Bulletin of the *Transilvania* University of Braşov
Series II: Forestry • Wood Industry • Agricultural Food Engineering • Vol. 10 (59) No.2 - 2017

EXPERIMENTAL STUDIES REGARDING THE PHYSICOCHEMICAL ASPECTS OF RAW MILK

Ioana A. FOLEA[1]

Abstract: *This paper presents the results of experimental studies conducted on 30 samples of cow, sheep and goat milk coming from 5 mixed farms specialized in animal breeding and exploitation and from 5 centres of milk collecting from smaller individual producers. The studies look upon the physicochemical aspects of milk, such as: antibiotics, aflatoxin, density and acidity. With the help of the diagrams the situations in which the milk is compliant (it meets the European and Romanian norms which are into force), but also some cases in which the analysed milk is not compliant are pointed out. The main purpose of these studies is to help the population realize the possible danger brought about by the acquisition of drinking milk from producers that commercialize milk that does not respect the above-mentioned rules and does not have the NSVFSA approval.*

Key words: *raw milk, experimental studies, physicochemical analyses*

1. Introduction

Milk is of high importance in human alimentation because it has all the necessary nutritional principles for the organism and it is easily digestible. From a physicochemical perspective, milk, is a mixture in which lactose and mineral salts are in soluble form, the fats in suspension and the proteins in colloidal form. In the geographical region in which Romania is situated, the main animal species that produce raw milk are cattle, sheep and goats and on restricted areas female buffaloes can also be found.

Alongside the development of farms specialized in animal breeding and exploitation, breeding in the small households still continues. The products obtained here are used in domestic consumption, but also commercialized with or without the approval of The National Sanitary Veterinary and Food Safety Authority, sometimes in really poor hygienic conditions. As a result, people from Romania are often confronted with issues such as food-borne diseases, caused by consumption of food infected with different pathogens, milk being a product which is easily perishable in these circumstances.

The important milk processors collect the raw material from extended geographical areas, as a result of contracts signed with specialized farms in animal breeding and exploitation and with the

[1] Department for Engineering and Management in Food and Tourism, *Transilvania* University of Braşov, Castelului Street no. 148, Braşov 500014, Romania;
Correspondence: Adelina I. Folea; e-mail: ioana.folea@unibv.ro.

small individual producers who do not process the milk on their own. The individual producers hand over the milk to collecting centres which have special equipment for its refrigeration and conservation in proper conditions until it is retrieved and taken to the processing units. At the time of milk reception in farms or collecting points it is only analysed from the point of view of the quantity and the fat content, other qualitative indicators being determined only at the reception made in processing units where there are accredited laboratories for this kind of analysis.

The physicochemical characteristics which are being researched in the paper (pH, the concentrations of antibiotics, aflatoxin, mass density and acidity) are of high interest especially when the analysed milk is consumed by children or enters in food prescriptions which are easily perishable.

2. Material and Method

The experimental researches regarding some qualitative aspects of raw milk have been done on 30 samples of cow, sheep and goat milk, coming from 5 mixed farms specialized in animal breeding and exploitation and from 5 collecting centres of milk from individual producers, as shown in Table 1.

Sources of the milk samples subject to the qualitative analyses Table 1

Unit type	Milk Type	Number of collecting farms/centres (samples)
Mixed farms specialized in animal growth and exploitation	Cow milk	5
	Sheep milk	5
	Goat milk	5
Regional collecting centres	Cow milk	5
	Sheep milk	5
	Goat milk	5

The research has been done in the specialized laboratory of a big milk processing unit from Braşov (S.C. Braşov Dairy S.A.), and the raw milk sources are located in locations from Braşov and near Braşov. The experimental researches have strictly followed the rules regarding the control of the analysed samples, the way of sampling them, receiving, depositing and handling the samples, the responsibilities of those involved in these researches and the criteria of rejecting them. The following documents were the basis of the rules:

- SR EN ISO/CEI 17025/2005 – General requirements for the competence of testing and calibration laboratories;
- STAS 10000-6 /1983 – The principles and methodology of standardization;
- SR ISO 8402/1995 – Quality-Vocabulary;
- SR EN 30012/1995 – Requirements regarding the assuring the quality of the equipment of testing/analysing;
- ISO 10011/1994 – Quality audit.

3. Results and Discussions

3.1. Determination of Antibiotic Concentration

The equipment consists of: a "HEAT SENSOR" incubator, a "READSENSOR" reader and a "TWISENSOR 40 °C" analysis kit. The equipment was set in a clean and dry place, easily accessible and comfortable for conducting the research.

The studies took place according to the following algorithm (Figures 1 and 2):

1. The device is connected to the electricity grid (the red light-emitting diode should light up) and there is a waiting period until the temperature of 40°C is reached- the red LED turns off.
1. As many recipients as the number of simultaneously analysed samples is prepared.
2. The optimum temperature of the milk sample is in-between 4°C and 20°C.
2. 4.200 µl from the milk sample is transferred in the socket with the help of the micropipette. The milk is mixed with the micropipette until the complete dissolution of the reagent.

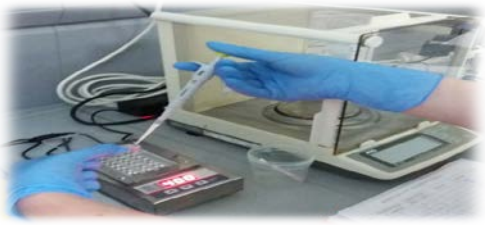

Fig. 1. *Determination of antibiotics*

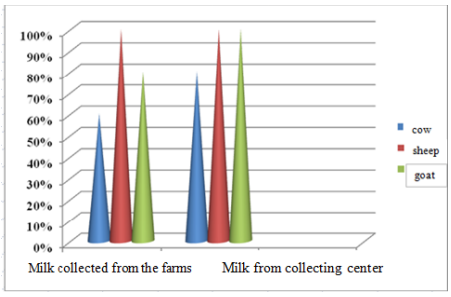

Fig. 2. *Antibiotic concentration results*

3. By pressing the START button the first incubation is started.
4. During the incubation, the flask with the stick tests is opened and as many tests as the number of analysed samples are removed from it. After that, the flask must be closed again.
5. After the first three minutes in each micro-reservoir a stick test is inserted.
6. After the second incubation is ended (by the sound signal) the process is stopped by pressing the START button and the stick tests are taken out from each container.

3.2. Determination of Aflatoxin

The AflaSensor (Fig. 3) is a competitive test in dipstick format and is used for a fast determination of the M1 Aflatoxin in the raw material (Fig. 4). The researches have been conducted according to the following algorithm:

1. The Heatsensor DUO incubator is spun. On the display it shows “AFL; current temperature; NOTOC’’.
2. It is waited until the temperature reaches 40 °C. On the display it will show ‘’AFL; current temperature; OK”.
3. The flask from the AflaSensor package is opened, with the reagent sockets and one of them or both are inserted into the incubator.
4. 4.200 µl from the milk sample is transferred into the socket. With the tip of the micropipette the milk is mixed with the reagent.
5. Immediately after this process the first minute of incubation must be started by pressing the START button.
6. The dipsticks are placed on some parts of the incubator above the sockets.
7. After three minutes, the incubator will automatically release the dipsticks in the sockets; the second 7 minutes incubation starts.
8. After the two incubations are done (the sound signal can be heard) the dipsticks are taken out and the results are read.
9. If no other tests are conducted, the package with the dipsticks and the sockets is sealed and kept a 2-8°C temperature.

Fig. 3. The *AflaSensor*

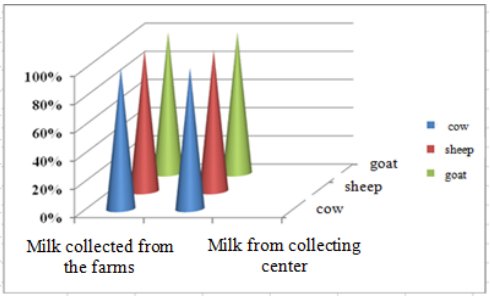

Fig. 4. *Aflatoxine concentration results*

3.3. Determination of the pH

In the factory's lab, the milk's pH is determined with the pH-meter InoLAb which has a high, fast and sure precision (Figures 5 and 6). In order to obtain more accurate results, at regular time intervals a calibration run is done.

The device is kept on a flat surface, protected from bright light and heat.

The electrochemical method is based on the measurement of the electromotive force of an electrochemical cell which consists of a sample, a glass electrode and a reference electrode.

The pH scale is in-between 0 and 14. Therefore:

- pH value < 7= **acidity** (it increases in intensity at a lower pH);
- pH value >7 = **alkalinity** (it increases in intensity at a higher pH);
- pH value =7 = **neutrality** of the measured solution.

In order to determine the pH, a part of the sample is decanted in a glass for a sample of 150- 200 ml. Before every series of measurements, the calibration run is done and only after that the actual measurement of the sample's pH is done, as it is further explained:

- The electrode is wiped with a soft tissue;
- The electrode is introduced in the milk sample, the pH-meter is put into operation, the indication is read, the sample is slowly stirred with the help of the electrode, carefully, in order for it to not touch the walls of the container – for a minute or until the indicated value becomes stable;
- The electrode is taken out of the milk sample, rinsed with distilled water, cleaned with soft tissue and inserted into the electrolyte liquid for conservation.

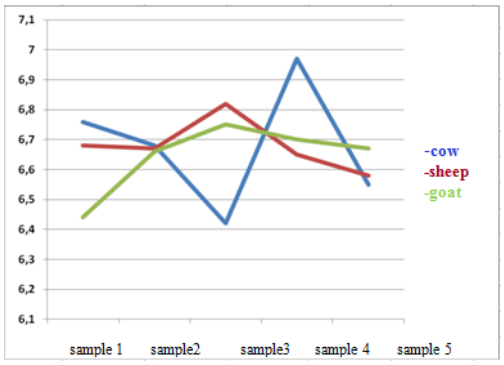

Fig. 5. *The pH of the milk obtained from the farms*

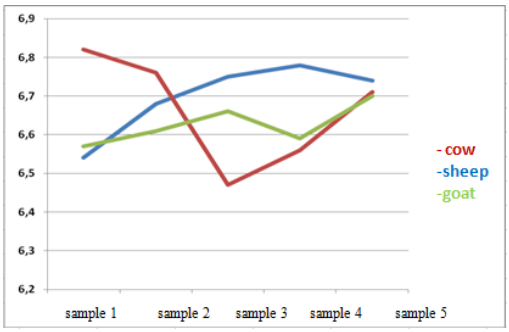

Fig. 6. *The pH of the milk obtained from the collecting centres*

3.4. Determination of Acidity (by Volumetric Titration)

Milk acidity (Figures 7 and 8) is provided by the mixture of free acids and salts with acid reaction and it indicates how fresh the milk is. The milk that is freshly milked has a slightly acid reaction, but its acidity increases as a result of the microbial fermentation of the lactose and of its transformation in lactic acid. The milk's acidity is expressed in Thörner (ºT) degrees which represent the number of 0,1N sodium hydroxide millilitres necessary for the neutralisation of 100 ml of milk with phenolphthalein as an indicator.

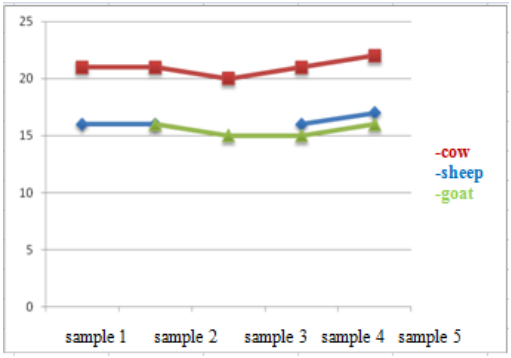

Fig. 7. *The acidity of the milk from the farms*

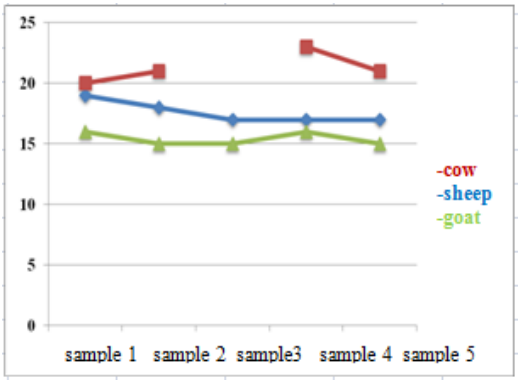

Fig. 8. *The acidity of the milk from the collecting centres*

Working process: 10 ml of milk are poured into an Erlenmeyer glass, 20 ml of distilled water are added with the same pipette with which the milk was measured, then 3-4 drops of phenolphthalein are added. The mixture is titrated with a solution of 0,1N sodium hydroxide 0,1N, stirring continuously until a pale pink colour appears, lasting for a minute. Acidity = 10V ºT, in which V is the NaOH 0,1 N volume used in the volumetric titration.

3.5. Determination of Mass Density with the Thermolactometer

Procedure

The milk is homogenized and brought at a temperature of 15-25ºC. It is carefully poured into the glass cylinder which is slightly tilted in order to avoid the formation of foam or gas bubbles.

The thermolactometer is introduced into the cylinder until the 1,030-gradation value is reached and it is let to float for a minute, after which the density value can be read at the superior side of the meniscus. If the milk temperature is other than 20ºC the value of the read density must be corrected in order to obtain the real density, as follows (Figures 9 and 10):

- When the milk temperature is above 20ºC, 0,0002g/cm3 is added for each degree.
- When the milk temperature is below 20ºC 0,0002 g/cm3 are removed for each degree.
    - Milk density for the cow and goat milk is of 1,029 – 1,033;
    - For the female buffalo milk it is of 1,029;
    - Milk density for the sheep milk is of 1,033.

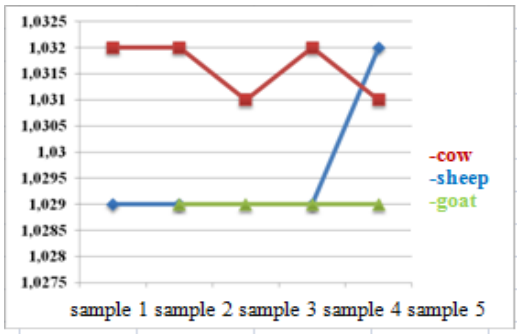

Fig. 9. *Density of the milk from the farms*

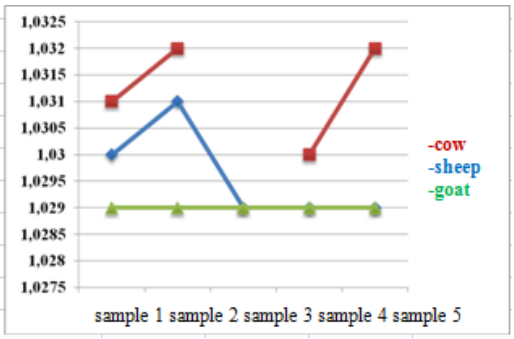

Fig. 10. *Density of milk from the collecting centres*

3.6. Determination of the Fat Content – Gerber Method

The method is based on dissolving the protein substance under the action of sulphuric acid and the separation of fat by centrifugation, in the presence of isoamyl alcohol.

Reagents and the required materials:

- Gerber lactobutyrometer with graduated rod (Fig. 11);
- 1 ml pipette with bulb or with metering unit for the sulphuric acid (H_2SO_4);
- 1 ml pipette with bulb or automatic metering unit for the isoamyl alcohol;
- 11 ml pipette for the milk;
- A rack for the lactobutyrometers;
- Gerber centrifugal pump with 800-1200 rot/per min;
- Sulphuric acid (H_2SO_4) with the mass volume of 1,820 (Fig. 12);
- Isoamyl alcohol with a density of 0,815
- Water bath (Fig. 13) for the temperature of 65-70ºC or centrifugal pump which is able to heat.

Fig. 11. *Gerber lactobutyrometer*

Fig. 12. *Sulphuric acid and isoamyl alcohol*

Fig. 13. *Water bath*

Procedure

The following are added, in this particular order, into the lactobutyrometer which is well-cleaned and dried:

- 1 ml of sulphuric acid (H_2SO_4) which is let to drain on the interior wall of the tilted lactobutyrometer;

- 10 of milk which is let to seep in the lactobutyrometer carefully in such a way in which the tip of the pipette leans on the interior wall of the lactobutyrometer and the milk does not mix with the acid (Fig. 14);
- 1 ml of isoamyl acid which is added without touching the neck of the lactobutyrometer (Fig. 15), so it does not become slippery;
- the lactobutyrometer is secured with a dry rubber stopper(Fig. 16);

Fig. 14. *Adding milk over the sulphuric acid*

Fig. 15. *Adding the isoamyl acid*

- the lactobutyrometer is introduced into the centrifugal with the stopper upside down in a water bath at 65 ºC, for 5 min (Fig. 17);
- the graduating rod is read, if the fat content is at 1 and the up level is at 4,2 it means that it has a fat content of 3,2% (Fig. 18);.
- the lactobutyrometer is wrapped in a cloth (to protect the hands) and it is stirred by overturning it, until the formed coagulum is completely dissolved.

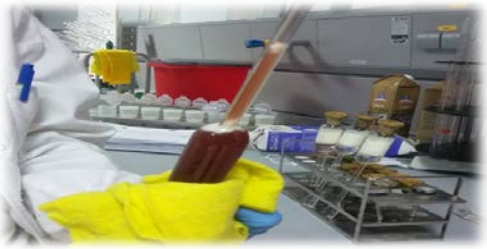

Fig. 16. *Dissolving the coagulum*

Fig. 17. *Introducing the butyrometer in the centrifugal*

Fig. 18. *Results reading*

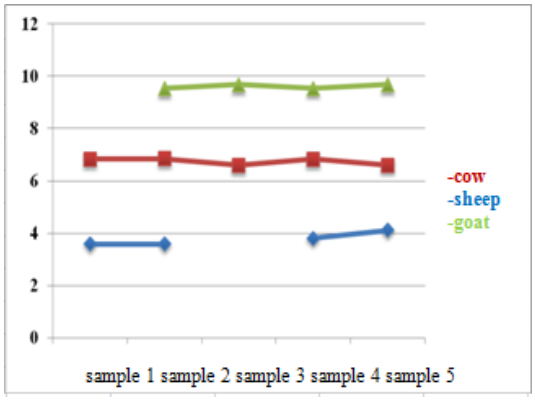

Fig. 19. *Fat content of milk from the farms*

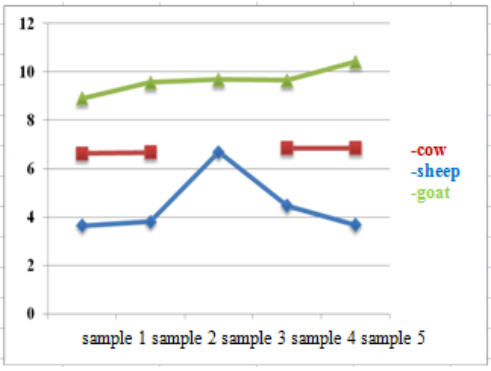

Fig. 20. *Fat content of milk from the collecting centres*

4. Conclusions

1. In order to protect the health of the consumers, the examination is one of the compulsory actions performed on all raw materials and the food products.
2. The analyses have been conducted in accordance with the rules of the Regulation (CE) No. 853/2004 of the European Parliament and the Council of 29 April 2004, regarding the specific hygienic norms applied to products of animal origins.
3. From a physicochemical perspective, the antibiotic can be felt in the cow milk which comes from both supply chains.
4. In what concerns the aflatoxin, the results of all the analyses have been compliant.
5. After determining the pH, at two of the 15 samples from the farms a value lower than 6.5 was obtained. Regarding the samples from the collecting centres a single value was lower than 6.5.

References

1. Albu, M., Argesiu, V., 1956. Milk and Dairy Products Technology (in Romanian). The Technic Publishing House Bucharest, Romania.
2. Banu, C., Vizireanu, C., 1998. The Industrial Processing of Milk (in Romania). The Technic Publishing House, Bucharest, Romania.
3. Jianu, I., Lucaci, L., 1996. Milk and Dairy Products Technology (in Romanian). Lito USAMVB Timişoara, Romania.
4. Necula, V., Babii, M., 2012. Analysis of Food and Food Products (in Romania). Transilvania University Publishing House, Braşov, Romania.
5. Puchianu, Gh., 2012. Microbiological Criteria for Food Safety and Hygienic Processing (in Romanian). Transilvania University Publishing House, Braşov, Romania.
6. Toma, C., Meleghi, E., 1963. Milk and Dairy Products Technology (in Romanian). The Didactic and Pedagogic Publishing House, Bucharest, Romania.