature was held at 100 °C for 5 min, then programmed to rise to 250 °C at 25 °C·min^{-1}, held for 1 min, then to rise to 290 °C at 3 °C·min^{-1}, and held for 20 min. The detector temperature was set to 300 °C. Hydrogen was used as a carrier gas at a flow rate of 1.5 ml·min^{-1}. 5α-Cholestane was used as the internal standard for sterol quantification. Identification was based on the retention data for compounds previously verified by mass spectrometry [21].

Determination of cholesterol oxidation products

COPs were determined according to the methodology described by Przygoński et al. [22]. Isolated fats were esterified with 10% sodium methylate in MTBE (4:6, v/v) and cholesterol together with its oxidation products, were extracted with chloroform and then fractionated on a SEP–PAK NH2 column (Waters, Milford, Connecticut, USA). Chromatographic analysis of silylated oxysterols was performed on a Hewlett-Packard 6890 apparatus (Hewlett-Packard, Palo Alto, California, USA). Separation was run on a DB-5 capillary column (30 m × 0.25 mm, with a stationary phase film of 0.25 μm; J&W Scientific), using a programmed temperature at a rate of 25 °C·min^{-1} from 60 °C to 270 °C, followed by 2.5 °C·min^{-1} until 290 °C was reached. Helium was used as a carrier gas at a flow rate of 1 cm^3·min^{-1}. The injector and detector were held at 300 °C and the split ratio at 1:40. 19-Hydroxycholesterol was used as an internal standard. Oxysterols were identified using retention data for compounds previously verified by mass spectrometry utilizing our library and published data [21].

Ferric reducing antioxidant power assay

Ferric reducing antioxidant power (*FRAP*) evaluates the ability of the analysed substance to reduce the complex of Fe(III)–2,4,6-tris(2-pirydyl)-*s*-triazine (TPTZ) to the form of Fe(II)–TPTZ [23]. The intensity of the blue colour, measured spectrophotometrically at 583 nm using apparatus RayLeigh UV-1601 (Beijing Rayleigh Analytical Instruments, Beijing, China) was linearly correlated with the reducing agent concentration. Results were presented as millimoles of Fe^{2+} per litre, based on a standard curve

$$y = 0.0001x + 0.0113;\ r^2 = 0.9938 \quad (3)$$

where y is absorbance, x is standard (Fe II) or evaluated sample concentration and r^2 is coefficient of determination.

DPPH radical-scavenging assay

The ability of the antioxidant to scavenge stable 1,1-diphenyl-2-picrylhydrazyl (DPPH) radicals was evaluated spectrophotometrically at 517 nm using RayLeigh UV-1601, in relation to the radical-scavenging ability of the reference substance, 6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox) [24]. The absorbance decrease is a result of the radical-scavenging ability of the substance. Results were presented as millimoles of Trolox equivalents (TE) per kilogram, based on a standard curve:

$$y = 83.8x;\ r^2 = 0.9635 \quad (4)$$

where y is percentage of reduced DPPH radical, x is standard (Trolox) or evaluated sample concentration and r^2 is coefficient of determination.

Statistical analysis

The obtained data ($n = 7$) were expressed as mean values and the respective standard deviations, and analysed by using repeated-measures ANOVA. The correlation coefficient significance tests were based on the assumption of normal distribution of the residual value of y variable and an equal residual value variation for all values of the x variable. In order to eliminate deviations from Pearson's distribution linearity, which could cause an increase of the square sum of deviations from the regression line, a scatter diagram analysis was performed. Paired *t*-tests were used for the calculations. For the verification of statistical hypotheses, a level of significance of $\alpha = 0.05$ was adopted. The statistical calculations were made using Statistica data analysis software system, version 10 (StatSoft, Tulsa, Oklahoma, USA).

RESULTS AND DISCUSSION

The buttermilk used to produce the cheese contained 0.4 % fat, 3.3 % protein and 3.8 % lactose. Titratable acidity was at the level of 0.4 %. WPC80 contained 78.8 % protein, 1.6 % lactose and 1.2 % mineral salts. Titratable acidity, pH, moisture, fat and fat in dry matter did not change significantly with WPC usage in fresh white cheese made from buttermilk with and without WPC.

An important result of the study was to demonstrate that it was possible to incorporate whey proteins into buttermilk cheese (ChB). The inclusion of WPC and $CaCl_2$ (ChB-WPC) significantly increased the total content of protein in the cheese by 2.1 %, $p < 0.05$ (total protein content in the ChB-WPC was 172.6 g·kg^{-1}) (Tab. 1). The ratio of casein to whey protein in ChB was only 20.3, while the ratio was 17.3 ($p < 0.05$) in ChB-WPC. Part of the whey proteins integrated in casein could have come from concentrate and the buttermilk itself.

The literature repeatedly emphasized the nutritional and structure-forming role of whey proteins [25–27] and the properties of buttermilk cheese [28–30]. After being long considered a low-value by-product, buttermilk is now widely used both as the basic ingredient in beverages and certain types of cheese, and as an ingredient of other foods. It is characterized by a high emulsifying capacity, it adds flavour and it provides certain functional and technological properties due to the high phospholipid content [31]. Buttermilk is higher in MFGM protein and phospholipids than skim milk, which may alter the functional properties of its WPC [14]. In the dairy industry, various techniques were developed for whey protein introduction [26]. Most of these are based on adding denatured and aggregated whey proteins to cheese milk. What may be problematic is to determine the size and water-holding capacity of such aggregated whey proteins [32]. Larger aggregates readily interfere with the casein network and are poorly retained in cheese curd. According to Saffon et al. [32], another issue associated with the incorporation of aggregated proteins is related to their effect on cheese moisture. Excessive cheese moisture is a common defect that limits the amount of whey protein that can be added to cheese milk. Whey proteins have the ability to bind, particularly with serum-phase κ-casein after the dissociation of κ-casein from micelles [33]. Ye et al. demonstrated that β-LG and α-LA associated with the MFGM by thiol–disulfide bonds in heated milk [34, 35]. Co-denaturation of cheese whey and buttermilk concentrates leads to the formation of protein aggregates. Higher proportions of buttermilk protein in the mixtures increased yields and decreased the water-holding capacities of the

Tab. 1. Physico-chemical characteristics and antioxidant activity of fresh white cheese made from buttermilk.

Parameters	Cheese without WPC	Cheese with WPC
Moisture [g·kg^{-1}]	782.5 ± 1.3^{a}	785.6 ± 1.6^{a}
Total nitrogen [g·kg^{-1}]	28.6 ± 0.3^{a}	30.3 ± 0.4^{b}
Non-protein nitrogen [g·kg^{-1}]	3.1 ± 0.7^{a}	3.2 ± 0.5^{a}
Non-casein nitrogen [g·kg^{-1}]	0.2 ± 0.7^{a}	4.5 ± 0.9^{a}
Total protein [g·kg^{-1}]	169.1 ± 0.5^{a}	172.6 ± 0.3^{b}
Casein [g·kg^{-1}]	142.3 ± 0.1^{a}	143.3 ± 0.4^{a}
Whey protein [g·kg^{-1}]	7.0 ± 0.4^{a}	8.3 ± 0.5^{b}
Total protein in dry matter [%]	77.8^{a}	80.5^{b}
Fat [g·kg^{-1}]	21.2 ± 0.5^{a}	20.8 ± 1.0^{a}
Fat in dry matter [%]	9.8^{a}	9.7^{a}
Titratable acidity [%]	1.6 ± 0.1^{a}	1.6 ± 0.1^{a}
pH	4.62 ± 0.02^{a}	4.60 ± 0.01^{a}
Salt [g·kg^{-1}]	0.8 ± 0.4^{a}	1.1 ± 0.3^{a}
Cholesterol [g·kg^{-1}]	12.32 ± 1.85^{a}	12.21 ± 1.32^{a}
FRAP [mmol·l^{-1}]	0.223 ± 0.010^{a}	0.687 ± 0.007^{b}
DPPH [mmol·kg^{-1}]	0.274 ± 0.042^{a}	0.334 ± 0.032^{b}

Values represent mean ± standard deviation ($n = 7$). Different small letters in superscript in a row indicate statistically significant differences at the level $\alpha = 0.05$.
WPC – whey protein concentrate. Titratable acidity is expressed as percentage of lactic acid. *FRAP* – ferric reducing antioxidant power expressed as millimoles of Fe^{2+}; *DPPH* – antiradical power expressed as millimoles of Trolox equivalents.

Tab. 2. Antioxidant activity of fresh white cheese made from buttermilk during storage.

Storage [d]	Cheese without WPC		Cheese with WPC	
	FRAP [mmol·l^{-1}]	*DPPH* [mmol·kg^{-1}]	*FRAP* [mmol·l^{-1}]	*DPPH* [mmol·kg^{-1}]
0	0.223 ± 0.010^{b}	0.274 ± 0.042^{b}	0.687 ± 0.007^{d}	0.334 ± 0.032^{c}
10	0.239 ± 0.022^{b}	0.216 ± 0.009^{a}	0.689 ± 0.013^{d}	0.290 ± 0.020^{b}
20	0.189 ± 0.019^{a}	0.209 ± 0.028^{a}	0.414 ± 0.029^{c}	0.285 ± 0.015^{b}
30	0.206 ± 0.036^{b}	0.186 ± 0.015^{a}	0.257 ± 0.017^{b}	0.269 ± 0.058^{b}

Values represent mean ± standard deviation (n = 7). Different small letters in superscript in columns indicate statistically significant differences at the level α = 0.05.
WPC – whey protein concentrate. *FRAP* – ferric reducing antioxidant power expressed as millimoles of Fe^{2+}; *DPPH* – antiradical power expressed as millimoles of Trolox equivalents.

aggregates. Preventing the formation of disulfide bonds between denatured whey proteins increased WHC of such aggregates [32].

The cholesterol content in ChB and ChB-WPC cheese did not differ (12.32 g·kg^{-1} and 12.21 g·kg^{-1} fat, $p > 0.05$). This was the condition for studying the effect of the addition of WPC on its oxidation. After considering the fat content in ChB and ChB-WPC cheese, average cholesterol content was 258 mg·kg^{-1}. Cholesterol content was much lower than indicated by Fletouris et al. [36] in mozzarella (714 mg·kg^{-1}), gouda (881 mg·kg^{-1}), feta (681 mg·kg^{-1}) or butter (2281 mg·kg^{-1}). Cholesterol levels in dairy products depend, among other factors, on fat content, heat treatment, milk homogenization and type of lactic acid bacteria. Milk contains approximately 120 mg of cholesterol per kilogram [36]. According to Kovacs et al. [37], low-fat dairy products have a larger share of cholesterol in fat than high-fat ones, as the small fatty beads, which accumulate cholesterol, have a relatively large surface area.

On Day 0 and Day 10 of storage, the cheese with WPC showed a three-fold higher ($p < 0.05$) antioxidant activity ($\Delta FRAP$ = 0.464 mmol·l^{-1} and $\Delta FRAP$ = 0.450 mmol·l^{-1}, respectively) than ChB cheese (Tab. 1, Tab. 2). After 30 days, *FRAP* of WPC cheese decreased by 62.6 %, but only by 7.6 % in the case of the cheese made from buttermilk. However, after the same time, their antioxidant activities did not differ significantly (0.206 mmol·l^{-1} and 0.257 mmol·l^{-1}, respectively). After production and during the entire storage time, WPC cheese had a higher antiradical power than the cheese made solely from buttermilk. After storage, antiradical power was decreased in WPC cheese ($\Delta DPPH$ = 32.1 %) as well as in ChB ($\Delta DPPH$ = 19.5 %).

Antioxidant activity of whey proteins had a significant impact on the resistance of cholesterol to oxidation. WPC cheese contained 4.5-fold ($p < 0.05$) more oxysterols, mainly 7α-OHC and α-epoxyC, than the buttermilk cheese (Day 0) (Tab. 3). When ChB-WPC cheese was stored longer, the amount of oxysterols decreased by 80 %. After 30 days of storage, only 7β-OHC and β-epoxyC were found in ChB-WPC cheese at a total level of 1.02 mg·kg^{-1} (fat basis). ChB buttermilk cheese, meanwhile, after the same period of time, contained the following: 7α-OHC, 7β-OHC, β-epoxyC, and α-epoxyC at a total level of 4.88 mg·kg^{-1} (fat basis). On Day 0 and Day 10, WPC-free cheese contained 25-OHC and 7-ketoC. A strong correlation was observed between the content of oxysterols in ChB-WPC cheese and *FRAP* (r = 0.793) and *DPPH* (r = 0.990). ChB cheese without whey protein had much smaller correlation coefficients of 0.215 and 0.789 for *FRAP* and *DPPH*, respectively. These coefficient values indicated that the quantity of oxysterols could not be compared with the size of the simultaneous antioxidant activity of whey proteins. The samples with WPC addition were characterized by higher *FRAP* values than the control cheese made from buttermilk. This higher activity was probably due to the presence of peptides in WPC. *DPPH* radical-scavenging activity was also higher in WPC-enriched cheese, but not that markedly as in the case of the *FRAP* assay results. Thus, the results showed a significant increase of antioxidant potential of samples with WPC addition over the 30 days of storage (Tab. 2). The expected antioxidant effect occured only in the subsequent period of storage. It seems that the method involving addition of $CaCl_2$ was effective in stabilizing cholesterol against oxidation. The addition of whey protein concentrate was also justified, although a better solution might be to introduce serum protein concentrate (SPC). SPC obtained by microfiltration from raw milk was referred to as native whey protein, milk microfiltrate protein, virgin whey protein or milk serum protein [38, 39]. Comparison of 80% WPC and SPC with commercial products showed higher levels of lipid oxidation in

Tab. 3. Levels of oxysterols in fresh white cheese made from buttermilk.

Storage [d]	7α-OHC [mg·kg^{-1}]	7β-OHC [mg·kg^{-1}]	β-epoxyC [mg·kg^{-1}]	α-epoxyC [mg·kg^{-1}]	triolC [mg·kg^{-1}]	25-OHC [mg·kg^{-1}]	7-ketoC [mg·kg^{-1}]	Σ COPs [mg·kg^{-1}]
Cheese without WPC								
0	0.12 ± 0.01^{a}	0.29 ± 0.01^{b}	0.28 ± 0.01^{a}	0.04 ± 0.01^{a}	nd	0.35 ± 0.02^{a}	0.09 ± 0.03^{a}	1.17
10	0.13 ± 0.02^{a}	0.10 ± 0.01^{a}	1.18 ± 0.04^{c}	1.56 ± 0.11^{d}	nd	0.37 ± 0.01^{a}	0.91 ± 0.01^{c}	4.25
20	nd	1.28 ± 0.02^{d}	nd	0.72 ± 0.07^{b}	nd	nd	nd	2.00
30	0.92 ± 0.07^{b}	1.25 ± 0.08^{d}	1.28 ± 0.04^{c}	1.43 ± 0.01^{d}	nd	nd	nd	4.88
Cheese with WPC								
0	1.21 ± 0.07^{c}	0.74 ± 0.08^{c}	0.90 ± 0.04^{c}	1.01 ± 0.01^{c}	nd	0.76 ± 0.01^{b}	0.53 ± 0.01^{b}	5.15
10	nd	1.90 ± 0.02^{e}	0.59 ± 0.01^{b}	nd	nd	nd	nd	2.49
20	nd	1.11 ± 0.08^{d}	nd	0.47 ± 0.03^{b}	nd	nd	nd	1.58
30	nd	0.62 ± 0.05^{c}	0.40 ± 0.05^{a}	nd	nd	nd	nd	1.02

Values represent mean ± standard deviation (n = 7). Different small letters in superscript in columns indicate statistically significant differences at the level α = 0.05.
WPC – whey protein concentrate. 7α-OHC – 7α-hydroxycholesterol; 7β-OHC – 7β-hydroxycholesterol; β-epoxyC – 5.6β-epoxycholesterol; α-epoxyC – 5.6α-epoxycholesterol; triolC – cholestanetriol; 25-OHC – 25-hydroxycholesterol; 7-ketoC – 7-ketocholesterol; Σ COPs – sum of cholesterol oxidation products. nd – not detected.

commercial WPC products [40]. SPC has unique functionalities, such as excellent solubility, gelling after heat treatment and foaming properties [41].

Cholesterol undergoes autoxidation in contact with air, the degree of formation of cholesterol oxidation products being mainly dependent on storage conditions [12]. SANDER et al. [42] showed that cheese spread, cottage cheese, evaporated milk, and whole milk did not contain any of the COPs. In cream cheese, only α-epoxyC and 7-ketoC were detected at 9 mg·kg^{-1} and 3 mg·kg^{-1}, respectively. In cheese spread, COPs were detected at 7 mg·kg^{-1} (fat basis) [43]. According to those authors, the probability of COPs being formed in fresh milk or fresh dairy products is very low since the medium is liquid and its oxygen content is low. Furthermore, milk has a low level of polyunsaturated fatty acids and a low level of pro-oxidant trace elements such as iron or copper.

CONCLUSIONS

This study showed that whey proteins can be introduced to cheese made from buttermilk. The ratio casein:whey protein in the buttermilk cheese containing the whey protein was smaller than in the case of cheese made from buttermilk only. The technological procedures applied could contribute to the reduction of oxycholesterol and the presence of bioactive compounds from whey protein in buttermilk cheese. The results demonstrated a significant increase in the antioxidant potential of samples with the added whey protein, which persisted during 30 days of storage. The addition of whey protein to cheese reduced the formation of 7α-OHC, 25-OHC and 7-ketoC during storage. The formation of β-epoxyC and α-epoxyC was reduced in the buttermilk cheese with added whey protein. At the end of the storage period, total content of cholesterol oxidation products was lower than in the control cheese. No triolC, which is the most toxic form of oxycholesterol, was found in the cheeses. This cheese, enriched with whey protein, can broaden the range of innovative and health-promoting dairy products.

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Received 20 November 2016; 1st revised 31 January 2017; 2nd revised 6 February 2017; accepted 15 February 2017; published online 13 March 2017.