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Study of the main microbiological traits in Romanian buffalo milk

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Abstract. In this study have been analyzed a number of 42 buffalo milk samples, which have been highlighted following parameters: coliform bacteria, total number of yeasts and moulds, total number of anaerobic mesophyl germs, *Salmonella*, coagulase-positive *Staphylococcus, Bacillus cereus*. Further, samples of buffalo milk were analyzed according to lactation to the Axio Observer microscope. These measurements were the basis for highlighting the quality of buffalo milk. *Salmonella,* coagulase-positive *Staphylococcus* and *Bacillus cereus* were absent.
Key Words: buffalo milk, coliform bacteria.

Introduction. Milk is a complete culture medium and it is a very favourable environment for many micro-organisms. It is also a convenient medium of micro-organisms survival which cannot multiply in the milk, but they can pollute (*Mycobacterium tuberculosis* (MTB), *Mycobacterium bovis*, rickettsia and viruses) (Bârzoi & Apostu 2002). Codex Alimentarius and the International Federation of Milk allowed in recent years a major importance of raw buffalo milk and milk products, including microbiological quality (Ganguli 1997). Ensuring the safety and the nutritional value of a particular standard for buffalo milk is dictated by the changing nature of quality requirements, requires depending by the many issues that it raises the mode of production, procurement, processing and recovery.

Ensuring the microbiological parameters of raw milk and milk products is a key step in quality control. In this way the consumers benefits by alimentary protection. The technological and economic losses in milk processing and longer shelf-life of foods are required.

Because milk has a complex chemical composition, the content-rich water, protein, carbohydrates, lipids, minerals and vitamins are a very favourable environment for growth and development of micro-contamination. Some of the micro-organisms may have deleterious effects by producing depreciation of organoleptic qualities of milk (Korn 1989). *Escherichia coli* grows on a variety of environments containing carbon mixed with other substances (Jensen & Pedersen 1990), but prefers the mediums with glucose. Glucose is than transported along the cytoplasmic membrane (Konings et al 1981). *E. coli* is the representative species of the genus *Enterobacteriaceae*, which is able to grow in both anaerobic and aerobic conditions (Baumberg et al 1981). Thus, *E. coli* can multiply and grow in mediums containing glucose, ammonium and mineral salts (Nagatani et al 1971).

In this paper, the main microbiological traits of Romanian buffalo milk were studied.

Material and Method. 42 Buffalo milk samples were collected in individual tubes. Romanian buffaloes from Mesendorf farm (Brașov County) and individual farms from Sălaj County (Buciumi, Agrij and Treznea villages) were selected function of total milk

production. The buffaloes were in different lactations (from I to X). The analyses were performed from raw buffalo milk, stored for a few hours to 4ºC.

In order to determine the coliform bacteria has been used Brilliant Green Bile Lactose Broth (BGBL) medium simply concentrated which has been inserted into Durham tubes in order to observe the release of gas. The homogenized product and its dilutions were seeded all 1 mL in a test tube with selective medium.

The product has been homogenized and from each dilution there was inoculated 1 mL in test tubes with three different mediums. The quantification has been done using MacCrady tables. Test tubes inoculated with medium were incubated at 37ºC for 24-48 h. To determine the number of yeasts and molds there were run the following steps: 1 mL of milk has been introduced into 10 mL physiological serum to obtain a dilution of 10^{-1}. After completion from each dilution has been taken 1 mL and placed in two Petri dishes each, including the undiluted sample and added melted and cooled medium to 45°C. After this step a sweep of the plates has been made. After solidification the samples were incubated at 20°C in the dark for five days. There has been calculated the arithmetic mean of the number of colonies found in Petri dishes and inoculated in the same dilution. The number found has been multiplied by the dilution factor and the number of colonies/mL product has been calculated. To highlight *Salmonella* sp. 25 mL of buffalo milk were weighed and placed into 200 mL BPW (buffered peptone water) and incubated at 35°C for 16 h.

The next step has been carried out on Malachite-green Broth (MGB) culture medium. Selective isolation phase has been realized on solid medium Kampelmacher. Step confirmation: the boards being presumptive, five characteristic colonies of *Salmonella* were selected and seeded on to a tilted tube Triple Sugar Iron (TSI) Agar and Lysine Iron Agar (LIA). Seeding has been done by pricking and slope corrugation, and after that step an incubation occurred at 37°C for 18 h. Determination of coagulated-positive *staphylococci* has been done by inoculation and homogenizing the product of each dilution (10^{-1}) in three tubes (all of 1 mL) with Chapman liquid enrichment medium. The samples were than incubated at 37°C for 24 hours.

The number of staphylococci has been made using MacCrady tables. From each test-tube where has been observed microbial growth we have made a surface biopsy for finding the staphylococci. Positive samples were inoculated through corrugation in medium and incubated at 37°C. Typical colonies of staphylococci were tested for pathogenicity (haemolysis test).

The evidence of *E. coli* from each sample of buffalo milk has been made from 10 mL milk which was distributed in tubes. These samples were seeded for 1, 2 and 3 mL of pure culture of *E. coli*. For quantitative assessment of *E. coli* Levine sheets of each positive dilution of coliform bacteria have been sowed in Agar Levin (AL) medium and incubated at 35-37ºC, 24 h. It has been determined than the number of dilutions at which positive results were obtained, respectively colonies with metallic green iridescence. The number of coliform bacteria has been performed using the McCrady tables.

Results and Discussion. Coliform bacteria in Romanian buffalo milk have an average value of 4.96±0.47/mL. Total number of yeasts and molds has a value of 633.47±0.01/g and for aerobic mesophyl germs the value was $4.46\pm0.11x10^5$/mL/ms. Regarding *Salmonella*, coagulase-positive *Staphylococcus* and *Bacillus cereus*, these were all absent in buffalo milk (see Table 1).

E. coli has been present in three samples from the 42 analyzed samples of buffalo milk and had the following amounts: $1.7X10^3$/mL *E. coli*, $1.3X10^3$/mL *E. coli*, $2.7X10^3$/mL *E. coli*. No correlations were observed between these samples. They were all from Agrij village, to an individual farm.

Coliform bacteria in the milk of Murrah breed buffaloes were 3.95±0.07, *Escherichia coli* 1.80±0.23, *Streptococcus aureus* 1.80±0.23 and fungi 1.33±0.46. For Nili-Ravi breed of coliform bacteria has an amount of 2.16±0.30, *Escherichia coli* 1.80±0.23, *Streptococcus aureus* 1.95±0.36 and fungi 1.33±0.46 (Han et al 2007). The average levels of coliform bacteria and *E. coli* in cow milk had a value of 2.76 and 1.63

log cfu/mL (to the base –10 logarithm of the number of colony forming units per mL) respectively (Lingathurai et al 2009).

Table 1

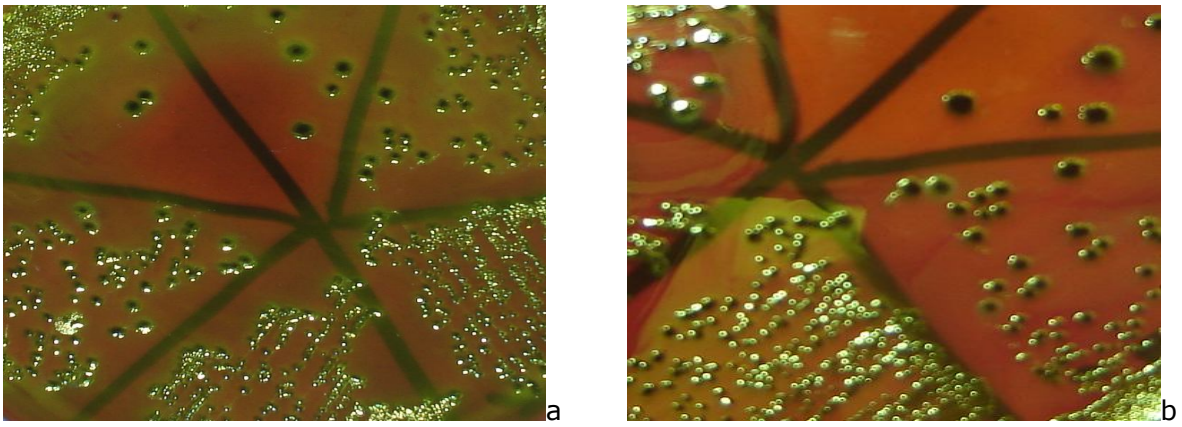
Results obtained on the microbiologic exam for the raw matter buffalo milk

n	*Statistical*	Coliform bacteria*/mL*	Total number of yeasts and moulds*/g*	Total number of aerobic mesophylic germs *x10^5/mL/ms*	*Salmonella*/25 mL	Coagulase-positive *Staphylococcu*	*Bacillus cereus*
42	$s \pm s_x$	4.96±0.47	633.47±20.01	4.46±0.11	Absent	Absent	Absent
	s	2.04	87.22	0.48			
	v	41.17	13.17	10.83			

Chye et al (2004) obtained in cow milk an average of 12x10^{-3} for *S. aureus,* 6.8x10^{-3} for *E. coli*, respectively 17x 10^{-4} for coliform bacteria. Han et al (2005) obtained an average of 8.9–9.5 log cfu/g in cow's milk for *S. aureus*.

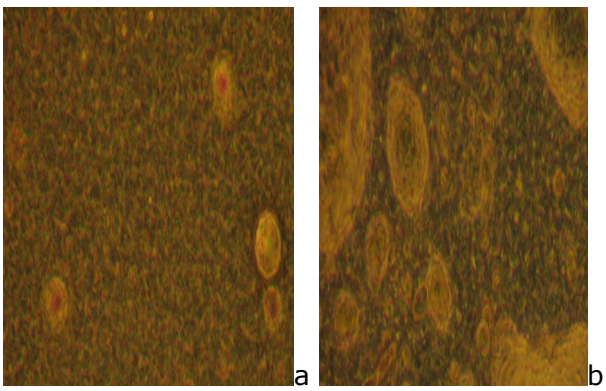
Salmonella is an enterobacter pathogen for humans and animals, there are over 2,000 serotypes. Toxi-infections caused by germs such as *Salmonella* are more common in warm weather because the high temperature is a contributing factor to their development and multiplication (Guş 2005).

Yeasts and moulds are widespread in nature; their presence indicates poor conditions during the technological process of production and during storage. Organoleptic characteristics of the food are degraded because yeasts and moulds action (Guş 2005).



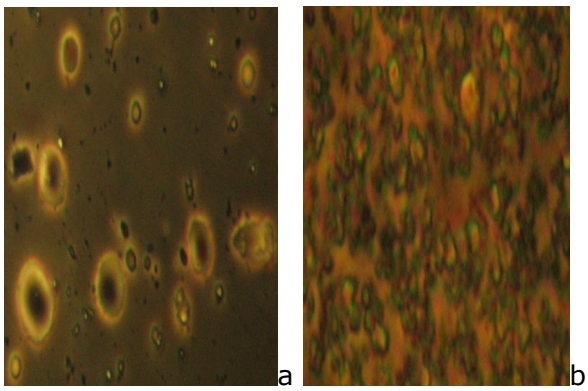
a b

Figure 1 - a, b - Selective isolation of different dilutions of pure culture on Levin agar; highlighting the environment of *Escherichia coli* Levin (Photo camera).



a b

Figure 2 - a, b - Emphasis of the buffalo milk microbiota on lactation I (Ob. 40X)



a b

Figure 3 - a, b - Emphasis of the buffalo milk microbiota on lactation II (Ob. 40X)

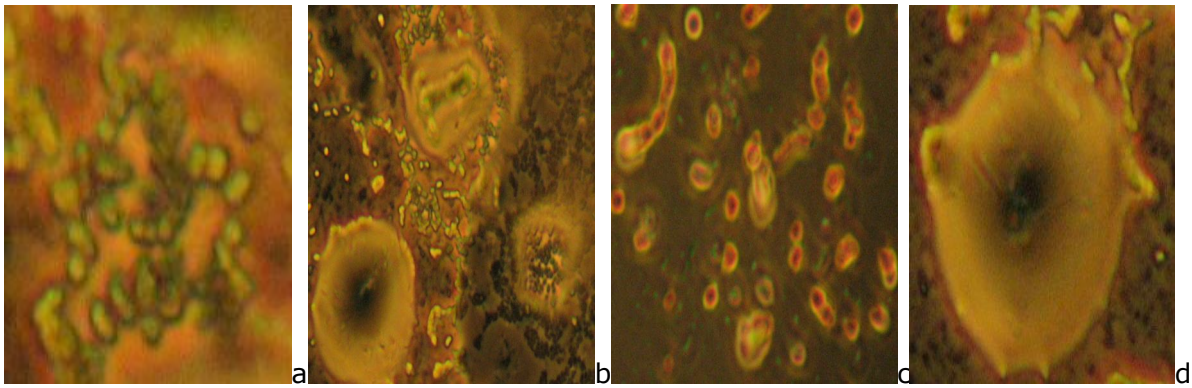


Figure 4 - a, b, c, d - Emphasis of the buffalo milk microbiota on lactation III (Ob. 40X)

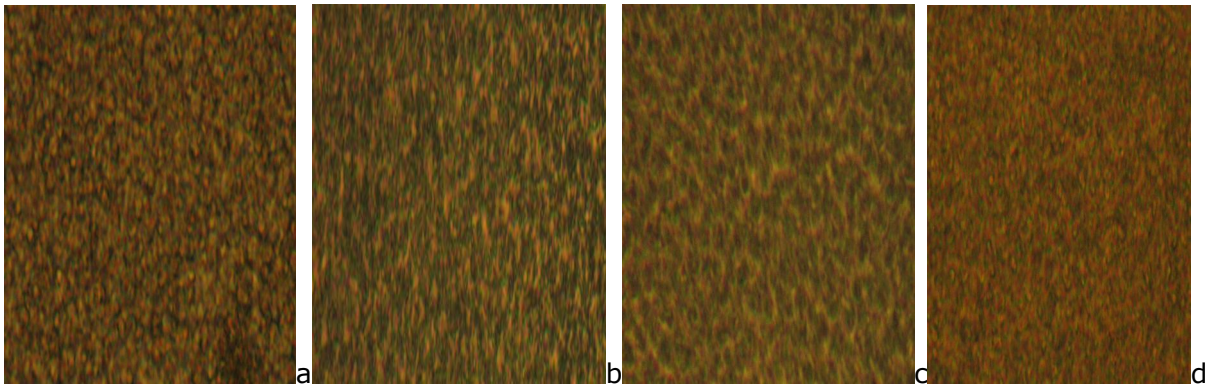


Figure 5 - a, b, c, d - Emphasis of the buffalo milk microbiota on lactation IV (Ob. 40X)

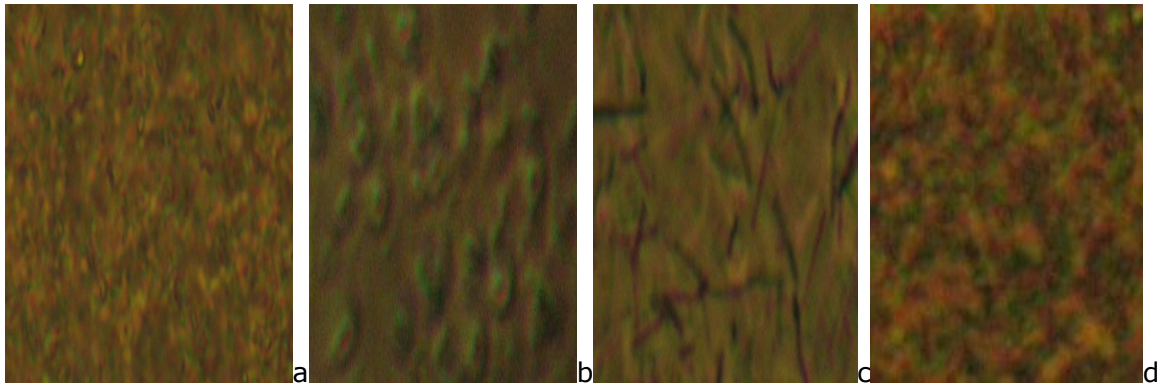


Figure 6 - a, b, c, d - Emphasis of the buffalo milk microbiota on lactation V (Ob. 40X)

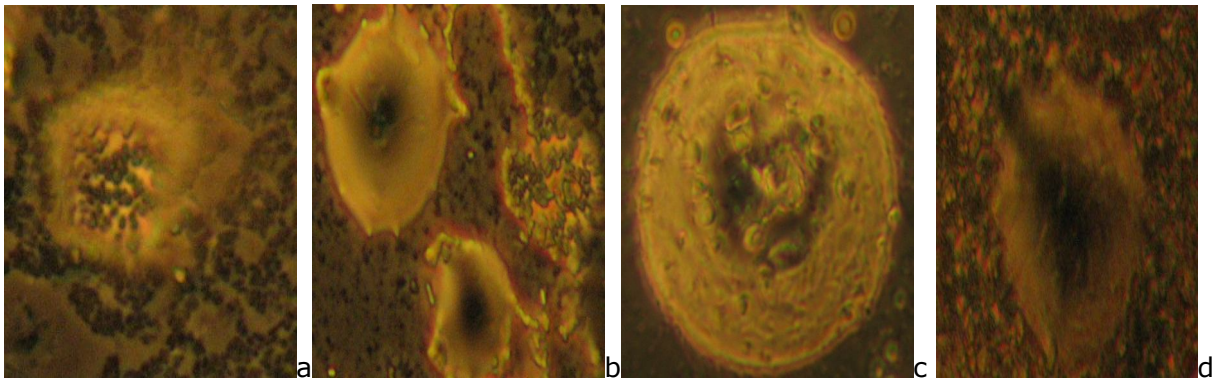


Figure 7 - a, b, c, d - Emphasis of the buffalo milk microbiota on lactation VI (Ob. 40X)

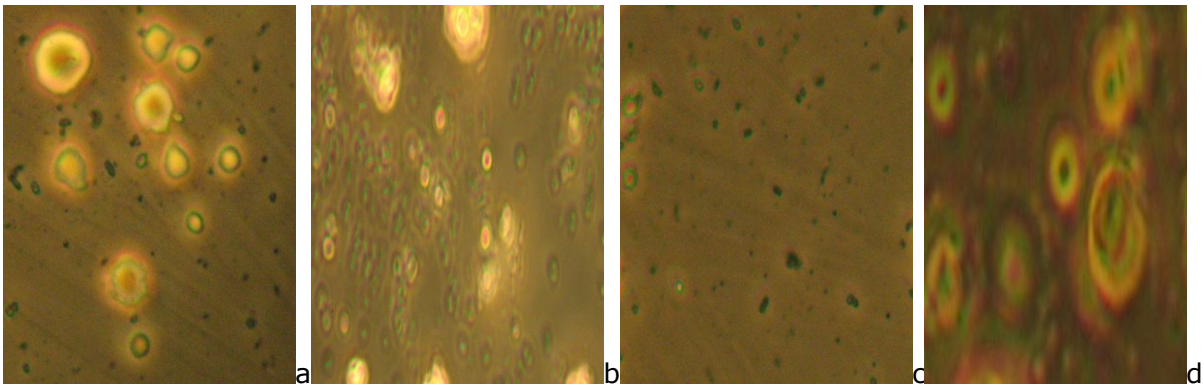


Figure 8 - a, b, c, d - Emphasis of the buffalo milk microbiota on lactation VII (Ob. 40X)

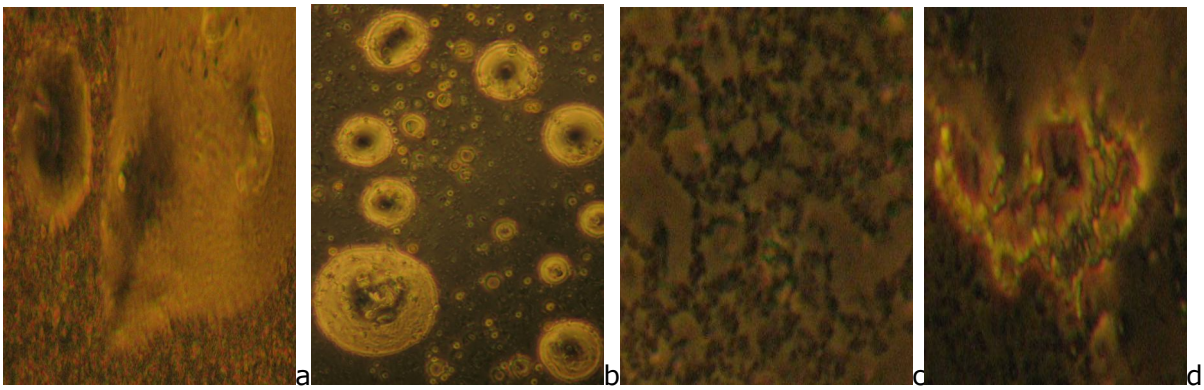


Figure 9 - a, b, c, d - Emphasis of the buffalo milk microbiota on lactation VIII (Ob. 40X)

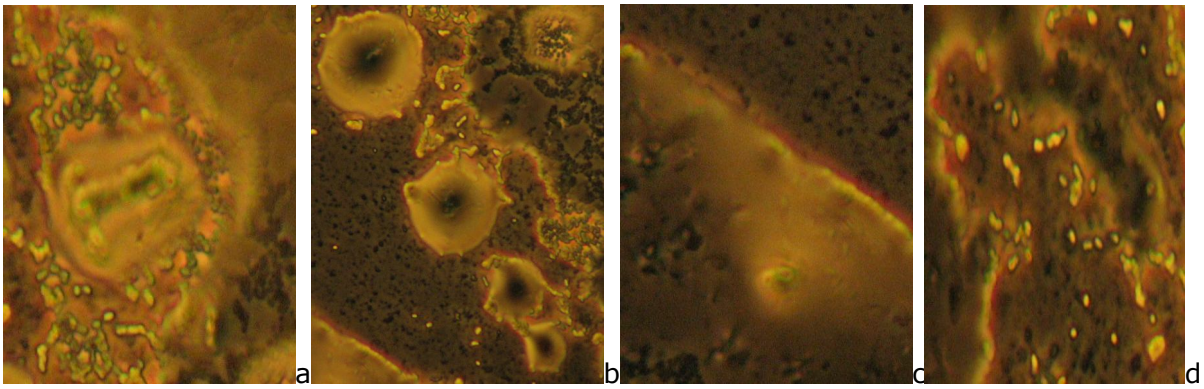


Figure 10 - a, b, c, d - Emphasis of the buffalo milk microbiota on lactation IX (Ob. 40X)

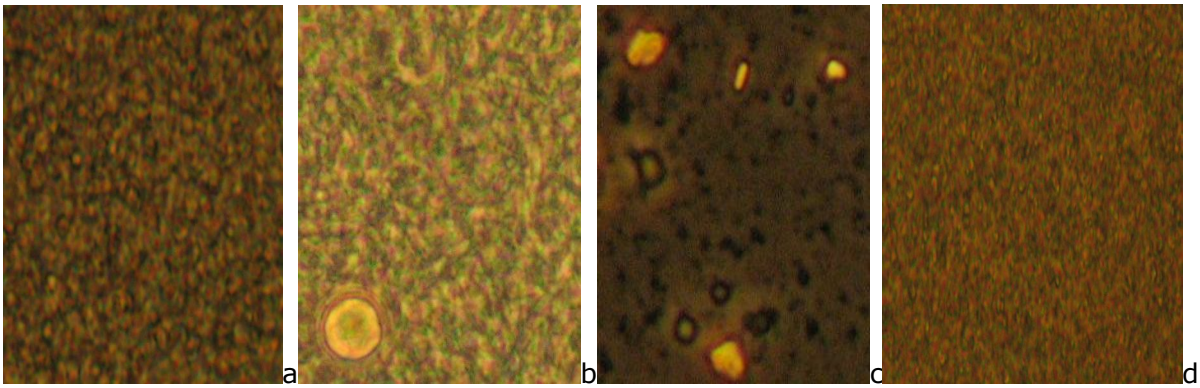


Figure 11 - a, b, c, d - Emphasis of the buffalo milk microbiota on lactation X (Ob. 40X)

Conclusions. The presence of coliform bacteria and *Escherichia coli* genus (considered health indicator) denotes serious hygiene deficiencies. This can come from water or from manipulative staff, indicating recent fecal contamination.

Some technological aspects are important to avoid milk contamination: garbage disposal during milking process, feeding buffaloes before or/and during milking process enhance air load in micro-organisms. Storage in the shelter of green fodder or silage increases the risk of milk contamination with anaerobic bacteria. Because of its composition, milk is a favorable medium for micro-organisms development. Some vegetable species can produce a desired flavor and physical changes of raw buffalo milk. For these reasons they are used in the production of various dairy products. Other plants may be pathogenic or toxigenic to humans in part, and therefore their use should be avoided. Some species can cause defects in color, texture, taste or smell and can lead to difficulties in processing.

Given the quality of components that gives buffalo milk quality, it is mandatory that each of the components mentioned above to be in a standard. A production of buffalo milk at a high quality is not just a constraint enacted - this is an opportunity to sell a product at a higher price. Buffaloes maintenance status influence both quantitative and qualitative milk production. Raw milk used for direct human consumption and raw milk used for manufacturing of which process does not include a heat treatment must correspond to a standard quality.

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