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Assessment of Water Buffalo Milk and Traditional Milk Products in a Sustainable Production System

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Abstract: Water buffalo (*Bubalus bubalis*) conservation in Serbia is under an in situ program, but additional efforts are needed to ensure the development of this animal's genetic resources biodiversity. This research aims to describe challenges and possible strategies for sustainable water buffalo milk production. In this study, the physicochemical characteristics of buffalo milk and buffalo dairy products (cheese, butter, and kajmak) were determined. Furthermore, amino and fatty acids composition and the related health lipid indices (atherogenic and thrombogenic) were assessed. The findings support the fact that buffalo milk is a reliable source of high-quality nutrients (dry matter: 16.10%, fat: 6.02%, protein: 4.61%). Leucine, lysine, and valine content were found to be high in buffalo milk and cheese. A substantial quantity of non-essential glutamic and aspartic amino acids was observed in milk, as well as glutamic acid and tyrosine in cheese. It was established that milk protein of buffalo cheese had a favorable proportion of essential and non-essential amino acids (61.76%/38.24%). The results revealed significant differences ($p < 0.05$) in fatty acid profiles among the three dairy products for saturated short-chain, n-3, and n-6 fatty acids. Conversely, no significant difference ($p < 0.05$) was observed in monounsaturated fatty acids content. Kajmak showed the most favorable anti-atherogenic and anti-thrombogenic properties due to lower saturated and higher polyunsaturated fatty acid content. These results confirmed that buffalo milk could be successfully used in producing high-quality traditional dairy products with added value and beneficial characteristics from the aspect of a healthy diet. Furthermore, it could actively contribute to the promotion of sustainable production of buffaloes and strengthen the agricultural production of rural areas and their heritage.

Keywords: sustainable livestock production; added value; buffalo milk; dairy products; chemical composition; nutritional properties

1. Introduction

Water buffaloes (*Bubalus bubalis*) are distributed worldwide, with a total population of about 180 million. The majority of populations are located in Asia (around 97%). It is considered that the water buffalo is an efficient converter of poor quality forages into high-quality products [1]. Buffaloes play an essential role in the heritage of the local rural populations and their economy in South-Western Serbia, where these animals are raised under extensive husbandry and sustainable principles. In previous decades, control of productivity and selection for milk production were insufficient. As a result, milk production is lower in comparison with selected populations of buffalo in some Asian countries [1]. The mainly conventional livestock production in Serbia and the popularization of imported, highly productive breeds of livestock contributed to a significant reduction in the number of autochthonous breed populations, and the genetic diversity is eroded. Buffalo population, as well, showed a tendency to decrease. Since the 2000s, a program for the in situ conservation of buffalo population started in Serbia [2], but still, according to the Domestic Animal Diversity Information System of the Food and Agriculture Organization of the United Nations (FAODAD-IS), the estimated population of buffalo is low, and their local risk status is “endangered”, with a decreasing trend.

As reported by the same database, there are about 30 bulls and 724 cows of buffalo evidenced in the breeding program in Serbia [3]. Furthermore, the main challenges facing the buffalo breeding sector is low profitability. On the other hand, in many countries, an increasing demand for buffalo dairy products has been noticed, and accordingly, most farms are undertaking a progressive intensification of rearing techniques [4,5]. Besides, non-cattle dairy systems, whether intensive or not, could be a sustainable alternative and potentially a significant added value for producers and the dairy sector [6]. Sheep and goats are essential species in non-cattle milk production. Thus, the polymorphism of selected genes affecting milk composition in small ruminants is well documented [7–9], but efforts are needed in the genetic assessment of buffalo populations.

Buffaloes of the Mediterranean region are phenotypically different from country to country because of the different environment and management practices. Still, the fundamental objective of breeding buffaloes in Europe and the Near East is milk production [5]. Dairy farms have valuable input to a resilient and sustainable food system. They possess the ability to provide not just basic nutrition, but also healthy nutrition. For developed and developing countries, dairy has the potential to reinvigorate rural economies, providing sustainable livelihoods for smallholder farmers and a resilient source of economic growth [10]. According to Borghese and Moioli [5], there is a need to more deeply study dairy products made from buffalo milk in the Mediterranean area as well the variability of their technologies because they represent an important part of global biodiversity.

Buffalo milk, like cow milk, can be used for the manufacture of a wide variety of dairy products [11]. It has long been valued for its fortunate chemical composition which determines its nutritive properties and suitability for the production of traditional as well as industrial dairy products [12]. Compared with cow milk, it contains less water and more fat and is especially useful for the production of fat-based dairy products such as butter and kajmak. Kajmak is an artisanal Serbian dairy product mainly produced from cow’s milk, and based on its physicochemical characteristics may be placed between cheese and butter [13]. The specific fatty acid composition of buffalo milk contributes to the unique features of its products. Butter, for example, made from buffalo milk due to its higher level of saturated fatty acids is much harder than that made from bovine milk [11].

Moreover, the high variability in triglyceride and fatty acid composition of buffalo milk makes it possible to separate milk fat into various fractions based on its melting characteristics [14]. Higher proportions of high-melting triglycerides in buffalo milk contribute to its higher density [15] and make it suitable for cheese making. Buffalo cheese is highly prized for its pure white appearance and smooth texture [11]. In Italy, fresh and Pasta Fialata cheeses, especially Mozzarella and Borelli cheeses, are traditionally prepared from buffalo milk. In Balkan countries, several types of white brined cheese and pickled cheese are made from buffalo milk [16]. Because of differences between buffalo and other ruminant milk in compositional and physicochemical properties, some modifications

of processing technology and equipment designed for different kinds of milk are necessary for buffalo milk processing [12].

The advantages of buffalo milk compared to cow milk are not only in terms of physicochemical, compositional, and sensory attributes, but also in its nutritional and health aspects [17]. Buffalo milk proteins are complete proteins of high biological value, and they contain all the essential amino acids in the proportions required by the human body [15]. Furthermore, buffalo milk and its products could represent a good source of favorable conjugated linoleic acid (CLA) in human nutrition [18]. In recent decades, many studies have been devoted to improving milk fatty acid (FA) composition by increasing the amount of FA with beneficial effects on human health and with more appropriate technological properties [19]. Findings of FA and triglyceride profiles of low melting fractions of milk fat have suggested that the therapeutic value of low melting fractions of buffalo milk fat was higher than native milk fat [14].

There are insufficient research data on the production potential and dairy product quality of water buffalo in sustainable farming systems in Serbia. In particular, the chemical characteristics of buffalo milk and its dairy products in Serbia have been studied in a limited fashion [2]. In this paper, the composition and physicochemical properties of major constituents of buffalo milk are presented, with particular emphasis on amino and fatty acids. Furthermore, buffalo dairy products are analysed, compared, and discussed in terms of their nutritional, technological, and health aspects to find the added value of the traditional products from sustainable production.

2. Materials and Methods

2.1. Sample Collection

Samples of bulk, raw buffalo milk and dairy products (cheese, butter, and kajmak) were taken in early spring 2017, from small households of the Rashka district (42.989400, 20.332684) in South-Western Serbia. Ten households participated in the study, with an average water buffaloes herd size of 5 milking buffaloes. All the herds were reared in a sustainable, extensive management system, grazing on pastures and meadows. The milking animals each received 0.5 kg oats daily as supplementary feed during milking.

2.2. Milk Chemical Analysis

The standard chemical composition and milk urea (MU) analysis of bulk, raw buffalo milk samples were conducted using a MilkoScan FT + analyzer for routine compositional raw milk analysis employing Fourier Transform Infrared “FTIR”. The MilkoScan™ FT + techniques comply with: ISO 9622/IDF 141:2013 [20] and the AOAC official method 972.16 [21]. The energy value of milk was estimated, according to Popović-Vranješ [22].

2.3. Buffalo Milk Dairy Products Physicochemical Analysis

Samples of buffalo dairy products (cheese, butter, and kajmak) were analyzed for chemical composition (dry matter, fat, protein, and moisture) following standard methods of the Association of Official Analytical Chemists [23]. The dry matter was determined with a standard method of measuring weight loss after drying (AOAC 926.08-1927). Protein content was measured using the Kjeldahl-Van Slyke method for the determination of total N (AOAC 2001.14) with a Kjeltec Auto Analyzer (Model 2400, Tecator, Hoganas, Sweden) and converted by a multiplication factor of 6.38. The fat content was quantified according to the Van Gulik method [24]. Fat in dry matter (FDM, %) was calculated as $\text{fat}/(100 - \text{moisture}) \times 100$, moisture in nonfat substance (MFFB, %) was calculated as $\text{moisture}/(100 - \text{fat}) \times 100$. The active acidity of the dairy product was measured applying a pH meter (WTW, tip inoLab pH 720), and titratable acidity was expressed in terms of Soxhlet-Henkel/°SH value.

2.4. Amino Acid Profile

Amino acid profile of buffalo milk and dairy products was performed following the protocol of Henderson [25]. The prepared samples were analyzed for the content of amino acids after acid hydrolysis using the HPLC method (High-Performance Liquid Chromatograph Chromaster, Zorbax Eclipse-AAA column (4.6 × 250, 5 μm) with DAD-3000 (diode array detector).

2.5. Fatty Acid Profile

To determine the milk fatty acids, a gas chromatograph (GC) with flame ionization detector (FID) was used. Determination of the free fatty acids (FA) in the buffalo milk and dairy products was done after methylation with boron trifluoride in methanol, using Shimadzu's gas chromatograph with FID on InterCap WAX (length 30 m, inner diameter 0.25 mm, film thickness 0.25 μm) column (AOAC 996.06). Atherogenic (AI) and thrombogenic indices (TI) were calculated according to Ulbricht and Southgate [26].

2.6. Statistical Analysis

Statistical analysis of the data was carried out using GraphPad Prism v6 (GraphPad, San Diego, CA, USA) software. All data was normally distributed (Shapiro-Wilk normality test $p > 0.05$). The homogeneity of variances was analyzed using Levene's test and then the parametric model of analysis of variances (ANOVA) followed by Tukey's multiple comparison test, and t test. Significance was established at $p < 0.05$.

3. Results

3.1. Milk Chemical Composition

The chemical composition of the buffalo milk samples is presented in Table 1. Results indicated that fat and ash were the most inconsistent components, whereas lactose and MU content showed minimum variation. As a result of a higher degree of fat variation, wide range of fat/protein ratio (CV: 28.36%) was also found. The analysis confirmed that buffalo milk is rich in fat, protein, and lactose. Therefore, the estimated energy value of buffalo milk varied from 81.81 to 106.62 (kcal/100 mL).

Table 1. Chemical composition of buffalo milk.

Parameters	$\bar{x} \pm SD$	CV (%)	Min–Max
Dry matter (%)	16.60 ± 1.12	6.75	14.91–17.80
Solid nonfat (%)	10.48 ± 0.52	4.96	9.97–11.51
Fat (%)	6.02 ± 1.27	21.10	4.26–7.16
Protein (%)	4.61 ± 0.52	11.28	4.12–5.62
Lactose (%)	5.36 ± 0.16	2.98	5.10–5.63
Ash (%)	0.60 ± 0.14	23.33	0.37–0.80
MU (mg/dL)	35.80 ± 0.88	2.46	33.90–36.70
F/P	1.34 ± 0.38	28.36	0.77–1.72
Energy value (kcal/100 mL)	96.90 ± 10.52	10.86	81.81–106.62
Milk density (g/cm ³)	1.037 ± 0.002	0.19	1.036–1.042

\bar{x} —arithmetic mean; SD—standard deviation; CV (%)—coefficient of variation; minimal X (min) and maximal X (max) values of variables; F/P—fat/protein ratio; MU—milk urea.

3.2. Milk Amino Acid Composition

Table 2 summarizes the composition of amino acids in buffalo milk. The results of the amino acid analysis revealed that leucine (0.41%) was the major amino acid, while lysine (0.33%) was second among all essential amino acids. A substantial quantity of other branched amino acids (valine and isoleucine) was also observed. Of the non-essential amino acids (NEAA), glutamic and aspartic acids

were found at a high level. Methionine is the first limiting amino acid. Higher variations of NEAA, especially of tyrosine content (CV: 36.93%), were also observed.

Table 2. Amino acids composition of buffalo milk (%).

Essential Amino Acid (EAA)	$\bar{x} \pm SD$	CV (%)	Min–Max	Non-Essential Amino Acid (NEAA)	$\bar{x} \pm SD$	CV (%)	Min–Max
Histidine	0.15 ± 0.02	13.33	0.13–0.17	Asparatic acid	0.32 ± 0.02	5.71	0.29–0.33
Threonine	0.14 ± 0.01	7.14	0.12–0.15	Glutamic acid	1.01 ± 0.01	0.99	0.99–1.02
Valine	0.26 ± 0.01	3.85	0.25–0.27	Serine	0.09 ± 0.02	22.22	0.07–0.11
Methionine	0.05 ± 0.004	8.00	0.05–0.06	Glycine	0.08 ± 0.003	3.75	0.08–0.09
Phenylalanine	0.21 ± 0.01	4.76	0.19–0.23	Arginine	0.11 ± 0.01	9.09	0.10–0.12
Isoleucine	0.24 ± 0.01	4.17	0.23–0.25	Alanine	0.14 ± 0.01	7.41	0.13–0.16
Leucine	0.41 ± 0.01	2.44	0.39–0.42	Tyrosine	0.11 ± 0.04	36.36	0.08–0.16
Lysine	0.33 ± 0.02	6.06	0.30–0.35	Total	1.86		
Total	1.79						

\bar{x} —arithmetic mean; SD—standard deviation; CV (%)—coefficient of variation; minimal X (min) and maximal X (max) values of variables.

3.3. Milk Fatty Acid Composition

Saturated (SFA), monounsaturated (MUFA), and polyunsaturated fatty acid (PUFA) content of buffalo milk was found to be 69.04%, 28.46%, and 2.50%, respectively (Table 3). The values found for fatty acid composition also showed that investigated samples contained a considerable amount of saturated long chain fatty acid (SLCFA) content (C16:0, C18:0, C20:0; in total: 45.99 g/100 g). The AI and TI amounted to 2.72 and 3.02, respectively, whereas the n-6/n-3 ratio was 2.42. Results also showed that SLCFAs were the most stable in buffalo milk and varied least (CV: 9.41%), while SSCFA and SMCFA possessed much higher coefficients of variation (CV: 33.05% and 27.48%, respectively).

Table 3. Fatty acid composition of buffalo milk (g/100 g).

Parameters	$\bar{x} \pm SD$	CV (%)	Min–Max
MUFA	25.43 ± 4.38	17.22	19.01–32.09
SSCFA	2.36 ± 0.78	33.05	1.21–3.31
SMCFA	13.32 ± 3.66	27.48	8.04–17.52
SLCFA	45.99 ± 4.33	9.41	40.98–53.58
SFA	61.68 ± 5.81	9.42	52.23–70.35
PUFA	2.23 ± 0.36	16.14	1.7–2.67
PUFA/SFA	0.04 ± 0.01	25.00	0.02–0.05
Total omega-6 fatty acids (n-6)	1.78 ± 0.22	12.36	1.37–2.06
Total omega-3 fatty acids (n-3)	0.77 ± 0.20	25.97	0.45–1.00
n-6/n-3	2.42 ± 0.59	24.38	1.85–3.69
Atherogenicity index (AI)	2.72 ± 1.05	38.60	1.56–4.71
Thrombogenicity index (TI)	3.02 ± 0.76	25.24	2.03–4.47

\bar{x} —arithmetic mean; SD—standard deviation; CV (%)—coefficient of variation; minimal X (min) and maximal X (max) values of variables; MUFA—monounsaturated fatty acids (C18:1); SSCFA—saturated short-chain fatty acids (C 4:0, C8:0); SMCFA—saturated medium-chain fatty acids (C10:0, C12:0, C14:0); SLCFA—saturated long-chain fatty acids (C16:0, C18:0, C20:0); SFA—total saturated fatty acids; PUFA—polyunsaturated fatty acids (C18:2, C18:3).

3.4. Chemical Composition of Buffalo Dairy Products

Mean composition values of cheese, butter, and kajmak produced from buffalo milk are shown in Table 4. According to Serbian regulations [27], buffalo cheese is classified as a semi-fat and semi-hard cheese, due to FDM and MFFB values of 43.5% and 60.1%, respectively. Moreover, kajmak samples met the Serbian requirements for mature kajmak, and contained 77.1% of FDM, on average, and appropriate values for pH and moisture (6.6 and 34.0%, respectively). On the other hand, butter did not meet Serbian regulations for solids non-fat (SNF) content (>2%), but fat and moisture contents were within the acceptable range. The results of chemical composition also showed that kajmak represents a special

and unique dairy product. It contains less fat compared with butter, as well as less protein than cheese. Cheese is the most varied regarding fat content (CV: 14.51%), while SNF content was the most inconsistent component in butter and kajmak (CV: 63.79% and 25.39%, respectively).

Table 4. Chemical composition of buffalo dairy products.

Parameters	Cheese		Butter		Kajmak	
	$\bar{x} \pm SD$	CV (%)	$\bar{x} \pm SD$	CV (%)	$\bar{x} \pm SD$	CV (%)
Moisture (%)	46.0 ± 1.20	2.61	14.2 ± 0.28	1.97	34.0 ± 1.30	3.82
Dry matter (%)	54.0 ± 1.20	2.22	85.8 ± 0.28	0.33	66.0 ± 1.30	1.97
Protein (%)	27.0 ± 1.62	6.0	0.9 ± 0.08	8.89	5.7 ± 0.34	5.96
Fat (%)	23.5 ± 3.41	14.51	83.0 ± 1.67	2.01	50.8 ± 2.56	5.04
FDM (%)	43.5 ± 5.22	12.0	96.7 ± 3.93	4.06	77.1 ± 5.39	6.99
MFFB (%)	60.1 ± 1.11	1.85	84.0 ± 8.12	9.67	69.3 ± 6.26	9.03
SNF	30.5 ± 2.21	7.24	2.9 ± 1.85	63.79	15.2 ± 3.86	25.39
pH	5.4 ± 0.12	2.22	6.7 ± 0.08	1.19	6.6 ± 0.12	1.82
°SH	60.0 ± 2.78	4.63	14.0 ± 0.44	3.14	14.0 ± 1.20	8.57

\bar{x} —arithmetic mean; SD—standard deviation; CV (%)—coefficient of variation; FDM—Fat on a dry matter basis; MFFB—Moisture on a fat-free basis; SNF—solids non-fat.

3.5. Amino Acid Composition of Buffalo Dairy Products

Buffalo cheese represents a good source of amino acids, and, as expected, it is significantly different ($p < 0.05$) compared with kajmak and butter (Table 5). In particular, buffalo cheese is abundant in essential amino acids: lysine, leucine, and valine (4.42%, 3.90%, and 2.45%, respectively). The primary non-essential amino acids were glutamic acid (5.09%) and tyrosine (1.97%). Results also revealed that EAA/NEAA ratio in buffalo cheese is very favorable (61.76%/38.24%) and together with a high amount of branched amino acids (leucine, valine, and isoleucine) confirmed the tremendous nutritional value of buffalo cheese. Kajmak and butter, on the other hand, as high-fat dairy products contain a low amount of protein and, consequently, amino acids. Compared with butter, kajmak contained a significantly higher ($p < 0.05$) amount of threonine, leucine, and arginine. However, minor differences were noticed in the isoleucine and glutamic acid content of butter and kajmak, whereas histidine, phenylalanine, valine, and threonine were not detected.

Table 5. Amino acid composition of buffalo dairy products.

Parameters	Cheese	Butter	Kajmak
	$\bar{x} \pm SD$	$\bar{x} \pm SD$	$\bar{x} \pm SD$
Essential Amino Acid (EAA)			
Histidine	0.75 ± 0.05	ND	ND
Threonine	1.13 ± 0.01 ^a	0.02 ± 0.004 ^c	0.13 ± 0.02 ^b
Valine	2.45 ± 0.17 ^a	ND	0.16 ± 0.04 ^b
Methionine	1.68 ± 0.08 ^a	ND	0.10 ± 0.01 ^b
Phenylalanine	1.81 ± 0.04 ^a	ND	0.12 ± 0.01 ^b
Isoleucine	2.03 ± 0.03 ^a	0.04 ± 0.004 ^c	0.13 ± 0.02 ^b
Leucine	3.90 ± 0.09 ^a	0.06 ± 0.007 ^c	0.26 ± 0.03 ^b
Lysine	4.42 ± 0.18 ^a	0.09 ± 0.07 ^b	0.26 ± 0.03 ^b
Total	18.17	0.21	1.16
Non-Essential Amino Acid (NEAA)			
Aspartic acid	1.46 ± 0.14 ^a	ND	0.23 ± 0.05 ^b
Glutamic acid	5.09 ± 0.20 ^a	0.19 ± 0.01 ^c	0.74 ± 0.05 ^b
Serine	0.99 ± 0.16 ^a	0.04 ± 0.01 ^b	0.17 ± 0.01 ^b
Glycine	0.42 ± 0.01 ^a	0.01 ± 0.002 ^c	0.07 ± 0.01 ^b
Arginine	0.90 ± 0.01 ^a	0.01 ± 0.001 ^c	0.20 ± 0.01 ^b
Alanine	0.42 ± 0.01	ND	ND
Tyrosine	1.97 ± 0.20 ^a	ND	0.11 ± 0.01 ^b
Total	11.25	0.25	1.52

\bar{x} —arithmetic mean; SD—standard deviation; ND—Non-detectable; ^{a,b,c}—different letters indicate statistical differences ($p < 0.05$), while same letters indicate no statistical difference.

3.6. Fatty Acid Composition of Buffalo Dairy Products

The fatty acid content of buffalo dairy products is presented in Table 6. Significant differences ($p < 0.05$) were established between butter and kajmak regarding all groups of saturated fatty acids. In particular, kajmak contained more SSCFA and SMCFA, whereas butter was more abundant in SLCFA. MUFA content did not show a significant difference ($p < 0.05$) between all investigated dairy products. The data showed that the nutritionally most beneficial fatty acids are found in kajmak. Thus, the fatty acid composition grouped as, SFA, MUFA and PUFA were: in cheese: 66.85%, 29.06%, 4.09%; in butter: 70.82%, 26.28%, 2.09%; and in kajmak: 65.30%, 30.52%, 4.18%, respectively. Concerning health-related factors (AI), significant differences ($p < 0.05$) were established between butter and kajmak. Furthermore, it was found that kajmak had the most favourable AI and TI (1.84 and 1.82, respectively). Furthermore, kajmak possessed the highest content of n-3 fatty acids, while cheese contained the highest amount of n-6 fatty acids and had the highest n-6/n-3 ratio.

Table 6. Fatty acid composition of buffalo dairy products (g/100 g).

Parameters	Cheese	Butter	Kajmak
	$\bar{x} \pm SD$	$\bar{x} \pm SD$	$\bar{x} \pm SD$
MUFA	26.85 ± 2.82 ^a	24.06 ± 0.17 ^a	26.89 ± 1.56 ^a
SSCFA	2.53 ± 0.02 ^c	3.5 ± 0.22 ^b	5.41 ± 0.23 ^a
SMCFA	13.35 ± 0.51 ^b	14.88 ± 1.08 ^b	21.48 ± 0.65 ^a
SLCFA	45.90 ± 2.62 ^b	46.46 ± 2.48 ^b	30.09 ± 0.73 ^a
SFA	61.78 ± 3.15 ^a	64.84 ± 3.78 ^a	57.52 ± 1.51 ^a
PUFA	3.78 ± 0.21 ^a	2.66 ± 0.10 ^b	3.68 ± 0.31 ^a
PUFA/SFA	0.06 ± 0.01 ^a	0.04 ± 0.001 ^b	0.06 ± 0.005 ^a
Total omega-6 fatty acids (n-6)	3.77 ± 0.21 ^a	1.92 ± 0.05 ^c	2.65 ± 0.26 ^b
Total omega-3 fatty acids (n-3)	0.31 ± 0.03 ^c	0.74 ± 0.05 ^b	1.30 ± 0.08 ^a
n-6/n-3	12.16 ± 0.54 ^a	2.60 ± 0.11 ^b	2.04 ± 0.07 ^b
Atherogenicity index (AI)	2.31 ± 0.34 ^b	3.01 ± 0.16 ^a	1.84 ± 0.06 ^b
Thrombogenicity index (TI)	2.99 ± 0.37 ^a	3.12 ± 0.11 ^a	1.82 ± 0.08 ^b

\bar{x} —arithmetic mean; SD—standard deviation. MUFA—monounsaturated fatty acids (C18:1); SSCFA—saturated short-chain fatty acids (C 4:0, C8:0); SMCFA—saturated medium-chain fatty acids (C10:0, C12:0, C14:0); SLCFA—saturated long-chain fatty acids (C16:0, C18:0, C20:0); SFA—total saturated fatty acids; PUFA—polyunsaturated fatty acids (C18:2, C18:3); ^{a,b,c}—different letters indicate statistical differences ($p < 0.05$), while same letters indicate no statistical difference.

4. Discussion

Dairy possesses an important role in the livestock sector and global healthy diets. It makes a significant contribution towards meeting the challenges of nutritional security, sustainability, and reduction in diseases related to poor quality diet [10]. On the other hand, it is very important to consider the economic sustainability of primary production in such a chain, as profitability is a precondition for the food chain to be relevant and sustainable [28]. To achieve economic sustainability dairy farms and producers should meet consumers demand. In particular, for the realization of sustainable non-bovine breeding in Serbia, it is necessary to take into account the habits and preferences of consumers to ensure market supply chains [29]. The study conducted by Paskaš et al. [30] examined the behaviour of consumers in Serbia and confirmed that healthiness and nutritional benefits are amongst the most important factors for consuming non-bovine milk and dairy products.

Even within the same species, milk composition can vary, due to genetics, physiology, nutrition, and environment [31]. According to Borghese and Moili [5], buffalo milk possesses a higher fat (6–9.5%) and protein (4–5%) content than cow milk. The present study has shown the mean content of fat (6.02%) to fall within this range, but it was considerably less compared with the results of Tiezzi et al. [32] and Liotta et al. [33] (7.56% and 8.78%, respectively). On the other hand, the obtained mean protein content (4.61%) was in agreement with those researches (4.69% and 4.61%, respectively).

The energy value of milk is closely related to the concentration of certain compounds in dry matter, especially the amount of fat [34]. Among the common dairy species, the energy density of buffalo milk is remarkably high [11]. In the research of Mane and Chatli [16], the energetic value of buffalo milk amounted to 117 kcal/100 g while presented results showed a lower value (96.90 kcal/100 mL). Furthermore, our findings have shown that lactose is the second major constituent of buffalo milk, with a minimum and maximum of 5.10% and 5.63%. On the contrary, Gantner et al. [31] reported that the content of lactose in buffalo milk is $\leq 5\%$. In general, buffalo milk is a richer source of lactose than cow, goat, sheep, and camel milk. From the health aspect, lactose could be a good source of energy for body functions, particularly for the brain and hormonal regulation [12]. Many factors influence milk quality, and for traditional cheese production milk fat, protein, and lactose content, as well as their ratio, are essential [35]. The optimal fat/protein ratio in buffalo milk is 2:1 [17], while the presented results showed an approximate value of 1.34.

Milk urea measured at the group level can be used to monitor the efficiency of nitrogen utilization in commercial buffalo herds and as an indicator of the protein feeding situation in buffaloes [36]. Observed MU levels in this study ranged between 33.90–36.70 mg/dL and were lower compared with the findings of Di Francia et al. [36] (40.8 mg/dL). Furthermore, Liotta et al. [33] recorded higher MU value in intensive (40.68 mg/dL) than in semi-intensive buffalo herds (37.50 mg/dL). In contrast, Santillo et al. [37] concluded that levels of MU were not different among buffalo groups that were fed low protein diets and diets with flaxseed supplementation.

Buffalo milk can be considered a good source of essential amino acids, and lysine content was found to be the highest, followed by valine and isoleucine. These findings correspond to Ren et al. [38]. Studies also have shown that buffalo milk contained methionine in traces, which is comparable with the findings of Barlowska et al. [34].

The majority of fatty acids in ruminant milk are saturated [19]. Obtained results especially showed a considerable amount of SLCFA (45.99 g/100 g). Buffalo milk contains almost three times more C14:0 (myristic) acid and two times less C16:0 (palmitic) acid than cow, sheep, or goat milk [34]. Regarding fatty acids content, the previous research works are very diverse. Bustamante et al. [39] reported a higher amount of MUFA (33.1% vs. 28.46%) and, at the same time, lower content of n-6 acids than the current study (0.96% vs. 1.78%). In contrast, Pegolo et al. [40] compared with our results, found in Mediterranean buffalo milk less amount of n-3 (0.46%) but approximately the same content of n-6 fatty acids (1.78%), whereas findings of Gantner et al. [31] were in line with our data. Comparative studies indicate that dairy breeds with a high milk fat content often have a less desirable milk fat composition, have higher levels of saturated and hypercholesterolaemic fatty acids, and a lower proportion of PUFA than breeds with a lower milk yield or fat content [41]. The importance of FA profile is reflected in terms of the technological quality of raw milk. It possesses the potential to contribute to the production of dairy products with added value. From a usability point of view, higher proportions of unsaturated fatty acids are preferred (as they increase the spreading ability of butter), but their milk fat content could also cause lower stability, oxidation, and possible sensory changes [19]. Nutritional effects of milk and dairy products are highly influenced by fatty acid profile [42], as well, and FAs possess diverse implications on human health [19]. Considering the two health-related indices, the ratio of essential FA (n-6/n-3), and AI estimated values were higher than those reported by Varricchio et al. [43] (2.42 and 2.71 vs. 2.15 and 2.61, respectively). According to Claeys et al. [44], the n-6/n-3 ratio varies from 1.0 to 4.0 in ruminant milk. Consuming large amounts of n-3 fatty acids is beneficial and contributes to a lower risk of coronary diseases and some types of cancer [34].

Considerable diversity traits of buffalo milk result in various directions of milk utilization. Each buffalo dairy product varies in composition, and the present study discussed their different production, composition, and potential health properties. Thanks to its specific characteristics, buffalo milk needs to be treated differently in the cheese-making process. It is less suitable for the manufacture of hard varieties of cheese, such as Cheddar cheese. Some problems that can occur are slow development of acidity, higher curd tension, shorter renneting period, hard, dry, crumbly, corky body and texture, and

slower proteolysis [16]. The buffering capacity, pH, and viscosity of buffalo milk are higher than those of cow milk, while the fermentation and ripening process of buffalo milk is generally slower [11]. Some procedural improvements are necessary for hard cheese production from buffalo milk, such as higher heat treatment, Mucor rennet, and a greater amount of *Streptococcus thermophilus* and *Lactobacterium bulgaricus* cultures [16]. Results of the present study showed the good quality of semi-hard buffalo cheese. Still, certain varieties including Mozzarella and white pickled Domiati cheese possess superior quality when made from buffalo milk [15]. One of the main problems in the production of buffalo semi-hard and hard cheese is the difficulty of converting milk into naturally ripened cheese [45].

Buffalo milk is also very desirable for the manufacture of fat-rich dairy products due to its higher fat content, the bigger size of the globule, and the higher proportion of solid fat [17]. Industrial butter is produced by the churning of cream, often after pasteurization. The homemade product is obtained simply by churning acidified milk. A peculiarity of buffalo butter is the colour, which is much whiter than cows' milk butter, due to the lack of carotenoids [5]. Furthermore, buffalo milk produces butter with a significantly higher yield, and displays more stability than that from cow cream, due to the more solid fat and a slower rate of fat hydrolysis in the former cream [17]. The results of this study confirm the results of [46,47] for butter chemical composition (pH, fat, and protein). Kajmak is a homemade product, it is produced based on the traditional manufacturing procedure [13]. Kajmak is a specific product, characterized by high-fat content, the presence of proteins, and its peculiar ripening process [48]. During the ripening process, which lasts 3–4 weeks, kajmak partly loses the continuity of its moisture phase, while limited fat phase continuity appears, and a specific flavor is developed [13]. Therefore, the ripened kajmak made from cow milk published by Pudja et al. [13] had values of chemical composition in the following ranges: moisture: 15–35%, fat: 50–70%, FDM: 75–90% and proteins: 2–7%, and do not differ from values in the present study.

The present investigation revealed that in cheese, lysine was the major amino acid but also a substantial quantity of branched-amino acids (valine, isoleucine, and leucine) was observed. Branched-amino acids promote protein synthesis in muscle cells, and they are metabolized to generate energy in muscles rather than in the liver [49]. Glutamic acid content was found to be the highest of non-essential amino acids in cheese. Similarly, glutamic acid was the predominant non-essential amino acid in butter made from cow milk, but in a much smaller amount. At the same time, leucine and isoleucine were the main essential amino acids [50]. On the contrary, leucine was not detected in the present study in buffalo butter.

Dairy products, such as butter, ghee, and cream, have been considered as basic nutrient-dense foods that can deliver many energy-rich nutrients [46]. It was reported by Popović et al. [47] that traditional Serbian dairy products, cheese, and kajmak, made from bovine milk, possessed a high content of SFAs (70%), mainly palmitic acid. In contrast, the present study showed lower SFA contents in buffalo milk cheese and kajmak and a more desirable fatty acid pattern. However, saturated fatty acids were the predominant fraction in buffalo butterfat (70.49%). Kwak et al. [50] reported that butter has double the concentration of saturated fat in comparison with that in cream and other dairy products. Therefore, dairy products such as butter very often have been criticized for their unfavorable FA profile [19]. However, stearic acid could be beneficial to health and contribute to the level of low-density lipoprotein (LDL) in the blood [50]. Compared with butter, cheese possesses a more favourable FA profile: in particular, higher proportions of MUFA and PUFA.

The manufacturing process could also affect the nutritional and health characteristics of the cheeses [51]. Even though ripened cheeses contain more fat on a wet basis, their fatty acid profile is more desirable. In particular, the Blu cheese shows a healthier fatty acid profile than Mozzarella and the cheese-making process, and ripening contribute to reducing atherogenic (C12:0 and C14:0) and increasing some beneficial fatty acids (C18:3 n-3, cis-9, trans-11 conjugated linoleic acid) [52]. Furthermore, it is supposed that milk fat with high AI and TI values may be more likely to contribute to the development of atherosclerosis or coronary thrombosis in humans [53]. The value of AI in milk and dairy products is around 2, whereas AI = 1.5 is considered as low, and 2.5 is high [54]. Accordingly,

our dairy products, kajmak, and cheese appeared to exhibit stronger anti-atherogenic activities compared with milk and butter. The thrombogenic indices take into account the relationship between the pro-thrombogenic (saturated) and anti-thrombogenic fatty acids (unsaturated) [26]. Despite being a high-fat product, kajmak also showed a low TI value (1.82).

5. Conclusions

The buffalo production under sustainable principles and extensive husbandry plays an essential role in the heritage of local rural populations and their economy in South-West Serbia. Buffalo milk is used for manufacturing of traditional milk products, such as cheese, butter, and kajmak. The results of the chemical composition of buffalo milk showed that it could be utilized for making a variety of good quality traditional dairy products with added value. Consequently, they are important for the dairy industry and especially useful for traditional artisanal production. The analysed parameters of milk and dairy products showed considerable diversity, and nutritional value varies from one product to the other. In particular, the nutritional content of ripened kajmak and cheese was the most favourable. Primarily, kajmak represents a valuable source of essential fatty acids, while cheese was abundant in important branched amino acids (leucine, valine, and isoleucine). These results can contribute to the promotion of the value-added buffalo dairy products from sustainable production systems. Although buffaloes are successfully bred with traditional methods on pastures, to achieve more consistent and sustainable milk production, some husbandry improvements should be required. In particular, more attention should be paid to the uniform quality of the raw milk, selective breeding, and productivity of animals. Thus, these would improve the farm economy and the quality of derived products, which could actively contribute to rural development of the region and more effective conservation of buffalo genetic resources. Furthermore, to enhance buffalo production in more sustainably and holistically, producers should be encouraged to access appropriate market information and take into account more sustainable initiatives in the dairy industry, such as the nutritional and health benefits of dairy products.

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