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PROTEIN CHARACTERIZATION OF ROMANIAN BUFFALO MILK COMPARED TO COW MILK

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ABSTRACT. Buffaloes have been studied in relation to the exclusive use of their milk for the manufacture of high-quality dairy products. Despite cow milk proteins having been extensively studied, there is still a substantial lack of characterisation on proteins fractions from Romanian buffalo milk. We report here a detailed protein analysis of buffalo skim milk, whey and whole milk protein fractions in comparison with values gathered from cow milk protein analysis. Major protein components, i.e. β- caseins, k-caseins, α-lactalbumin and β-lactoglobulin, were characterized through HPLC with the subsequent amino acids (GS) providing a scientific basis to coagulation/cheese making processes used in dairy productions. The mean data collected from the samples of buffalo milk were compared using the Origin 8.5 program, ANOVA test. The Romanian buffalo milk proved to be a high value raw material for further processing into dairy products, with more essential amino acids than cow milk. It is further suggested that buffalo milk should be considered as functional food through protein input in human diet.

Keywords: *buffalo, proteins*, *k-caseins, β- caseins, amino acids.*

INTRODUCTION

Milk is the most important food for young mammals and a common source of proteins and microelements for adults. Its main components, namely caseins, lactoglobulins, have specific biological functions that have been already reviewed [1]. The knowledge of protein chemistry has been used by the dairy manufacturing industry to develop/optimize more modern technologies [2] and also it has been indispensable for detection of adulteration in raw materials used for high-quality products [3]. Despite cow milk proteins having been extensively studied in a number of studies [4,5], there still is a substantial lack of characterization on the proteins from Romanian buffalo. The Romanian

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buffalo breed stems from the Common European water buffalo, Mediterranean type. Because of its economic value in our country it has been studied in regard to protein polymorphism detection [6] but a full proteomic evaluation in regard to cow milk has not yet been established. The main goal of this work was to obtain new data on the quali-quantitative composition of the major proteins and amino acids in Romanian water buffalo milk, useful for animal selection purposes.

The field literature has established that the protein content of buffalo milk is higher than in cow milk, with 80% of total proteins being caseins and whey proteins being higher in colostrum [7]. Almost all casein of buffalo milk is present in the micelles form (90–95% in cow milk) [8]. The size of the micelle in buffalo milk ranges from 80–250 nm with the majority being 110–160 nm compared to 70–110 nm in cow milk [9]. The voluminosity of the buffalo casein micelle is 2.7–3.7 ml/g in the temperature range of 25–37° C [10]. Solvation of the casein micelle as calculated from voluminosity is 2.6–2.9 g water/g casein.

RESULTS AND DISCUSSION

The results obtained after analyzing the mean concentration of proteins in buffalo milk were higher (Table1) than the one stated in the field literature [11]. These differences can be explained through the fact that in the determination the skimmed milk was used and not the whole one like in the literature mentioned data.

Table 1. Total protein concentration (mg/ml milk) in skimmed cow and buffalo milk

Samples	Absorbtion at λmax = 595nm	Total proteins (mg/ml skimmed milk)
Skimmed cow milk	0,5932	28,97
	0,6269	36,50
	0,7084	54,71
Average – cow milk		**40,06**
Skimmed buffalo milk	0,6988	52,61
	0,700	52,88
	0,7409	62,03
Average – buffalo milk		**55,84**

The protein concentration in lactoserum was higher in buffalo milk (14.13 mg/ml) than the one found in cow milk (11.79 mg/ml). Also the casein profile and soluble proteins varies according to the specie. The lowest content of casein was found in cow milk, while the highest in buffalo milk (Figure1). At other buffalo species like the African buffalo [12] the values obtained were lower than in our study.

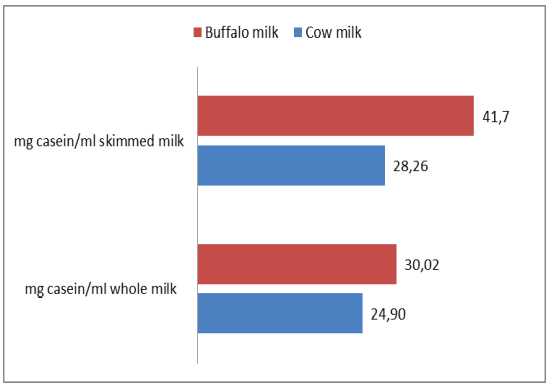

Figure 1. Casein concentration (mg/ml) in whole and skimmed cow and buffalo milk

After the total protein content was established the protein fractions were evaluated through HPLC separation both from skimmed and whole milk (Figure 2).

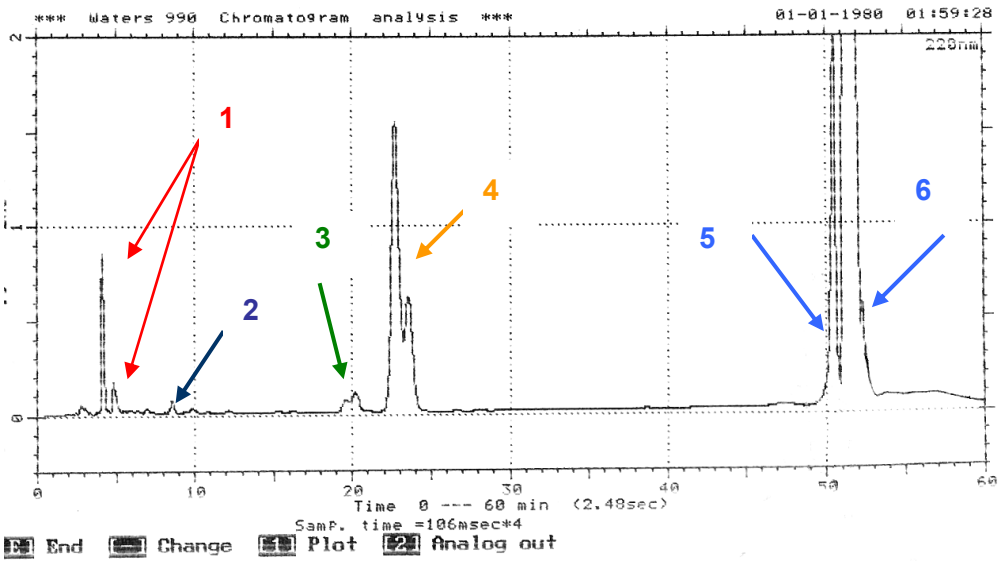

Figure 2. Identification of separated fractions from whole milk:1 - k cazeine; 2 - αs2 cazeine; 3 - αs1 cazeine; 4 - β cazeine; 5-αlactalbumin; 6 - β lactoglobuline

Recent proteomic studies are concerned on the polymorphisms of these β or k caseins, lactalbumins given the fact that it influences their quantity and product quality.

The buffalo k casein is similar to the cow's k casein and has 7 main fractions of which k4 and k5 show two more (a and b) and k7 four more (a, b, c and d). Fraction k1 represents respectively 40% of the total k-casein in buffalo and is very similar to kB1-casein in the cow, where it accounts for only 25% of total k-casein [13]. The amino acid composition of the k-casein of the two species differs in the quantity (mole/mole protein) of N-acetylgalactosamine (0–4.3 and 0–6.7 respectively in buffalo and cow) and sialic acid (5.5–8.5 and 3.5–4.3 in buffalo and cow).

As casein is constituted by α_{s1} and α_{s2} fraction, α_{s1} casein we found out that it does not differ much in the two species and it consists of fractions α_{s0} [14], α_{s1}-II and α_{s1}-I which are differentiated respectively by the presence of eight, seven and six phosphate groups. Buffalo's β casein is similar to that of the cow and has two variants. Of these, A has been found only in Venezuelan buffalo and differs from B in three amino acids [15]. The two variants A and B closely resemble cow's βA2 casein, differing from the latter by four and five amino acids respectively. The β and α_{s1} casein fractions make up 70% of the micelle network of proteins.

In figure 3 there are revealed the chromatograms obtained in the separation of the amino acids (two different extractions), being also revealed the retention time. The quantitative analysis results are shown in table 2, these representing the average of 10 parallel determinations.

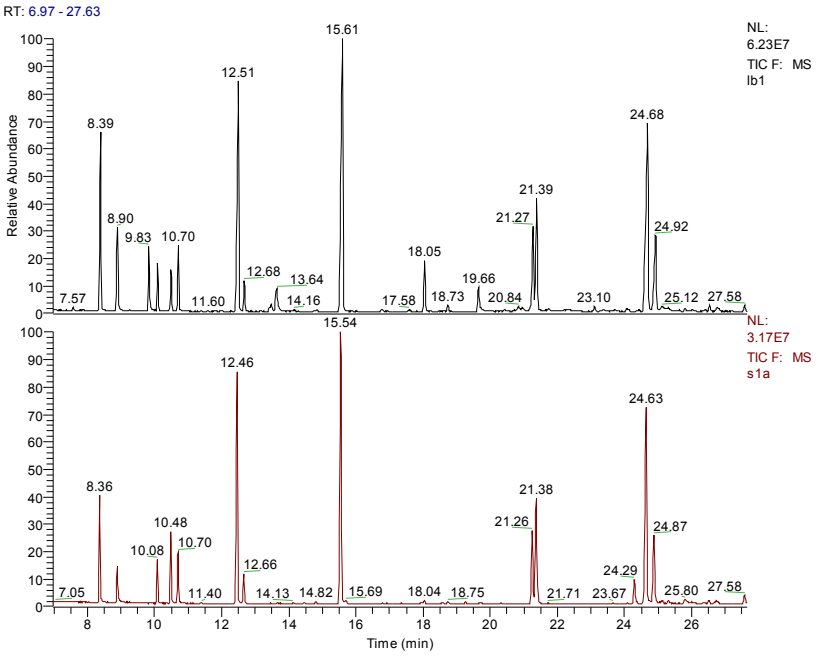

Figure 3. The comparative chromatograms of amino acids' separation in two cow milk samples obtained by two different extraction protocols

In the case of buffalo milk there was a very similar amino acids profile to the one revealed in the cow milk samples. The quantitative numbers are shown in table 2. Also, in figure 4 there is a representative chromatogram obtained through gas-chromatographic separation of the amino acids in buffalo milk.

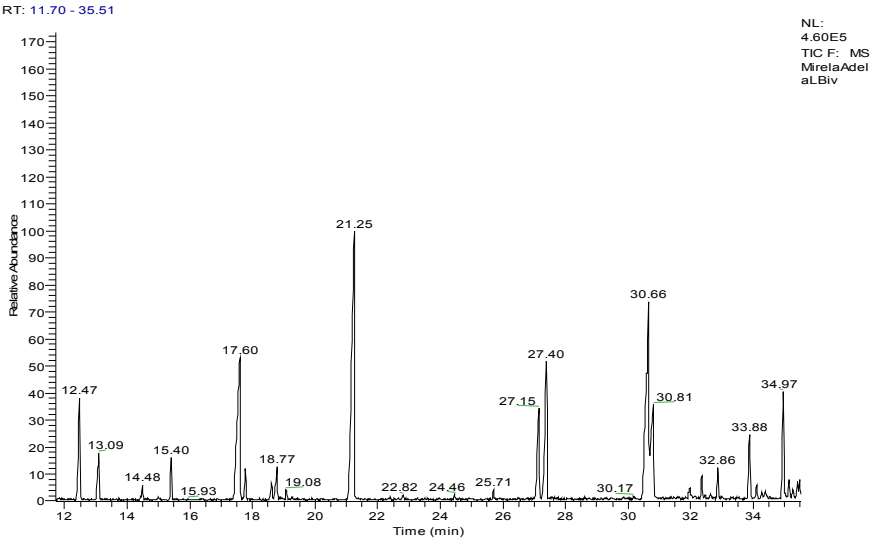

Figure 4. Representative chromatogram of gas-chromatographic separation of the amino acids in buffalo milk

There are high variations in the amino acids profile at these species. Some amino acids like cysteine, aspartic acid, arginine, histidine have been high lightened only in cow milk, whereas ornithine has been revealed in significant quantities only in buffalo milk, varying from 20.8 – 27.6 g%. significant differences have been shown in the quantity aspect also at other amino acids: alanine, glycol, glutamic acid and proline are found in two or even three times higher amounts than in cow milk; tyrosine, methionine, isoleucine, serine are found in lower quantities in buffalo milk.

Analyzing the amino acids composition in the protein fractions investigated, αS-casein, β-casein, K-casein, we revealed a high amount of glutamic acid in the β- casein fraction (Table 3) and also in K-casein fraction of buffalo milk. These results were in contrast with Addeo et al. 1977 and Ganguli, 1976 which obtained lower amounts. Given the fact that these studies were made on Murrah buffalo milk we consider that the results obtained serve as data base for Romanian buffalo with these particularities in the amino acids composition. Also the some of the essential amino acids for human nutrition were found in high amounts, like leucine and phenylalanine in β-casein and threonine in K – casein (table 3).

Table 2. Amino acids content in cow and buffalo milk

AMINO ACIDS	Average quantity (g/100g)		SEM	ANOVA
	Cow milk	Buffalo milk		
Alanine	7,58	14, 84	6.23	***
Glycol	2,05	7, 34	3.2	***
Serine	3,84	3, 00	0.34	*
Valine	5,11	5, 99	0.21	NS
Leucine	20,29	23, 68	1.09	*
Isoleucine	2,20	1, 83	0.56	**
Cysteine	6,81	-	-	-
Proline	25,55	47, 86	5.4	***
Methionine	1,13	0,56	0.44	*
Aspartic acid	3,39	-	-	-
Phenylalanine	5,65	12, 52	4.34	***
Tyrosine	5,32	0,56	3.20	***
Glutamic Acid	19,88	51, 64	5.32	***
Lysine	9,59	23, 79	6.3	**
Arginine	7,62	-	-	-
Histidine	4,45	-	-	-
Ornithine	-	25, 99	-	-

Significance, NS, not significant P>0,5; * P< 0,5; ** P<0.01; *** P<0.001; Data is presented as least square mean. SEM, standard error of the mean.

Table 3. Average amino acids composition in αS-casein, β-casein, K-casein of buffalo and cow milk

Amino acid	αS-casein		β-casein		k-casein	
	Buffalo	Cow	Buffalo	Cow	Buffalo	Cow
Aspartic acid	7.7	7.5	4.4	4.8	7.8	7.1
Tyrosine	5.8	6.2	6.5	7.6	7.8	7.2
Serine	6.1	6.5	6.9	7.4	5.7	6.4
Glutamic acid	22.7	22.5	30.6	26.8	21.2	18.2
Proline	7.9	8.4	15.9	15.8	10.1	8.6
Glycine	2.8	2.9	1.6	2.7	0.3	1.5
Alanine	3.1	3.3	1.7	1.8	5.8	5.5
*Valine	5.2	4.9	8.7	10	7.5	5.2
*Methionine	2.3	3.1	3.6	3.2	1.5	0.8
*Isoleucine	5.8	6.4	5	5.5	7.4	6.8
*Leucine	8.9	8.7	12.6	10.7	5.9	6
*Threonine	2.3	3.2	3.6	5.6	10.2	5.9
*Phenylalanine	5.4	5	6	5.3	3.3	4.1
*Lysine	8.6	9	6.7	6.5	5.7	5.8
*Tryptophan	2.2	2	0.5	0.6	1.4	1

* Essential amino acids in human nutrition

CONCLUSIONS

Buffalo milk is an important source of proteins with higher levels of amino acids like alanine, glycol, glutamic acid and proline which are found in two or even three times higher amounts than in cow milk. The Romanian buffalo should be exploited better, even on an international level, given the fact that this particular breed produces a high quality product from the protein content point of view. In regard to human nutrition buffalo milk should be considered as functional food through protein input in human diet.

EXPERIMENTAL SECTION

Samples

The samples were gathered from a semi–subsistence buffalo dairy farm situated in north Transylvania, and a dairy farm situated in the same region. Each month 10 samples were collected from buffaloes and cows fed on the same dietary regiment, including green forages (constitutes 70% of dietary intake during spring and summer) and a mixture of crop residues.

The analysis of milk proteins

Casein concentrations were revealed the volumetric method was made in accordance to the protocol initiated by Ciurdaru et al. (2001) [18], the etalon curve and the protein dosage according the Bradford method [19], and their separation using the HPLC method. The separated proteins were identified using the protocol described by Miranda et al. (2004) [20]. The only alteration made in our protocol was the type of the column used: the INRA research group from Paris used the Vydac column type C4, while we used the Vydac column type C8, which explains the slightly delay (2-3 minutes) of the retention time.

Another protocol used for casein identification was the one presented by Morra-Gutierrez et al. (1991) [21] and applied by us in accordance. The protein identification from the whey was done according to Trujillo et al. (1999) [22] and Moatsou et al. (2005) [23] who described it on goat and ewe milk, both using the same technique, RP-HPLC, their final isolation using a high tech method – mass spectrometry.

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