

## Fermented Buttermilk-Based Beverage: Impact on Young Volunteers' Health Parameters

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### Abstract

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The technology of a functional fermented buttermilk-based beverage was developed and the impact of beverage consumption on the health parameters of young volunteers was studied. Three functional beverages were made on the basis of buttermilk, which differed in the composition. The beverage containing the buttermilk-skimmed milk-milk protein concentrate (0.3%) as a product with the preferred quality characteristics (synaeresis, viscosity, sensory properties, and acceptability) was used for further studies regarding the impact on human health. The medical nutrition experiments did not show any statistically significant effect on the biochemical blood parameters of 25 young volunteers after a 21-day period of the consumption of fermented buttermilk beverage with milk protein concentrate, although some of them (e.g. total, low- and high-density cholesterol, triacylglycerol concentrations, etc.) slightly decreased. The anthropometric and body composition, arterial blood pressure, and pulse evaluation indicators of recipients did not change significantly.

**Keywords:** functional beverages; lipid metabolism; milk protein concentrate

The use of functional foods with high nutritional value and positive physiological effect on the body could reduce the incidence of diseases which are heavily influenced by nutrition problems. The beneficial impact of these healthy products, including beverages is determined by active substances incorporated into these products. Food scientists and food producers pay more and more attention to milk proteins because there is an advanced understanding of the physiological properties and biological activity of proteins. Proteins help to build and preserve muscle mass, are important to maintain normal bone health, and affect the sense of satiety (DARLING *et al.* 2009; WESTERTERP-PLANTENGA *et al.* 2012).

Particularly high scientific interest is focused on buttermilk due to its unique composition as components of high biological value are released from destroyed milk fat globules during churning of butter. Buttermilk is rich in polar lipids (phospholipids and sphingolipids) and, in much lower concentrations, neutral lipids such as mono-, di-, and triglycerides, cholesterol, and its esters (DEWETTINCK *et al.* 2008). Recently, using modern research methods, more than 130 specific proteins have been isolated and identified from milk fat droplet membrane material (AFFOLTER *et al.* 2010). Despite their small amounts, membrane-specific proteins can play an important role in human nutrition.

Evidence of cholesterol lowering, anti-inflammatory, chemotherapeutic, and anti-neurodegenerative effects of milk fat globule membrane lipids, mainly through the action of phospholipids, was established by many authors (DEWETTINCK *et al.* 2008; EL-LOLY 2011; KÜLLENBERG *et al.* 2012; CONTARINI & POVOLO 2013). The cholesterol-lowering action of milk phospholipids in humans is one of the most frequently studied health benefit related to the consumption of buttermilk minor components (CONWAY *et al.* 2010, 2013; BAUMGARTNER *et al.* 2013).

Buttermilk contains milk proteins (caseins and whey proteins), lactose, vitamins, and minerals in the same proportion as skimmed milk, meanwhile its amount of phospholipids is about nine times higher (MACGIBBON & TAYLOR 2006). Excellent emulsifying properties of buttermilk and above-mentioned positive influence of its constituents on human health make it as one of the most suitable food matrixes for functional beverages.

Besides buttermilk products, milk protein concentrates (MPCs) obtained by spray-drying retentates of ultrafiltered skim milk are prospective in terms of functional food production. In MPCs the casein is in a micellar form, similar to that found in milk, casein and whey protein ratio remains as in milk, but a large part of lactose is removed (MARTIN *et al.* 2010). The MPCs provide a concentrated source of protein for enhanced nutritional, sensory, and functional properties in final application (MARTIN *et al.* 2010; AGARWAL *et al.* 2015).

The aim of this study was to develop a technology of a functional fermented buttermilk-based beverage enriched with MPC of the product and determine its influence on human health.

## MATERIAL AND METHODS

**Material.** Buttermilk obtained from the manufacturing of sweet cream butter (0.63% of fat, 2.80% of proteins, 4.21% of lactose), skimmed milk (0.65% of fat, 3.30% of proteins, 4.95% of lactose) (JSC Rivona, Alytus, Lithuania), and milk protein concentrate MPC 85 (1.2% of fat, 86.5% of proteins, 3.5% of lactose) (JSC MG Baltija, Medeikiai, Lithuania) were used for beverage preparation.

The mesophilic culture FD-DVS Flora Danica Normal (*Lactococcus lactis* subsp. *cremoris*, *L. lactis* subsp. *lactis*, *Leuconostoc mesenteroides* subsp. *cremoris*, *L. lactis* subsp. *lactis* biovar *diacetylactis*) (Chr. Hansen, Hoersholm, Denmark) was used.

**Production of fermented beverages.** Three different formulations of fermented beverages were prepared: (1) control from buttermilk, (2) from buttermilk and 0.3% of MPC, and (3) from buttermilk-skim milk mixture (60 : 40) and 0.3% of MPC. MPC was dissolved in buttermilk or in buttermilk-skim milk mixture. The obtained solutions or buttermilk for the control sample were pasteurised at  $85 \pm 2^\circ\text{C}$ , cooled down to fermentation temperature ( $24 \pm 2^\circ\text{C}$ ), fermented with added starter (0.025%) up to pH 4.5–4.6, stirred, distributed into plastic bottles (500 ml), and stored at  $4 \pm 2^\circ\text{C}$ .

**Viscosity.** Viscosity was measured with a Rheotest-2 rotational viscometer (VEB Kombinat Medizin und Labortechnik Kombinatbetrieb, Berlin, Germany), using the S/S<sub>2</sub> cylinder measuring system at  $10^\circ\text{C}$ . Viscosity was recorded at a deformation rate of  $27 \text{ s}^{-1}$ .

**Synaeretic properties.** Synaeretic properties were determined by centrifugation. The amount of serum discharged after centrifugation ( $2000 \text{ min}^{-1}$ , 20 min, 20 g of sample) was measured and expressed in %.

**Sensory analysis.** A quantitative descriptive analysis (QDA) was carried out by a group of 6 trained assessors (age 20–60 years) having previous experience in the assessment of sensory properties of beverages. The beverage temperature before test was equilibrated to room temperature ( $21 \pm 2^\circ\text{C}$ ), and samples of approximately 20 ml were presented to the assessors in 40 ml plastic cups, coded with three digital numbers. A 9-point scale (where 1 equal to low intensity/absent, and 9 equal to high intensity) was used to evaluate each sensory attribute.

The consumer acceptance test was performed using a five-point hedonic scale (where 5 was equal to extremely like and 1 was equal to extremely dislike). For each beverage, consumers scored their degrees of liking in the following order: odour, appearance, consistency, taste, and overall liking. A total of 50 consumers, aged 22–65, participated in the test.

**Design of medical study.** The study protocol was approved by the Lithuanian Bioethics Committee (2012-11-29; Order No. 158200-12-227-158).

Initially, 25 healthy volunteers (21 women and 4 men) between 20 and 24 years of age were recruited for the time of the study. The participants modified their diet by 500 ml of the beverage consumed daily over a period of 21 days, at the same time they were encouraged not to change their dietary habits.

The participants were invited to arrive at the hospital between 7:30 a.m. and 9:00 a.m. after having fasted for 12 hours. Blood pressure was measured

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twice with an Omron M6W automatic blood pressure manometer, pulse rate was measured once after resting supine for 5 min, body composition monitor was performed with Omron BF 511 (both OMRON Healthcare, Hoofddorp, The Netherlands). All measurements and blood samples were taken on the first visit before the beverage was consumed and on the second visit after the last dose of the beverage (on the 22<sup>nd</sup> day).

**Biochemical analyses.** Cholesterol and triglyceride concentrations in serum were analysed by enzymatic colorimetric methods (Architect ci8200; Abbott Diagnostics, Chicago, USA). Low-density lipoprotein cholesterol concentration was calculated using the Friedewald formula. High-density lipoprotein cholesterol was analysed by the accelerator selective detergent method (Architect ci8200).

Plasma glucose concentration was analysed by the hexokinase enzymatic method (Architect ci8200). Serum insulin was measured by a chemiluminescent microparticle immunoassay (Architect ci8200).

Oxidised low-density lipoproteins (OxLDL) in plasma were detected by ELISA (Mercodia, Uppsala, Sweden) based on the direct sandwich technique in which monoclonal antibodies are directed against separate antigenic determinants on the oxidised apolipoprotein B molecule (HOLVOET *et al.* 2007).

Fibrinogen concentration in blood plasma was analysed by the Clauss coagulometric method (STA-Compact; Diagnostica Stago, Paris, France).

C-reactive protein was analysed by a latex enhanced immunoturbidimetric assay (Architect ci8200)

**Statistical analysis.** The data were analysed by ANOVA, if significant interactions were determined,

multiple comparisons were made. The differences were classified by Duncan's multiple comparison test ( $P \leq 0.05$ ). SPSS software, version 15.0 (2006) (SPSS, Chicago, USA) was used for the statistical analysis of the data.

## RESULTS AND DISCUSSION

**Buttermilk beverage enriched with milk protein concentrate.** The results of the quality characteristics such as viscosity, synaeresis, and sensory properties of beverages that differ in their composition are presented in Table 1.

It was found that the enrichment of fermented buttermilk beverages with MPC and partly buttermilk replacement by skimmed milk (40%), which is characterised by a slightly higher amount of dry matter, resulted in an increase in viscosity that was instrumentally measured and sensory evaluated. Increased viscosity had a positive impact on the synaeretic properties of beverages, as lower separation of the serum in centrifuged samples was noticed. However it should be noted that changes in viscosity did not have any significant influence on the acceptability of consistency of beverage samples (Table 2).

The beverages with different composition differed in some sensory properties (Table 1). The beverage prepared from buttermilk, skimmed milk, and MPC showed significantly higher overall odour intensity and lactic acid taste when compared with two other beverage formulations.

Sensory properties of beverages should fulfil consumers' expectations. Mean consumer ratings of

Table 1. Effect of composition on the quality characteristics of beverages

Characteristics	Buttermilk	Buttermilk + 0.3% MPC	Buttermilk + skimmed milk + 0.3% MPC
Synaeresis (%)	54.9 ± 0.8 <sup>b</sup>	52.4 ± 0.6 <sup>a</sup>	52.1 ± 0.4 <sup>a</sup>
Viscosity (mPa)	210.6 ± 9.2 <sup>a</sup>	230.7 ± 4.5 <sup>b</sup>	258.5 ± 8.6 <sup>c</sup>
<b>Sensory properties</b>			
Overall odour intensity	8.2 ± 0.2 <sup>a</sup>	8.5 ± 0.3 <sup>a</sup>	8.8 ± 1.0 <sup>b</sup>
Lactic acid odour	8.2 ± 1.3 <sup>a</sup>	7.7 ± 1.2 <sup>a</sup>	7.9 ± 1.7 <sup>a</sup>
Non typical odour	1.0 ± 0.1 <sup>a</sup>	1.0 ± 0.5 <sup>a</sup>	1.0 ± 0.6 <sup>a</sup>
Viscosity (visually)	6.5 ± 0.4 <sup>a</sup>	6.8 ± 0.8 <sup>ab</sup>	7.2 ± 0.6 <sup>b</sup>
Taste intensity	6.8 ± 1.3 <sup>a</sup>	6.8 ± 1.5 <sup>a</sup>	7.2 ± 0.9 <sup>a</sup>
Lactic acid taste	7.7 ± 0.3 <sup>bc</sup>	7.5 ± 0.1 <sup>ab</sup>	7.8 ± 0.2 <sup>c</sup>
Non typical taste	5.8 ± 0.2 <sup>a</sup>	6.5 ± 0.3 <sup>a</sup>	6.7 ± 0.4 <sup>a</sup>

<sup>a-c</sup> mean values within each row with different superscripts are different at  $P < 0.05$

Table 2. Effect of composition on the acceptability of beverages

Characteristics	Buttermilk	Buttermilk + 0.3% MPC	Buttermilk + skimmed milk + 0.3% MPC
Odour	4.0 ± 0.5 <sup>a</sup>	4.4 ± 0.2 <sup>b</sup>	4.4 ± 0.2 <sup>b</sup>
Appearance	4.7 ± 1.2 <sup>a</sup>	4.7 ± 0.7 <sup>a</sup>	4.8 ± 1.5 <sup>a</sup>
Consistency	4.2 ± 0.4 <sup>a</sup>	4.4 ± 0.9 <sup>a</sup>	4.4 ± 0.4 <sup>a</sup>
Taste	3.5 ± 0.2 <sup>a</sup>	3.7 ± 0.4 <sup>ab</sup>	4.0 ± 0.4 <sup>b</sup>
Overall	3.7 ± 0.4 <sup>a</sup>	4.0 ± 0.7 <sup>ab</sup>	4.1 ± 0.5 <sup>b</sup>

MPC – milk protein concentrates

the acceptability of sensory properties are shown in Table 2.

It can be noted that the majority of the respondents liked the product and all formulations received positive ratings with regard to its odour, taste, or appearance. None of the evaluated sensory properties had a mean rating below 3 (which corresponds to neither like nor unlike point), which shows that the beverages were acceptable to consumers.

The buttermilk-skimmed milk-MPC composition of the beverage was characterised as having the best quality characteristics and acceptable sensory properties in comparison with other beverages. This particular beverage of increased nutritional value was selected and produced in larger quantities for further studies regarding the impact on human health.

**Medical nutrition experiment.** All 25 volunteers who modified their diet by 500 ml of the fermented buttermilk-skimmed milk-MPC beverage for a period of 21 days successfully completed all aspects of the study. The biochemical data of their blood serum

before and after diet supplementation with functional beverage are presented in Table 3.

Our study showed that dietary intervention with MPC-enriched beverage did not change significantly the total cholesterol, LDL-cholesterol, HDL-cholesterol, and triacylglycerol concentrations, although reductions in the mean values of these parameters were by 5.6, 8.3, 1.3, and 6.0%, respectively.

The increased total cholesterol, LDL-cholesterol, and triacylglycerol levels in the blood are among the main risk factors for atherosclerosis. The lipid accumulation in the body creates the initial conditions for the emergence of vascular lesions (atheroma formation). Whereas the analysed lipid levels in plasma during the study showed no significant effect and remained within the normal range, the slight reduction of these parameters shows that the protein-enriched beverage had a positive impact on lipid metabolism. The absence of significant changes can be associated with a young, healthy group of persons under investiga-

Table 3. Serum levels of lipids, carbohydrates and other characteristics in the group of healthy volunteers before and after diet supplementation with buttermilk beverage ( $n = 25$ )

Blood characteristics	Recommended values	Supplementation with buttermilk		$t$	$P$
		before	after		
Total cholesterol (mmol/l)	< 5.2	4.67 ± 0.83	4.41 ± 1.18	1.362	0.280
HDL-cholesterol (mmol/l)	> 0.91 for men > 1.2 for women	1.51 ± 0.27	1.49 ± 0.41	0.149	0.882
LDL-cholesterol (mmol/l)	2.6–3.5	2.78 ± 0.74	2.55 ± 0.85	1.974	0.059
Triglycerides (mmol/l)	< 1.8	0.83 ± 0.45	0.78 ± 0.41	0.874	0.435
OxLDL (U/l)	26–117	67.91 ± 33.62	66.73 ± 28.34	0.138	0.716
Insulin (pmol/l)	43–210	48.50 ± 19.96	48.46 ± 21.33	0.08	0.675
Fasting glucose (mmol/l)	3.33–5.55	4.90 ± 0.35	4.86 ± 1.04	0.16	0.109
Fibrinogen (g/l)	2–4	2.79 ± 0.36	2.83 ± 0.67	–0.318	0.753
C reactive protein (mg/l)	< 5	0.57 ± 0.60	0.71 ± 0.77	–1.29	0.361

HDL – high-density lipoproteins; LDL – low-density lipoproteins; OxLDL – oxidised low-density lipoproteins;  $t$  (degrees of freedom) –  $t$ -value;  $P$  – significance level

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Table 4. Anthropometric, body composition measurements, blood pressure, and pulse data in the group of volunteers before and after diet supplementation with buttermilk beverage ( $n = 25$ )

Parameters	Recommended values	Supplementation with buttermilk		$t$	$P$
		before	after		
Weight (kg)	–	68.00 ± 8.65	64.97 ± 16.08	1.031	0.695
Height (cm)	–	171.69 ± 8.65	171.70 ± 8.70	0.979	0.325
Body mass index (kg/m <sup>2</sup> )	18.5–24.9	23.06 ± 2.44	22.04 ± 5.17	1.094	0.312
Body fat (%)	8–19.9 for men 21–32.9 for women	28.46 ± 9.53	27.83 ± 10.89	0.515	0.399
Skeletal muscle (%)	33.3–39.3 for men 24.3–30.3 for women	31.34 ± 7.10	30.15 ± 8.95	0.872	0.385
Visceral fat (relative) (%)	3–4	3.84 ± 1.14	3.72 ± 1.31	0.768	0.527
Systolic blood pressure (mm Hg)	< 120	125.52 ± 12.83	118.96 ± 26.56	1.286	0.230
Diastolic blood pressure (mm Hg)	< 80	72.68 ± 10.28	67.44 ± 15.52	1.832	0.126
Pulse (rate/min)	60–100	73.40 ± 12.94	74.76 ± 22.61	–0.314	0.486

 $t$  (degrees of freedom) –  $t$ -value;  $P$  – significance level

tion, when the body is able to maintain stable and normal metabolic indicators.

Many scientific studies have indicated that the increased level of oxidised LDL is a hallmark of atherosclerosis. OxLDL plays a direct role in the initiation stage and progression of cardiovascular diseases (ANSELM *et al.* 2006; HOLVOET *et al.* 2007). Undoubtedly, it is important to search for components which are able to reduce the blood level of OxLDL. Our study showed an insignificant decrease in the concentration of OxLDL.

The insulin and glucose level (reflection of the carbohydrate metabolism) in blood plasma was almost unchanged after diet supplementation. The concentration of C-reactive protein, which is associated with acute-phase inflammatory proteins, was increased in response to inflammation. However, the protein concentration in the blood of healthy young individuals can vary from 30% to 60%. The same acute-phase inflammatory protein group includes the coagulation protein fibrinogen (range 2–4 g/l). It is obvious that after 3 weeks of taking the beverage, the insignificant increase in C-reactive protein and fibrinogen concentration in serum (respectively 24.5 and 1%) did not exceed the upper normal range and did not show any medical disorder.

In general, the impact of the beverage consumption over a 21-day period did not show any statistically significant effect on many biochemical blood parameters of the volunteers. The detected small variations in volunteers' biochemical characteristics of blood after diet supplementation were in the normal clinical range and could not have an adverse health effect.

Anthropometric data, body composition, blood pressure, and pulse evaluation findings are presented in Table 4. The results showed that these indicators did not change significantly, although some of them inclined to decrease (weight, body mass index, body fat, and blood pressure).

## CONCLUSIONS

The enrichment of fermented buttermilk beverage with 0.3% MPC increased its viscosity, improved synaeresis, sensory properties, and acceptability.

The consumption of buttermilk beverage with 0.3% MPC over a 21-day period did not show any statistically significant effect on biochemical blood parameters of the volunteers. However, it should be noticed that a reduction in the mean values of lipid levels in plasma was registered: concentrations of total cholesterol, LDL-cholesterol, HDL-cholesterol, and triacylglycerol decreased by 5.6, 8.3, 1.3, and 6.0%, respectively. The results of anthropometric, body composition measurements, blood pressure, and pulse data showed that these indicators did not change significantly.

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