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# STUDY ON THE CHEMICAL COMPOSITION AND NITROGEN FRACTION OF MILK FROM DIFFERENT ANIMAL SPECIES

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***Abstract***

*The dairy industry occupies a very important place in the economy of our country. In order to obtain high quality dairy products, it is necessary to use a good quality raw material milk, which involves determining its chemical composition in particular. At present, on the shelves of our stores in the country there are products obtained from the processing of cow's milk, buffalo's milk, goat's milk and, last but not least, sheep's milk. For these reasons, in this paper we set out to perform a chemical analysis of milk from these species of interest. Following the determinations, the sheep's milk proved to be with the highest percentage of fat (7.70 ± 0.06%), SNF (11.40 ± 0.09%), TS (19.10 ± 0.04%), TP (4.98±0.04%) and in the casein content (3.65 ± 0.04%) being followed by the milk collected from the buffaloes. This study can support processors, especially those who process only cow's milk but also consumers who will be able to evaluate their products.*

***Key words***: *milk, casein, quality, sheep.*

## INTRODUCTION

Consumers have become more concerned about the consistency and protection of food items in recent years, and they have developed a strong interest in learning more about food authenticity and food fraud. In other words, customers want more detailed facts about their food, such as what they're purchasing, where it came from, and where and how it was made (McGrath et al., 2018).

World milk production derives from cows, buffaloes, goats, sheep, and camels, with buffalo milk being the second most consumed type after cow's milk (FAO, 2000).

In Romania, the most consumed types of milk are cow's milk, goat's milk, buffalo's milk and sheep's milk.

Cow milk is composed of different components including water, fats, proteins, ash, and lactose. The nitrogen-containing milk proteins can be classified into three main categories: caseins, whey (serum) proteins, and nonprotein nitrogen, which are also subdivided into several fractions (Urgu et al., 2019).

Milk is a biotic substance that animals have evolved to feed their newborns and provide essential nutrition for growth and development. Lactoferrin and lactoperoxidase are two milk proteins that have antimicrobial and immune-modulatory properties (Chen et al., 2019).

Goat's milk is an essential contribution to human nutrition, especially for people who are lactose-intolerant or sensitive to cow's milk. Goat's milk has been associated with low allergenic reactivity, antioxidant and anti-inflammatory effects, and prevention of atherosclerosis and cardiovascular diseases (Haenlein, 2004; O'Shea et al., 2004; Russell et al., 2011; Lad et al., 2017).

Buffalo milk is thought to contain almost all of the protective compounds present in other milks, such as proteins, peptides, fatty acids, vitamins, and other bioactive compounds. Total calcium, medium chain fatty acids, CLA, and retinol and tocopherol content are all higher in buffalo milk than in cow milk. Specific groups of gangliosides, for example, can only be found in buffalo milk (Berger et al., 2005).

As for sheep's milk, it is more used in obtaining cheeses, products that are in Romania are quite appreciated by consumers. Typical products have been developed according to local resources available. The production of cheese with particular characteristics can be carried out only if genetic diversity in sheep rearing is retained. Milk composition, and especially proteins and fat, may vary according to genetic diversity of the animals and different feeding systems, giving peculiar features to the milk utilized to make typical milk products. Most sheep milk produced in the world is processed into cheese, yogurt and other dairy products. The specific composition of sheep milk makes it especially valuable nutritionally and for consumer health. The nutritional importance of sheep milk is due to its higher total solids and major nutrient contents than goat and cow milk (Ospanov and Toxanbayeva, 2020).

Although milk is a food appreciated by all consumers, its composition differs from species to species. We can also have differences in the case of milk from the same species, the influencing factors being given by the breeding system, diet, age of the animals, etc. (Kittivachra et al., 2007).

The largest group of milk proteins is caseins and whey proteins, which are present in varying ratios in various milk organisms. The casein to whey protein ratio in human milk is 40:60, in quine milk it is 50:50, and in cow, pig, goat, and buffalo milk it is 80:20 (Fox et al., 2000).

Proteins are the most essential components of the human diet, providing major chemical, biochemical, and functional properties. These proteins are considered high-quality proteins because of their high biological importance, high digestibility (97–98%), and fast absorption and utilization in the body. Casein, in particular, is an extraordinarily versatile food source because it provides a steady and gradual release of amino acids into the bloodstream (Schaafsma, 2000).

Present research set out to perform a study on the quality of milk composition from species of interest in our region, including cow, buffalo, goat, and sheep milk.

## MATERIALS AND METHODS

Collecting milk samples

For each type of milk analyzed (cow's milk, buffalo, goat's and sheep's milk) ten samples were collected in sterile bottles, the milk coming from farms located in the NE region of Romania as follows: cow's milk was collected from a female located in Iași county, the buffalo milk was brought from a farm in Neamț county, the goat's milk came from a farm in Vaslui county and the sheep's milk from a farm in Suceava county.

The samples were brought in special bags provided with ice boxes and in the laboratory were stored in a refrigeration system at + 4ºC. Qualitative analyzes were performed on five samples within 24 hours in the Qualitative Milk Analysis laboratory at USAMV-Iași.

Physicochemical analysis

The pH value was determined with an electronic pH meter (WTW InaLab).

The AOAC method no. 925.23 (AOAC, 2005) was used to assess solids (TS) by dehydration in a Memmert UFE 700 forced air oven Water (W) content resulted from the difference, according to the relation: Water (%) = 100% - DM (%).

Fat of milk was determined by following Gerber method according to Dick et al., 2001.

Regarding the non-fat solid (SNF) content, this acetate was calculated by difference: SNF = TS (%) - Fat (%).

Crude ash content was assessed via incinerating at 550ºC, in a Super Therm C311 oven after prior combustion with a Bunsen funnel, until samples ceased to smoke, in accordance with AOAC 945.46 specification (AOAC, 2005).

The crude protein (CP), true protein (TP), casein, noncasein- nitrogen (NCN), whey proteins and non proteinnitrogen (NPN) contents were determined by using Kjeldahl method applied on a Velp Scientifica DK 6 digestion and UDK 7 distillation system according to standard protocol of IDF (1993). The total nitrogen content was multiplied by 6.38, which generated the crude protein content. The TP in the milk sample were determined by treating with 12% trichloroacetic acid. The nitrogen (%) was converted to NPN and NCN contents by using the conversion factor 3.60 and 6.25, respectively. Protein (nitrogen) fractions were calculated using the formulas described by Rafiq et al. (2016):

TP = CP–NPN,

Casein (N %) = Total protein (N%)–NCN (N %)

Whey protein = NCN–NPN.

Quantitative determination of amino acids was performed using the method described in the literature and using hight performance amino acid analyzer for the separation of amino acids, while tryptophan was determinated colorimetrically according to the method of (Opienska-Blauth et al., 1963; Ratu et al, 2017).

## RESULTS AND DISCUSSIONS

The pH value is a very important qualitative parameter used especially in the milk processing industry. As can be seen in Table 1 with regard to the analysis of this parameter, no very large differences were observed between the milk samples analyzed.

Regarding the chemical composition of the analyzed milk samples, it can be seen that we have differences from one species to another. Therefore, for cow's milk the dry matter content was 12.45 ± 0.04% lower by 6.65% than that of sheep's milk and 4.84% lower than that of buffalo milk. For goat's milk the average value of fat content was 12.38 ± 0.18% (Table 1).

Regarding the fat content, the milk that had the highest value was the one from sheep, the average value being 7.70 ± 0.06%. Buffalo milk registered a fat content of 6.97 ± 0.04% and cow's milk of 3.86 ± 0.02% being also the lowest percentage in terms of this quality parameter. It was also considered necessary to calculate the non-fat dry matter, an index for which the highest average value was for goat's milk (11.40 ± 0.09%) followed by buffalo milk with an average value of 10.32 ± 0.04% of the milk. cow's milk (8.59 ± 0.05%) and goat's milk where the mean was 8.13 ± 0.18%.

When we talk about the protein level in milk, we must keep in mind that this is one of the most important parameters. According to the results obtained by the new parameters CP, TP, Casein, WP, NCN and NPN highlighted different values for the milk from each analyzed species.

For example, the highest values were obtained for sheep's milk where the average value for TP was 4.98 ± 0.04% followed by buffalo milk with an average of 3.98 ± 0.04%, after that from cow where the mean was at a level of 3.24 ± 0.02% and goat's milk where the mean value for TP was only 3.02 ± 0.04% (Table 2).

Table 1. The chemical composition of different milk species

| Species | pH | W (%) | TS (%) | Fat (%) | SNF (%) | Ash (%) |
|---|---|---|---|---|---|---|
| **Caw** | 6.51±0.04 | 87.55±0.04 | 12.45±0.04 | 3.86±0.02 | 8.59±0.05 | 0.69±0.004 |
| **Buffalo** | 6.62±0.01 | 82.70±0.02 | 17.29±0.02 | 6.97±0.04 | 10.32±0.04 | 0.84±0.02 |
| **Goat** | 6.48±0.01 | 87.62±0.18 | 12.38±0.18 | 4.25±0.02 | 8.13±0.18 | 0.81±0.003 |
| **Sheep** | 6.48±0.01 | 80.90±0.04 | 19.10±0.04 | 7.70±0.06 | 11.40±0.09 | 0.85±0.01 |

W, water content; TS, total solids; SNF, solid non-fat; SD, standard deviation.
All values are ±SD which represent data average of five sample

Table 2. The fractions protein of different milk species

| Species | CP (%) | TP (%) | Casein (%) | WP (%) | NCN (%) | NPN (%) |
|---|---|---|---|---|---|---|
| **Caw** | 3.57±0.02 | 3.24±0.02 | 2.48±0.01 | 0.44±0.02 | 0.76±0.02 | 0.32±0.004 |
| **Buffalo** | 5.20±0.05 | 3.98±0.04 | 3.07±0.04 | 0.51±0.01 | 0.90±0.01 | 0.39±0.01 |
| **Goat** | 3.42±0.04 | 3.02±0.04 | 2.10±0.04 | 0.51±0.01 | 0.92±0.01 | 0.40±0.004 |
| **Sheep** | 5.63±0.03 | 4.98±0.04 | 3.65±0.04 | 0.67±0.004 | 1.32±0.01 | 0.65±0.01 |

CP, crude protein; TP, true protein; WP, whey proteins; NCN, non-casein nitrogen; NPN, non- protein nitrogen; SD, standard deviation. All values are mean±SD, representing data average of five samples.

Another very important parameter regarding the milk processing part, especially when we talk about cheese processing is represented by the casein content of milk, the main protein in it, being also the protein that remains in the cheese. Therefore, the milk that recorded the highest value in terms of casein content was sheep's milk where the average value was 3.65 ± 0.04% followed by buffalo milk where the average was 3.07 ± 0.04% and that of a cow for which the average casein content was 2.48 ± 0.01%.

Analyzes were also performed to determine the content of the main essential and non-essential amino acids. Therefore, for cow's milk, the highest level of essential amino acids was found in the case of leucine, namely 324.02 ± 0.32 mg /100 g followed by lysine where the content was 261.38±0.25 mg/100 g. Among the non-essential amino acid content, the highest level was found in the case of glutamic acid (717.2 ± 0.20 mg/100 g) followed by proline where the average value was 302.23 ± 0.19 mg/100 g with variation limits between 302 mg/100 g and 303 mg/100 g.

In the case of non-essential amino acids determined for cow's milk, the lowest level was found in the case of Arginine (190.92 ± 0.33 mg/100 g) (Table 3).

For milk from buffaloes in terms of content of essential amino acids - leucine recorded a higher content compared to the content from cow's milk, namely 398.00 ± 0.32 mg/100 g.

Differences were also noted in the lysine content, where the mean value for buffalo milk was 310.20 ± 0.73 mg/100 g (Table 4).

Table 3. The main amino acid content of proteins in COW milk

| Specification | n | $\overline{X} \pm s_{\overline{X}}$ | V% | Min. | Max. |
|---|---|---|---|---|---|
| **Essential amino acids (mg/100g milk)** | | | | | |
| Valine | 5 | 190.92±0.33 | 0.38 | 190 | 192 |
| Isoleucine | | 189.60±0.24 | 0.29 | 189 | 190 |
| Leucine | | 324.02±0.32 | 0.22 | 323 | 235 |
| Lysine | | 261.38±0.25 | 0.21 | 260.9 | 262 |
| Threonine | | 153.34±0.19 | 0.28 | 153 | 154 |
| Phenylalanine | | 171.34±0.19 | 0.25 | 171 | 172 |
| **Non-essential amino acids (mg/100g milk)** | | | | | |
| Arginine | 5 | 122.07±0.32 | 0.58 | 121 | 123 |
| Asparagic acid | | 218.23±0.19 | 0.20 | 218 | 219 |
| Glutamic acid | | 717.2±0.20 | 0.06 | 716.9 | 718 |
| Proline | | 302.23±0.19 | 0.14 | 302 | 303 |
| Serine | | 187.01±0.32 | 0.38 | 186 | 188 |
| Threonine | | 185.05±0.32 | 0.38 | 184 | 186 |

Table 4. The main amino acid content of proteins in BUFFALO milk

| Specification | n | $\overline{X} \pm s_{\overline{X}}$ | V% | Min. | Max. |
|---|---|---|---|---|---|
| **Essential amino acids (mg/100g milk)** | | | | | |
| Valine | 5 | 240.20±0.37 | 0.35 | 239 | 241 |
| Isoleucine | | 210.80±0.37 | 0.40 | 210 | 212 |
| Leucine | | 398.00±0.32 | 0.18 | 397 | 399 |
| Lysine | | 310.20±0.73 | 0..53 | 308 | 312 |
| Threonine | | 195.60±0.51 | 0.58 | 194 | 197 |
| Phenylalanine | | 278.60±0.51 | 0.58 | 194 | 197 |
| **Non-essential amino acids (mg/100g milk)** | | | | | |
| Arginine | 5 | 129.40±0.51 | 0.88 | 128 | 131 |
| Asparagic acid | | 362.80±0.58 | 0.36 | 361 | 364 |
| Glutamic acid | | 561.20±0.86 | 0.34 | 559 | 564 |
| Proline | | 370.00±0.71 | 0.43 | 368 | 372 |
| Serine | | 269.80±0.86 | 0.71 | 267 | 272 |
| Threonine | | 199.00±0.71 | 0.79 | 197 | 201 |

Regarding the analysis of the results obtained for non-essential amino acids from buffalo milk, it can be seen that in the case of Glutamic acid the average level obtained by us was 561.20 ± 0.86 mg/100 g and that of proline recorded an average value of 370.00 ± 0.71 mg/100 g. The lowest content was also found in the case of Arginine, where the mean value was 129.40 ± 0.51 mg/100 g slightly higher compared to the level of cow's milk (Table 4).

For goat's milk in the case of essential amino acids we had average values of 308.8 ± 0.37 mg /100 g for Leucine, 234.60 ± 0.51 mg/100 g for Lysine and only 137.80 ± 0.58 mg/100 g for Phenylalanine. In the case of non-essential amino acids, the mean level was 107.00 ± 0.71 mg/100 g for Threonine and 595.60 ± 0.51 mg/100 g for Glutamic acid (Table 5).

Table 5. The main amino acid content of proteins in GOAT milk

| Specification | n | $\overline{X} \pm s_{\overline{X}}$ | V% | Min. | Max. |
|---|---|---|---|---|---|
| **Essential amino acids (mg/100g milk)** | | | | | |
| Valine | 5 | 192.60±0.51 | 0.59 | 191 | 194 |
| Isoleucine | | 173.00±0.71 | 0.91 | 171 | 175 |
| Leucine | | 308.8±0.37 | 0.27 | 308 | 310 |
| Lysine | | 234.60±0.51 | 0.49 | 233 | 236 |
| Threonine | | 144.60±0.51 | 0.79 | 143 | 146 |
| Phenylalanine | | 137.80±0.58 | 0.95 | 136 | 139 |
| **Non-essential amino acids (mg/100g milk)** | | | | | |
| Arginine | 5 | 111.01±0.71 | 1.42 | 109 | 113 |
| Asparagic acid | | 249.8±0.37 | 0.33 | 249 | 251 |
| Glutamic acid | | 595.60±0.51 | 0.19 | 594 | 597 |
| Proline | | 273.00±0.71 | 0.58 | 271 | 275 |
| Serine | | 155.20±0.58 | 0.84 | 154 | 157 |
| Threonine | | 107.00±0.71 | 1.48 | 105 | 109 |

The last type of milk analyzed was the one from sheep, milk for which the protein level was the highest, which is also noticeable in the case of the level of essential and non-essential amino acids. Therefore, the average value obtained for valine was 371.00 ± 0.32 mg/100 g, much higher than the average value of milk from other species. A strong difference is also noted in the case of glutamic acid, for which the mean value was 1166.00 ± 0.71 mg/100 g (Table 6).

Table 6. The main amino acid content of proteins in SHEEP's milk

| Specification | n | $\overline{X} \pm s_{\overline{X}}$ | V% | Minima | Maxima |
|---|---|---|---|---|---|
| **Essential amino acids (mg/100g milk)** | | | | | |
| Valine | 5 | 371.00±0.32 | 0.19 | 370 | 372 |
| Isoleucine | | 278.8±0.37 | 0.30 | 278 | 280 |
| Leucine | | 519.40±0.51 | 0.22 | 518 | 521 |
| Lysine | | 572.20±0.58 | 0.23 | 571 | 574 |
| Threonine | | 233.40±0.51 | 0.49 | 232 | 235 |
| Phenylalanine | | 269.80±0.58 | 0.48 | 268 | 271 |
| **Non-essential amino acids (mg/100g milk)** | | | | | |
| Arginine | 5 | 207.4±0.51 | 0.55 | 206 | 209 |
| Asparagic acid | | 272.6±0.51 | 0.42 | 271 | 274 |
| Glutamic acid | | 1166.00±0.71 | 0.14 | 1164 | 1168 |
| Proline | | 537.00±0.71 | 0.29 | 535 | 539 |
| Serine | | 321.40±0.51 | 0.35 | 320 | 323 |
| Threonine | | 193.00±0.71 | 0.82 | 191 | 195 |

## CONCLUSIONS

Following the analyzes performed, regarding the chemical composition of milk samples from different species, we can conclude that fat was the most inconsistent component while the ash content showed minimal variations between milk samples. Therefore, it can be seen that sheep's milk has the highest levels in terms of fat, SNF, TS and ash content, followed by buffalo milk.

Regarding the protein fractions such as CP, TP, casein, whey proteins, NCN and NPN content, the results obtained showed that there are differences between milk from different species.

As a final conclusion, taking into account that the chemical composition of milk is a very important aspect in terms of its processing, it is appropriate that those working in this field be informed about the raw material used.

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