Journal of Food and Nutrition Research, 2019, Vol. 7, No. 2, 114-121
Available online at http://pubs.sciepub.com/jfnr/7/2/3
Published by Science and Education Publishing
DOI:10.12691/jfnr-7-2-3

Composition and Processing Characteristics of Milk from Lithuanian Black and White Cows

Milda Keršienė[1], Ina Jasutienė[1], Marija Kubiliūtė[1], Tomas Ruzgas[2], Daiva Leskauskaitė[1,*]

[1]Department of Food Science and Technology, Kaunas University of Technology, Kaunas, Lithuania
[2]Department of Applied Mathematics, Kaunas University of Technology, Kaunas, Lithuania
*Corresponding author: daiva.leskauskaite@ktu.lt

Received October 13, 2018; Revised January 02, 2019; Accepted February 10, 2019

Abstract The goals of this study were to understand how seasonal variations in composition of milk from native breed cows influence the technological properties of milk and how these changes might be beneficial for the production of cultured dairy products. Milk samples from native Lithuanian Black and White dairy cattle breed (LBW) were collected and profiled for different compositional and technological traits over a 1-year period. In general, insignificant differences were observed in overall milk composition between seasons. However, protein content was significantly affected by season, which was reflected in protein composition. The contents of individual milk proteins in LBW milk during the winter months were significantly lower ($p < 0.05$) in comparison with the summer period. The technological properties of LBW milk demonstrated considerable variations. A significant relationship was found between milk protein profiles and acid-induced gelation properties. Milk samples with strong acid-induced gelation properties had significantly higher β-lg content ($p < 0.05$) and lower α_{S1}- and κ-Cn content in comparison with milk samples with weak acid-induced gelation properties. Further analysis of yoghurt produced from LBW milk showed that the production of yoghurt with strong physicochemical properties was possible even when the milk had weak acid-induced gelation properties. Lithuanian Black and White dairy cattle milk can thus be considered a good raw material for the production of cultured dairy products.

Keywords: *milk, casein fractions, acid-induced gelation, vitamin D, vitamin A*

Cite This Article: Milda Keršienė, Ina Jasutienė, Marija Kubiliūtė, Tomas Ruzgas, and Daiva Leskauskaitė, "Composition and Processing Characteristics of Milk from Lithuanian Black and White Cows." *Journal of Food and Nutrition Research*, vol. 7, no. 2 (2019): 114-121. doi: 10.12691/jfnr-7-2-3.

1. Introduction

The Lithuanian Black and White (LBW) is an old native dairy cattle breed with significant regional and cultural value. To improve production of LBW cow milk, US and Canadian Holsteins have been used for 25 years because of the low quantity of this native breed (1975 animals in 2016), whose contribution to total milk production is marginal in Lithuania. However, new knowledge about the importance of genetic resources for food security and Lithuania's obligations in international agreements such as the Convention on Biological Diversity highlighted the need to focus on milk from native Lithuanian dairy cattle breeds.

Recent studies have shown that there are differences in the genetic composition of milk proteins in the native North European dairy cattle breeds [1]. A high variance in milk protein allele frequencies between five commercial Nordic cattle breeds and seventeen native breeds have been described [2]. At the same time there is a large variability in the protein profile of milk which is crucial for milk's technological properties, such as rennet and acid coagulation, gel strength, etc [3-5]. Studies indicate that genetic polymorphisms are significantly associated with rennet coagulation time and curd firming rate [6]. Several studies have suggested that milk protein composition and content, κ - casein concentration, ionic calcium and total calcium content and casein micelle size are the most important factors responsible for the rennet gelation properties of milk [7,8]. In addition, it was found that the native breeds of Swedish cows displayed superior milk coagulation properties compared to Swedish Red cows [9].

Studies relating the effects of milk protein profile to the properties of fermented milk are still limited and have mainly used milk from the commercial cattle breeds: Swedish Red [10,11,12] and Norwegian Red [13]. It was reported that several properties of fermented milk products, such as the yield stress of curd and the concentrations of organic acids, have been affected by milk protein genetic polymorphism in β-lactoglobulin [14]. Reference [11] presented an association between milk acid gelation time and β-lactoglobulin genotype. However, no previous studies have analysed the acid gelation of milk from native dairy cattle breeds.

Understanding how variations in milk protein profiles from native breed cows influence the properties of acid-induced gels might be beneficial in the production of

cultured dairy products for technological purposes. The aim of this study was to investigate variations in the composition, protein profile and technological properties of milk from native breed LBW cows destined for the production of cultured dairy products. Seasonal variations in the composition and technological properties of milk were also considered in this study. The potential of milk to produce cultured dairy products was characterised by the acid-induced gelation time and gel strength of the milk. Moreover, the rheological properties and degree of syneresis of yoghurt produced from LBW milk were evaluated.

2. Materials and Methods

2.1. Milk Samples

Monthly individual cow milk samples were obtained from 20 cows (native Lithuanian black and white cattle) from six farms in the Alytus district (Lithuania) between November 2016 and November 2017. The total quantity of milk samples was 220. Fresh milk samples were transported to the laboratory of Kaunas University of Technology for further analysis.

For the analysis of acid-induced gelation properties, 50 mL of each fresh milk sample was centrifuged at 500 x g for 20 min at a temperature of 4°C. After centrifugation, the separated cream layer was removed, and the skim milk was pasteurised at 95°C for 30 minutes. After heating, the milk was cooled to 4-6°C. The pasteurized milk was subsequently analysed the next day.

All reagents and chemicals were of analytical grade.

2.2. Milk Composition Analysis

2.2.1. Gross Composition of Milk

Fat (g/100g), protein (g/100g), lactose (g/100g) and urea (mg/100g) content in the individual fresh milk samples were determined using the LactoScope FTIR (Delta Instruments, Drachten, the Netherlands) in the central milk testing laboratory in Pieno Tyrimai (Lithuania).

The analysis of total calcium content in the milk samples was conducted according to official AOAC analysis method [15], employing an AAnalyst 400 Spectrophotometer (Perkin Elmer, Waltham, USA) and using a calcium lamp at a wavelength of 422.7 nm.

2.2.2. Vitamins D_3 and a Content in Milk

Standard solutions of vitamin D_2 (Sigma Aldrich, Praha, Czech Republic) and D_3 (Sigma Aldrich, Poznan, Poland) in ethanol were prepared according to EN standard 12821:2009 [16]. The vitamin D_2 solution (0.02 mg/mL) was used as an internal standard, and reference solutions of ergocalciferol and cholecalciferol (concentration of each vitamin: 0.02 mg/100mL) were used for the quantification of vitamin D_3. Vitamin A was determined according to the EN 12823-1 standard [17], and the calibration curve of retinyl palmitate (Sigma Aldrich, Buchs, Switzerland) was used for the quantification of vitamin A.

For the sample preparation 2 g of milk, 0.2 g of ascorbic acid (Reachem, Petrzalka, Slovakia), a spatletip of pyrogallol, 3 mL of KOH solution (Reachem, Petrzalka, Slovakia; 50 g KOH dissolved in 100 mL H_2O), 8 mL ethanol (96 % by volume), (Riedel-de-Haën, Seelze, Germany) and 1 mL of D_2 internal standard solution were added to a dark flask. The flask was sprayed with a nitrogen stream, in order to avoid oxidation, and left for saponification under shaking (GFL No-1092, Germany) in the dark at a temperature of 20°C until the next day. The next day, the samples were mixed with 9 mL H_2O and 10 mL hexane (Avantor Performance Materials, Gliwice, Poland), transferred to a separating funnel, shaken vigorously for 2 min and allowed to stand until the two layers were clear. The aqueous (lower) phase was transferred to a second separating funnel and extraction with 10 mL of hexane was repeated twice. Collected organic phases were evaporated under vacuum (IKA RV 10 Rotary Evaporator, Germany) until dry. Before HPLC analysis, the dry residue was dissolved in 1 mL of mobile phase and filtered through a 0.22 µm pore size filter. Separation by analytical RP-HPLC was carried out as described in [18]. Vitamin D was recorded at a wavelength of 265 nm, and vitamin A at 325 nm.

2.2.3. Quantification of Milk Proteins

Purified α_s- (purity 97 %), β- (purity 99 %) and κ- (purity 99 %) caseins, and β-lactoglobulin (purity 99 %) from bovine milk were used as standards to obtain a four-level calibration curve (Sigma Aldrich, St. Louis, USA). Samples and standard solutions for the calibration curve were prepared as described by [19].

Separation by analytical RP-HPLC was made using the Shimadzu Prominence analytical HPLC system with a diode array detector set at 220 nm, an autosampler and LC Solutions software (Shimadzu Corp., Tokyo, Japan). The Vydac 218TP C18 column (5µm 300A, 250x4.6 mm, Grace Alltech, Maryland, USA) with guard column (5 µm, 7.5x4.6 mm, Grace Alltech, Maryland, USA) was used with a temperature of 35°C. The analyses were carried out applying a binary gradient: eluent A was 0.1 % (by volume) trifluoroacetic acid in pure water, eluent B was acetonitrile containing 0.07 % (by volume) trifluoroacetic acid. Programme of gradient: 0 – 5 min isocratic elution 4 % B; 5 – 15 min linear gradient from 4 to 25 % B; 15 – 29 min linear gradient from 25 to 45 % B; 29 – 32 min linear gradient from 45 to 90 % B; 32 – 35 min isocratic elution 90 % B; 35 – 40 min return linearly to the starting conditions of 4 % B. The flow rate was 1 mL/min. The injection volume was 20 µL. Calibration curves of casein fractions and β-lactoglobulin were used for the quantification of milk protein fractions.

2.3. Milk Technological Properties Analysis

2.3.1. Thermal Stability and Buffering Capacity

Thermal stability of the milk samples was determined by measuring heat coagulation time (HCT). A heat-resistant screw-cap test tube containing 10 mL aliquot samples was immersed in the oil bath (Haake AC200; Thermo Fisher Scientific, Newington, NH, USA) maintained at 140°C. HCT was defined as the time necessary to observe the visible coagulation of milk at 140°C. The reported HCT values were an average of six measurements of the sample.

The buffering capacity (BC) of the milk samples was determined by adding 4.0 mL of 0.1 M HCL solution to 25 mL of fresh milk sample. The pH difference before and 1 hour after acid addition was considered the buffering capacity and was expressed in pH units. The reported BC values were an average of three measurements of the sample.

2.3.2. Acid-induced Gelation Properties

The acid-induced gelation properties of milk were measured rheologically using a Physica MCR rheometer (Anton Paar GmbH, Ostfildern-Scharnhausen, Germany) equipped with a cone-plate measuring system (60 mm diameter, 2° cone angle and 0.05 mm gap) and a temperature-controlling unit (Peltier plate). Oscillation mode was used with a frequency of 1 Hz and a constant strain of 0.003. Each milk sample (10 mL) was pre-heated at 30 °C for 30 min and acidified by adding 0.03 g of glucono-δ-lactone (GDL). The thoroughly mixed sample was transferred to the cone-plate measuring system of the rheometer. The acid-induced gelation was followed at 30 °C for 60 min from the addition of GDL. *G'* (storage modulus) and *G"* (loss modulus) were plotted against time. Acid-induced gelation time (AGT) was defined as the time from GDL addition until *G'*=*G"*. For acid-induced gel firmness, G'_{40} was recorded as *G'* at 40 min. All measurements were made in duplicate.

2.4. Production of Yoghurt

Seven yoghurt samples were produced from individual LBW cows' milk samples. Skim milk was obtained by separating the cream from milk samples preheated to 50 °C using a batch electrical cream separator (Milky FJ 125 EAR., Althofen, Austria). Protein content in the skim milk samples was in the range of 3.45-3.52 g/100g. 1 L of skim milk was heated in a water bath at 95°C for 5 min and subsequently cooled to 42°C. The commercial frozen yoghurt starter (Chr. Hansen Yo-Flex, Hoersholm, *Danmark*) containing *Lactobacillus delbrueckii* ssp. bulgaricus and *Streptococcus thermophilus* strains was added into the milk at 42°C (0.1 g/100g) and mixed thoroughly. The inoculated milk was divided into 200 mL of aliquots in 5 sterile glass jars with lids and incubated at 42°C with a temperature-controlled thermostat (KB8182 Termaks, Bergen, Norway) until a pH of 4.5 ± 0.05 was reached. The fermentation process was stopped by cooling the yogurt samples to 4°C. All yogurt samples were stored at 4°C for 1 and 10 days for further analysis.

2.5. Physical Properties of Yoghurt

2.5.1. Rheological Properties

Static shear rheology tests were performed using the Physica MCR101 rheometer (Anton Paar GmbH, Ostfildern-Scharnhausen, Germany) equipped with a cone-plate measuring system (60 mm diameter, 2° cone angle and 0.05 mm gap). Each sample was loaded on the measuring plate of the rheometer and left at rest in the measurement system at 20°C for at least 1 min to obtain thermal equilibrium. Any extra quantity of the sample was trimmed by a spoon. Silicone oil was used to prevent moisture evaporation. The measuring device was equipped with a temperature-controlling unit (Peltier plate). Shear stress (Pa) as a function of shear rate (s^{-1}) was monitored at 20°C. The range of shear rate was from 0.001 s^{-1} to 500 s^{-1}. Data points were regressed and fitted in the Herschele Bulkley model. The fitting coefficient (R^2) shows the fitting accuracy of the equation, which is as follows:

$$\tau = \tau_0 + K(\gamma)^n \quad (1)$$

where τ is the shear stress (Pa), τ_0 is the yield stress (Pa), K is the consistency factor (Pa·s^n), γ is the shear rate (1/s), and n is the fluid index.

The test was conducted in triplicate.

2.5.2. Degree of Syneresis

The method described by [20] was adopted with some modification to determine the degree of yoghurt syneresis. Briefly, 20 g of the sample was weighed into 50 mL Falcon tubes which were placed in a 50 mL conical tube bucket connected to an angle rotor (ref No. 11746 30°, MPW MED. Instruments, Poland). After centrifugation at 500 x g for 20 min at 4°C using an MPW-260/R/RH centrifuge (MPW MED. Instruments, Warszawa, Poland) the supernatant was drained off and weighed on an analytical balance. The degree of syneresis was calculated according to the following equation:

$$Synerysis(\%) = \frac{W_1}{W_2} \times 100 \quad (2)$$

where W_1 is the weight of the supernatant and W_2 is the weight of the sample (20 g).

The test was conducted in triplicate.

2.6. Statistical Analysis

Seven yoghurt samples were produced from individual Mean values, seasonal variations and correlation coefficients between the composition and technological properties of milk were calculated using an SAS 9.4 package. The seasonal effect was demonstrated by performing statistical analysis with the Mann-Whitney-*Wilcoxon* test. The $p < 0.05$ threshold level of significance was used in the analysis.

3. Results and Discussion

3.1. Composition of Milk from LBW Dairy Cattle

Table 1 shows the average values, range and seasonal variation in composition of milk collected from individual LBW cows. The fat, lactose and total protein content were in line with the Lithuanian national statistics for 2017, which were 4.48 g/100g for fat, 3.52 g/100g for protein and 4.41 g/100g for lactose. Furthermore, fat and total protein content results agreed well with the data from earlier studies on milk composition from LBW cows [21].

The fat content of raw milk ranged from 2.08 to 5.93 g/100g, although there was no significant seasonal

variation. Compared with fat, the variability in lactose content was less, ranging from 3.87 to 5.00 g/100g. Raw milk had significantly higher lactose content ($p < 0.05$) in autumn than in spring, but there were no significant variations in lactose content between the summer and the winter seasons. Protein and total casein content in LBW milk were influenced by the season. Raw milk produced in the autumn period had significantly higher protein content in comparison with the spring and summer periods. The total casein content followed a similar trend; however, significant differences in milk were recorded during the summer and winter periods. Urea content fluctuated greatly over the study, but significantly lower urea content of – 1.0±7.67 mg/100g, was observed in the winter months.

Reference [3] studied seasonal variation in Dutch cows' milk. Authors found that the lactose content in milk was constant throughout the season; the lowest protein content was found in June (3.21 g/100 g) and the highest in December (3.38 g/100 g), and milk fat concentration increased from 4.10 g/100 g in June to 4.57 g/100 g in January. Reference [22] analysed Holstein cows' milk in a dairy farm from June 2012 to May 2013. They found that the lowest values for all the main milk components were found in the summer period. [23] conducted another study on the seasonal variations in the composition of raw milk. According to the results, milk from the pasture-fed Friesian cows (summer period) had significantly higher concentrations of fat, protein, and casein.

Generally, the levels of calcium in LBW cows' milk were within the normal range found in cows' milk. The seasonal effect was recorded only during winter months, when Ca content was significantly higher than during the spring, summer and autumn periods. It was also reported that the calendar month affected milk's Ca content, which dropped in the spring during grazing turnout and was lower when cows were fed fresh and conserved grass rather than corn silage [24].

The average concentrations of fat-soluble vitamins A and D in LBW cows' milk were 78.51±31.37 and 1.13±0.66 µg/100g, respectively. However, with the exception of vitamin D during the summer months, no significant variations in fat-soluble vitamins content in raw milk was observed in the current study. The effect of fat content on the amounts of vitamins A and D was not found to be significant in this study either. Currently, there are limited data about seasonal effects on fat-soluble vitamin content in cows' milk. There are data that vitamin D_3 levels in milk range from a winter low to a summer high [25]. The studies of seasonal variation in individual milk components regarding the local breeds in Pakistan showed the highest vitamin A and E contents in the summer months, as compared to winter months, due to the high consumption of fodder, which provides an excess of vitamins in summer [26].

The analysis of protein profiles of raw LBW milk showed that the contents of all studied milk proteins were influenced by the season. Milk contained more β-Lg, α_{S1}-, α_{S2}-, β- and κ-Cn during summer months. The contents of individual milk proteins in LBW milk during winter months were significantly lower ($p < 0.05$). This is in general agreement with Joudu et al. (2008), who showed that the contents of all milk proteins were significantly influenced by the sampling month. However, the study of milk protein fractions in Holstein cows demonstrated that all casein fractions showed the lowest values in the summer and the greatest values in the winter [22]. The β-Lg content trend was similar with our study – it was greater in summer than in the winter and spring.

Significant seasonal variations were found for the protein content and protein profile of LBW cows' milk. Unexpectedly, other compositional parameters of LBW cows' milk, such as fat, fat-soluble vitamins, and lactose content demonstrated less seasonality. Much of the data about the average values, range and seasonal variation in composition of milk collected from individual LBW cows were consistent with data from other studies. The set differences can be explained by differences in experimental design, such as the number of dairy cows, sampling frequency, farming practices, and peculiarities of geographical region.

Table 1. Composition of raw milk from LBW dairy cattle collected over the period April 2016 to April 2017

Composition	Mean	Range	Seasonal variation*			
			Spring	Summer	Autumn	Winter
Fat, g/100g	4.48±0.83	2.08÷5.93	4.5±0.73^{a}	4.46±0.95^{a}	4.37±0.88^{a}	4.54±0.75^{a}
Lactose, g/100g	4.41±0.16	3.87÷5.00	4.45±0.16^{a}	4.35±0.18^{ab}	4.34±0.11^{b}	4.33±0.19^{ab}
Protein, g/100g	3.52±0.41	2.69÷4.63	3.36±0.35^{a}	3.51±0.35^{c}	3.71±0.45^{b}	3.63±0.37^{bc}
Total casein, g/L	29.91±3.82	21.50÷42.80	28.43±2.54^{ac}	30.68±3.45^{b}	30.01±4.00^{cb}	27.53±2.96^{a}
β-Lg, g/L	7.64±1.36	4.46÷11.42	7.70±1.44^{ab}	7.86±1.26^{a}	7.73±1.57^{a}	7.04±0.81^{b}
Casein fractions						
α_{S1}-Cn, g/L	9.98±1.33	5.58÷13.74	9.60±1.00^{ab}	10.02±1.41^{a}	10.00±1.45^{ab}	9.50±1.06^{b}
α_{S2}-Cn, g/L	2.15±0.56	1.11÷4.37	2.03±0.44^{a}	2.44±0.67^{b}	2.29±0.46^{b}	1.66±0.27^{c}
β-Cn, g/L	14.06±1.89	13.50÷13.71	13.44±1.15^{a}	14.43±2.05^{b}	13.94±1.95^{ab}	13.08±1.18^{a}
κ-Cn, g/L	3.58±0.92	1.93÷8.04	3.40±0.66^{ab}	3.63±0.82^{a}	3.61±0.84^{ab}	3.20±0.84^{b}
Urea, mg/100g	20.0±8.59	4.0÷34	19.0±8.89^{a}	17.0±9.64^{a}	20.0±8.59^{a}	10.0±7.67^{b}
Ca^{+2}, mg/100g	117.12±13.35	100÷168	115±10,58^{a}	114±16,08^{a}	120±13,39^{ab}	121±9,60^{b}
Vitamin D, µg/100g	1.13±0.66	0.42÷2.54	0.86±0.41^{a}	1.91^{b}±0.79^{b}	1.11±0.54^{ab}	0.76±0.40^{a}
Vitamin A, µg/100g	78.51±31.37	33.53÷164.09	84.88±35.55^{a}	79.66±17.00^{a}	73.27±46.76^{a}	85.27±27.44^{a}

*Results of seasonal variations are mean ± standard deviation; different superscript letters within a row for each compositional property show the significant differences ($p < 0.05$).

3.2. Technological Properties of Milk from LBW Dairy Cattle

Table 2 presents the average values, range and seasonal variation in technological properties of milk collected from individual LBW cows. The average buffering capacity of raw milk was 0.63±0.15, which was slightly smaller than that reported by [27]. The seasonal effect was recorded only during the spring months, when the buffering capacity of LBW milk was significantly lower than that during the summer, autumn and winter periods.

The average HCT was 14±5 min. This parameter is used as a measure of the heat stability of raw milk. HCT is determined as the time passed between placing the sample at 140 °C and the first visible symptoms of coagulation. In our study, seasonal trends were not observed for this technological property. The literature reveals contradictory data on seasonal variations of raw milk heat stability. A study on thermal stability of bulk milk found a significantly greater heat stability of milk in autumn and winter than in spring and summer [28], while other authors reported that spring milks had lower values of heat stability than autumn milks [29]. In addition, data from our study showed that 45 % of LBW milk samples had low heat stability, i.e. < 15 min, and only 23 % of raw milk samples withstood heat treatment for more than 20 min. Heat stability is an important parameter for milk that will be used for sterilization, UHT pasteurization or evaporation. Low heat stability is a serious problem in the dairy industry, although [30] found that the correlation between the low heat stability (<15 min) properties of raw milk and the commercial sterilization process is weak.

The acid-induced gelation properties of milk were characterised by acid-induced gelation time (AGT) and acid-induced gel strength determined after 40 min of gelation (G'_{40}). Both parameters displayed a broad range: AGT changed from 16 to 42 min and G'_{40} from 0.02 to 99.7 Pa. However, average AGT values did not show seasonal variation. Average G'_{40} values followed a different trend with significantly higher values in the winter and spring, compared to summer and autumn.

Table 2. Technological properties of raw milk from LBW dairy cattle collected over the period April 2016 to April 2017

Properties	Mean	Range	Seasonal variation*			
			Spring	Summer	Autumn	Winter
BC	0.65±0.15	0.30÷0.85	0.51±0.14^{a}	0.70±0.08^{b}	0.69±0.10^{b}	0.70±0.04^{b}
HCT, min**	14±5	4÷20	14.20^{a}	14.35^{a}	14.24^{a}	15.10^{a}
AGT, min**	26.75±7.70	16.00÷42.00	25.14±3.27^{a}	27.33±12.45^{a}	28.26±6.22^{a}	25.50±4.42^{a}
G'_{40}, Pa**	45.02±33.03	0.02÷99.7	60.94±28.07^{ac}	1.07±0.04^{b}	12.19±18.72^{ab}	64.83±23.86^{c}

*Results of seasonal variations are mean ± standard deviation; different superscript letters within a row for each technological property show the significant differences ($p < 0.05$).
**HCT - Heat coagulation time, min; AGT- acid-induced gelation time, min; G'_{40} – acid-induced gel strength after 40 min of gelation, Pa.

Table 3. Milk proteins content as a function of acid-induced gelation of milk from LBW dairy cattle collected over the period April 2016 to April 2017

Composition	Mean	Range	Seasonal variation*			
			Spring	Summer	Autumn	Winter
Strong acid-induced gelation						
β-lg, g/L	7.96±1.27^{A}	5.31÷10.66	6.75±1.30^{a}	7.87±1.48^{b}	8.09±1.51^{b}	6.99±0.72^{a}
Total casein, g/L	28.86±3.83^{A}	21.88÷38.44	27.72±2.84^{a}	31.29±4.76 ^{a}	28.35±3.97^{a}	27.75±2.23^{a}
Casein fractions:						
α_{S1}-Cn, g/L	9.69±1.41^{A}	5.58÷12.60	9.64±0.86^{a}	10.05±2.03 ^{a}	9.51±1.46^{a}	9.58±0.90^{a}
α_{S2}-Cn, g/L	2.17±0.73^{A}	1.34÷5.14	1.76±0.18^{a}	2.77±0.92^{b}	2.32±0.62^{b}	1.66±0.18^{a}
β-Cn, g/L	13.61±1.89^{A}	9.01÷13.66	13.11±1.43 ^{a}	14.83±2.41 ^{a}	13.13±1.81^{a}	13.27±1.23^{a}
κ-Cn, g/L	3.38±0.64^{A}	2.15÷4.67	3.22±0.63^{a}	3.63±0.82^{a}	3.38±0.49^{a}	3.23±0.59^{a}
Week acid-induced gelation						
β-lg, g/L	7.52±1.33^{B}	6.47÷10.29	7.28±0.98^{a}	8.43±0.38^{b}	8.20±1.75^{ab}	6.73±0.55^{a}
Total casein, g/L	30.96±3.37^{A}	25.35÷35.01	28.69±2.34^{a}	34.10±1.82^{a}	31.65±3.03^{a}	27.25±2.68^{a}
Casein fractions:						
α_{S1}-Cn, g/L	11.11±1.85^{B}	8.84÷13.74	9.93±0.97^{a}	13.36±0.53^{a}	10.96±1.95^{a}	9.80±0.50^{a}
α_{S2}-Cn, g/L	2.20±0.70^{A}	1.24÷3.27	1.72±0.16^{a}	2.96±0.01^{a}	2.36±0.56^{a}	1.28±0.06^{a}
β-Cn, g/L	13.49±1.37^{A}	11.64÷15.9	13.34±1.38^{a}	12.35±1.00^{a}	14.14±1.40^{a}	13.08±1.73^{a}
κ-Cn, g/L	4.17±0.94^{B}	2.81÷6.37	3.70±0.73^{a}	5.43±1.34^{a}	4.19±0.28^{a}	3.09±0.40^{a}

*Results of seasonal variations are mean ± standard deviation; different superscript letters within a row for each protein compositional property show the significant differences ($p < 0.05$); different superscript letters within a column for each protein compositional property show the significant differences ($p < 0.05$).

We classified the LBW cows' milk samples as demonstrating good or weak acid- induced gelation properties. The milk samples with strong acid-induced gelation properties were defined as having a low AGT (> 25 min) and high G'_{40} (< 50 Pa), thus forming strong gels over short periods of time. Samples were defined as showing weak acid-induced gelation when high AGT and low G'_{40} values were determined. The distribution between strong and weak acid-induced gelation milk samples was dependent on the season. 86 % of spring milk samples and 84 % of summer milk samples were defined as having strong acid-induced gelation properties, compared to 72 % of autumn samples and 80 % of winter milk samples. To explain the importance of this observation, we showed the different protein contents of the milk from individual LBW dairy cattle, representing different classes of acid-induced gelation properties over the seasons (Table 3).

Data presented in Table 3 revealed that milk samples with strong acid-induced gelation properties had significantly higher β-lg content ($p < 0.05$) and lower α_{S1}- and κ-Cn content in comparison with milk samples with weak acid-induced gelation properties. A similar trend was observed for the total casein content of α_{S1}- and κ-Cn; however, the differences were not significant. The positive effect of β-lg content on the gelation time and strength of acid gels was reported in other studies as well [31,32].

From data presented in Table 3 we can see that the season had no effect on the milk protein content for either strong or weak acid-induced gelation samples. This contradicts our previous results (Table 1), which showed that the contents of all milk proteins studied in raw LBW milk were influenced by the season.

3.3. Interrelationship between Composition and Acid-induced Gelation of Milk from LBW Dairy Cattle

The correlations between the milk components and acid-induced gelation properties are demonstrated in Table 4. The current results showed that the content of major milk components were not correlated with the acid-induced gelation properties of milk. Only Ca content had a significant positive effect on acid gelation time ($p < 0.01$). However, milk samples with higher concentrations of Ca produced weaker acid gels ($p < 0.05$).

As shown in Table 4, the higher contents of the studied milk protein fractions were associated with acid-induced gelation properties, since they were positively correlated with AGT value and negatively correlated with G'_{40}. Acid gelation time was reduced, and a weaker gel was formed, along with the increase in total casein content. The relative α_{S1}-Cn concentration showed a significant positive correlation with acid gelation time ($p < 0.05$) and negative correlation with acid gel strength ($p < 0.05$). The increase in κ-Cn content was associated with improved (shorter) acid-induced gelation time, while gel strength was impaired with the increase of a relative amount of κ-Cn. The positive correlation between β-lg content and AGT was demonstrated ($p < 0.01$), as well as their negative correlation with G'_{40} ($p < 0.05$).

Results reported from this study on the relationship between the main composition and acid-induced gelation properties of LBW cows' milk disagree with the results reported for other dairy cattle breeds [12,13]. [14] have shown positive correlations between protein content, (including casein content) and acid gel firming rate and gel firmness. Higher lactose content in this study was associated with improved acid gelation properties, while samples with higher fat content gelled in a shorter period of time and formed weaker acid-induced gels. Differences in milk sampling could be the reason for these inconsistencies. Norwegian Red cattle milk samples in the referred study were collected once or twice in the second and fourth months of lactation. In our study, LBW cows' milk was sampled monthly during a one-year period. The differences in the milk protein polymorphism of Norwegian Red cattle and LBW cattle could be another reason for the observed inequalities.

Table 4. Correlation matrix between milk compositional characteristics and milk acid-induced gelation parameters[a]

Milk compositional characteristics	Acid-induced gelation parameters	
	AGT	G'_{40}
Major milk constituents		
Total fat	NS	NS
Total protein	NS	NS
Total lactose	NS	NS
Ca	0.50**	-0.63*
Urea	NS	NS
Protein composition		
Total casein	0.36*	-0.75**
α_{S1}-Cn	0.36*	-0.67*
α_{S2}-Cn	NS	-0.72*
β-Cn	NS	-0.67*
κ-Cn	0.49**	-0.65*
β-lg	0.68**	-0.62*

[a]Numbers in the table indicates the coefficients of correlation: NS – non significant; ** $p < 0.01$; * $p < 0.05$.

3.4. Physical Properties of Yoghurt from LBW Dairy Cattle Milk

In order to determine the suitability of LBW cow's milk for the production of certain dairy products, the most popular fermented dairy beverage – yoghurt – was produced under semi-industrial conditions. For this experiment, individual milk from 7 different LBW cows, collected in February - March of 2017, was used. Yoghurt was made from skim milk. It is important to emphasize that the protein content in the milk samples was in the narrow range of 3.45-3.52 g/100g. The acid-induced gelation properties of each milk sample were first evaluated. Then, the yoghurt was made from each milk sample. The products were characterized by pH, syneresis and rheological indexes, which were determined on the first day and after the 10th day of storage.

Several differences in the acid-induced gelation properties of individual milk samples were observed (Table 5). Three samples (No. 1, 2 and 3) showed weak acid gelation properties; their acid-induced gelation time was determined between 30.0 and 38.0 min, while G'_{40} was recorded in a range of 17.2 – 28.7 Pa. Another four samples (No. 4, 5, 6 and 7) could be characterised as showing good acid-induced gelation properties, when low AGT values (22.0 – 24.0 min) and high G'_{40} values (59.6 – 98.7 Pa) were determined.

Table 5. Physicochemical characteristics of yogurt produced from individual milk samples of LBW cows

No	Acid-induced gelation properties		Characteristics of yogurt at the end of technological process			
	AGT, min	G'_{40}, Pa	pH	Degree of syneresis %	K, consistency factor (Pa·s^n)	n, fluid index
1	30.0±1.0	27.2±0.1	4.58±0.01	36.88±0.19	2.36±0.15	0.38±0.01
2	38.0±2.0	17.2±3.9	4.60±0.00	35.00±0.25	2.01±0.10	0.41±0.01
3	33.0±1.0	28.7±5.4	4.61±0.02	36.63±0.12	2.63±0.17	0.32±0.01
4	24.0±0.0	59.6±3.2	4.56±0.01	36.00±0.08	3.57±0.19	0.36±0.01
5	22.0±1.0	98.7±2,7	4.59±0.01	35.75±0.14	3.54±0.14	0.36±0.01
6	23.0±0.0	76.9±2.7	4.56±0.00	35.98±0.11	3.12±0.15	0.35±0.01
7	24.0±1.0	61.1±1.4	4.60±0.01	35.63±0.09	3.32±0.15	0.32±0.01

Syneresis and low viscosity are the main defects of yoghurt. The reason for syneresis is the weak structure of the protein network, which causes the release of whey from the body of yoghurt [33]. Normally the increase in whey separation is recorded during yoghurt storage [34]. Table 5 illustrates the influence of storage on the syneresis of yoghurt produced from LBW cows' milk. No substantial difference between the synereses of yoghurt samples was found on the first day of storage. There was a slight increase in syneresis values during storage of yoghurt produced from milk with weak acid gelation properties, while no change in the syneresis values of the yoghurt produced from milk with strong acid gelation properties was observed.

In the present study all samples of yoghurt produced from LBW cows milk exhibited non-Newtonian fluid behaviour, including shear rate thinning. On the first and 10th days of storage, all samples showed a decrease in apparent viscosity during shearing. The consistency factor of the yoghurt samples ranged from 2.01 to 3.57 Pa s^n on the first day of storage (Table 5). After 10 days of storage, the increase in the consistency index of all the samples was determined. This could be a consequence of the further strengthening of the structure formed by protein network during storage. It should be mentioned that the highest consistency factor values belonged to the yoghurt produced from milk with strong acid-induced gelation properties after 10 days of storage. The lowest consistency factor (4.64 Pa s^n) was recorded for the yoghurt produced from milk samples with the highest AGT values and the lowest G'_{40} values.

Considering the beneficial properties of LBW cows' milk, it seems use for the production of yoghurt with strong physicochemical properties is possible even when the milk exhibits weak acid-induced gelation properties. LBW cows' milk therefore has potential for yoghurt production.

4. Conclusions

Gross composition of milk obtained from native Lithuanian Black and White breed cows demonstrated considerable variation, which were apparently unrelated to the seasons. However, protein content was significantly affected by the season, as reflected in protein composition. The concentration of individual milk proteins in LBW milk during the winter months was significantly lower ($p < 0.05$) in comparison with those in the milk from the summer period. Evaluation of the technological usability of LBW cows' milk for the production of cultured dairy products revealed that milk samples with strong acid-induced gelation properties had significantly higher β-lg content ($p < 0.05$) and lower α_{S1}- and κ-Cn content in comparison with milk samples with weak acid-induced gelation properties. However, further analysis of yoghurt produced from LBW milk showed that the production of yoghurt with strong physicochemical properties was possible even when milk had weak acid-induced gelation properties. Lithuanian Black and White dairy cattle milk can be considered a good raw material for the production of cultured dairy products.

Acknowledgements

The authors received financial support for the research from Lithuania Ministry of Agriculture.

References

[1] Kantanen, J., Olsaker, I., Holm, L.E., Lien, S., Vilkki, J., Brusgaard, K., Eythorsdottir, E., Danell, B., Adalsteinsson, S., "Genetic diversity and population structure of 20 North European cattle breeds", *Journal of Heredity* 91(6), 446-457. 2000.

[2] Lien, S., Kantanen, J., Olsaker, I., Holm, L.E., Eytorsdottir, E., Sandberg, K., Dalsgard, B., Adalsteinsson, S., "Comparison of milk protein allele frequencies in Nordic cattle breeds", *Animal Genetics* 30(2), 85-91. 1999.

[3] Heck, J.M.L., van Valenberg, H.J.F., van Hooijdonk, A.C.M., "Seasonal variation in the Dutch bovine raw milk composition", *Journal of Dairy Science* 92(10), 4745-4755. 2009.

[4] Jensen, H.B., Holland, J.W., Poulsen, N.A., Larsen, L.B., "Milk protein variants and isoforms identified in bovine milk representing extremes in coagulation properties", *Journal of Dairy Science* 95(6), 2891-2903. 2012.

[5] Gustavsson, F., Glantz, M., Buitenhuis, A.J., Lindmark-Månsson, H., Stålhammar, H., Andren, A., Paulsson, M., "Factors influencing chymosin-induced gelation of milk from individual dairy cows: Major effects of casein micelle size and calcium", *International Dairy Journal* 39, 201-208. 2014.

[6] Poulsen, N.A., Bertelsen, H.P., Jensen, H.B., Gustavsson, F., Glantz, M., Lindmark, Månsson, H., Andrén, A., Paulsson, M., Bendixen, C., Buitenhuis, A.J., Larsen, L.B., "The occurrence of noncoagulating milk and the association of bovine milk coagulation properties with genetic variants of the caseins in 3 Scandinavian dairy breeds", *Journal of Dairy Science* 96(8), 4830-4842. 2013.

[7] Tyriseva, A.M., Ikonen, T., Ojala, M., "Repeatability estimates for milk coagulation traits and non-coagulation of milk in Finnish Ayrshire cows", *Journal of Dairy Research* 70(1), 91-98. 2003.

[8] Joudu, I., Henno, M., Kaart, T., Pussa, T., Kart, O., "The effect of milk protein contents on the rennet coagulation properties of milk from individual dairy cows", *International Dairy Journal* 18(9), 964-967. 2008.

[9] Poulsen, N.A., Glantz, M., Rosengaard, A.K., Paulsson, M., Larsen, L.B., "Comparison of milk protein composition and rennet coagulation properties in native Swedish dairy cow breeds and high-yielding Swedish Red cows", *Journal of Dairy Science* 100(11), 8722-8734. 2017.

[10] Allmere, T., Andrén, A., Lindersson, M., Björck, L., "Studies on rheological properties of stirred milk gels made from milk with defined genetic variants of κ-casein and β-lactoglobulin", *International Dairy Journal* 8 (10-11), 899-905. 1998.

[11] Hallen, E., Allmere, T., Naslund, J., Andren, A., Lunden, A., "Effect of genetic polymorphism of milk proteins on rheology of chymosin-induced milk gels", *International Dairy Journal* 17(7), 791-799. 2007.

[12] Glantz, M., Gustavsson, F., Bertelsen, H.P., Stalhammar, H., Lindmark-Mansson, H., Poulson, M., Bendixen, C., Gregersen, V.R., "Bovine chromosomalr egions affecting rheological traits in acid-induced skim milk gels", *Journal of Dairy Science* 98(2), 1273-1285. 2015.

[13] Ketto, I.A., Knutsen, T.M., Øyaas, J., Heringstad, B., Ådnøy, T., Devold, T.G., "Effects of milk protein polymorphism and composition, casein micelle size and salt distribution on the milk coagulation properties in Norwegian Red cattle", *International Dairy Journal* 70, 55-64. 2017.

[14] Ketto, I.A., Øyaas, J., Ådnøy, T., Johansen, A.G., Schüller, R.B., Narvhus, J., Skeie, S.B., "The influence of milk protein genetic polymorphism on the physical properties of cultured milk", *International Dairy Journal* 78, 130-137. 2018.

[15] AOAC Official method 991.25: (2002): Calcium, Magnesium and Phosphorus in Cheese, Atomic Absorption Spectrophotometric and Colorimetric Method - Official final action. Gaithersburg, MD, USA: AOAC International.

[16] LST EN 12821:2009. Foodstuffs. Determination of vitamin D by high performance liquid chromatography. Measurement of cholecalciferol (D3) or ergocalciferol (D2). Vilnius, Lithuania: The Lithuanian Standards Board (in English).

[17] LST EN 12823-1:2014. Foodstuffs. Determination of vitamin A by high performance liquid chromatography. Measurement of all-E-retinol and 13-Z-retinol. Vilnius, Lithuania: The Lithuanian Standards Board (in English).

[18] Leskauskaite, D., Jasutiene, I., Malinauskyte, E., Kersiene, M., Matusevicius, P., "Fortification of dairy products with vitamin D3", *International Journal of Dairy Technology* 69(2), 177-183. 2016.

[19] Bonizzi, I., Buffoni, J.N., Feligni, M., "Quantification of bovine fractions by direct chromatographic analysis of milk. Approaching the application to a real product context", *Journal of Chromatography A* 1216(1), 165-168. 2009.

[20] Zhang, L., Folkenberg, D.M., Amigo, J.M., Ipsen, R., "Effect of exopolysaccharide-producing starter cultures and post-fermentation mechanical treatment on textural properties and microstructure of low fat yoghurt", *International Dairy Journal* 53, 10-19. 2016.

[21] Šernienė, L., Kabašinskienė, A., "Analysis of reproductive performance, milk composition and quality of indigenous cows: Lithuanian light grey and white backed", *Veterinarija ir Zootechnika* 72(94), 30-36. 2015.

[22] Bernabucci, U., Basiricò, L., Morera, P., Dipasquale, D., Vitali, A., Cappelli, F.P., Calamari, L., "Effect of summer season on milk protein fractions in Holstein cows", *Journal of Dairy Science* 98 (3), 1815-1827. 2015.

[23] O'Callaghan, T.F., Hennessy, D., McAuliffe, S., Kilcawley, K.N., O'Donovan, M., Dillon, P., Ross, R.P., Stanton, C., "Effect of pasture versus indoor feeding systems on raw milk composition and quality over an entire lactation", *Journal of Dairy Science* 99(12), 9424-9440. 2016.

[24] Gaignon, P., Gelé, M., Hurtaud, C., Boudon, A., "Characterization of the nongenetic causes of variation in the calcium content of bovine milk on French farms", *Journal of Dairy Science* 101(5), 4554-4569. 2018.

[25] Gill, B.D., Zhu, X., Indyk, H.E., "The determination of vitamin D3 and 25-hydroxyvitamin D3 in early lactation and seasonal bovine milk", *International Dairy Journal* 63, 29-34. 2016.

[26] Yasmin, A., Huma, N., Butt, M.S., Zahoor, T., Yasin, M., "Seasonal variation in milk vitamin contents available for processing in Punjab, Pakistan", *Journal of the Saudi Society of Agricultural Sciences*11, 99-105. 2012.

[27] Chen, B., Lewis, M.J., Grandison, A.S., "Effect of seasonal variation on the composition and properties of raw milk destined for processing in the UK", *Food Chemistry* 158, 216-223. 2014.

[28] Chen, B., Grandison, A.S., Lewis, M.J., "Effect of seasonal variation on some physical properties and heat stability of milk subjected to ultra-high temperature and in-container sterilisation", *Food Chemistry* 181, 227-234. 2015.

[29] Gaucher, I., Boubellouta, T., Beaucher, E., Piot, M., Gaucheron, F., Dufour, E., "Investigation of the effects of season, milking region, sterilisation process and storage conditions on milk and UHT milk physico-chemical characteristics: a multidimensional statistical approach", *Dairy Science and Technology* 88(3), 291-312. 2008.

[30] Singh, H., "Heat stability of milk", *International Dairy Journal* 57, 111-119. 2004.

[31] Lucey, J.A., Singh, H., "Formation and physical properties of acid milk gels: A review", *Food Research International* 30(7), 529-542. 1997.

[32] Tamime, A.Y., Skriver, A., Nilsson, L.E., "Starter cultures", In: Tamime AY, editor. Fermented milks. Oxford, UK: Blackwell Science 11-52. 2006.

[33] Hassan, L.K., Haggag, H.F., El Kalyoubi, M.H., Abdel-Aziz, M., El-Sayed, M.M., Sayed, A.F., "Physico- chemical properties of yogurt containing cress seed mucilage or guar gum", *AOAS* 60(1), 21-28. 2015.

[34] Salvador, A., Fiszman, S.M., "Textural and sensory characteristics of whole and skimmed flavored set-type yoghurt during long storage", *Journal of Dairy Science* 87(12), 4033-4041. 2004.