FUNCTIONAL MILK AND DAIRY PRODUCTS PROBIOTICS AND PREBIOTICS

Elena HILMA[1], Georgiana Raluca CRAINIC[2]

[1] University of Oradea, Faculty of Environmental Protection, 26 Gen. Magheru St., 410048 Oradea, Romania,
[2]University of Oradea, Faculty of Environmental Protection, 26 Gen. Magheru St., 410048 Oradea, Romania

Abstract

In this study the cow milk from a particular farm in Bihor County was analyzed. The physical-chemical properties and biological and nutritional value of the milk were studied. The raw milk was heated at room temperature and the nutritional quality of the raw material milk was monitored, as well as the presence of inhibitors. The microbiological quality of the milk, the quality of the lactic bacteria from the spontaneous composition of the raw milk, were also appreciated.

The physio-chemical characteristics, resulting from the analyses of the raw material milk are: total dry matter - 13.7%; nonfat dry matter - 8.6% and fat percentage - 5.1%. Also, acidic dairy products: yogurt, sane and kefir, were manufactured in the laboratory by inoculating boiled milk with specific microbiologic culture for each type of products. The products obtained were sensorially, physio-chemically and microscopically analyzed. The microflora analyzed microscopically was vigorous in raw milk and specific in acidic milk products.

Keywords: cow milk, raw milk composition, acidic dairy products, yogurt, sane, kefir

INTRODUCTION

Raw milk is a complex, nutritious, liquid, yellowish-white food product obtained from mammals during lactation period. It is rich in carbohydrates (lactose), proteins (casein and whey protein), fats, minerals, essential amino-acids and vitamins and due to its high content of water (87,5%) it is beneficial environment for the development of microorganisms, thus having increased perishability. (Lisa Quigley, 2013) (Harjinder Singh, 2002)

In cows' milk, triglycerides represent the major lipidic compound in 98,3% percent. Also, the 2% percent is represented by diglycerides (0,3%), monoglycerides (0,03%), cholesterol (0,32%), phospholipids (0,8%), cerebrosides (0,1%), gangliosides (0,01%) and free fatty acids (0,1%). (Harjinder Singh, 2002)

In the milk's composition are also present complex lipids which may act as emulsion stabilizers and constituents of fat globules. These fatty acids can be: saturated, such as Butyric, Caproic, Caprylic, Capric, Lauric, Myristic, Pentadecanoic, Palmitic, Stearic, Arachidilic; monounsaturated, such as Myristoleic, Palmitoleic, Oleic; and polyunsaturated, suc as Linolenic, Arachidonic and Diene. (Guetouache Mourad, 2014)

Fat level in milk may vary between cows' breeds, as in (Harjinder Singh, 2002) is mention that Jersey breed milk has a 5,2% level of fat, Friesian breed has a 4,2% level of fat and Holstein breed has a 3,5% level of fat. In (Vasile Cighi, 2021) is mentioned that Bălțata Românească breed has a fat level of 3,8%. Also, the feeding of cows may influence the fat level content in milk, as is mentioned in (Harjinder Singh, 2002).

Milk proteins are considered valuable biopolymers with adequate thermal stability, non-toxicity, preventing loss of moisture and flavors, protective against gases. These qualities could allow their use in obtaining packaging based on milk proteins as a promising alternative to petroleum-based polymers. The disadvantages would be sensitivity to moisture, fragility, low elasticity. However, selection of the correct source of milk proteins in addition to suitable fortifying agents and procedures based on the nature of the food could guarantee a well-protected food with an extended shelf life and the least environmental impact (Farhad Garavand et al. 2022).

Milk contains a high level of quality proteins as they contain all the essential amino acids that human body cannot produce. These proteins are, as it is mentioned in (Guetouache Mourad, 2014), crude protein with a level of 100 g/L, protein (94 g/L), insoluble proteins or whole caseins (84 g/L), soluble proteins (18 g/L), α-lactoglobulin (45 g/L), β-lactoalbumin (25 g/L), immune globulins (12 g/L) and protein peptones (13 g/L).

Cow's milk protein is one of the main food allergens. It has been shown that by fermentation with lactic bacteria of the genus Lactobacillus Helveticus and Lactobacillus

Plantarum, a combination of both strains produces a hydrolysis of α-casein and β-lactoglobulin by the proteases of the bacterial strains (Lina Zhao et. al, 2021).

The main carbohydrate from milk is lactose. This represents a union between D-galactose and one molecule of D-glucose. It has a concentration level between 4,5 and 5,2 g/100g milk. Also, even if it is considered sugar, lactose's flavor is not sweet. It has an important role in milk fermentation as it is used as a feeding substrate for lactic bacteria. (Guetouache Mourad, 2014)

The most important minerals present in milk are Calcium, Magnesium, Potassium and Sodium. Their main role in milk composition is to realize structural organisation of casein micells. (Guetouache Mourad, 2014)

The vitamins that are present in milk are those that are fat-soluble. These vitamins are A, D, E and their concentration level can be variable depending on the season. Also, the milk contains vitamin C, which is water soluble and can be easily destroyed by the air after milking or by technological processing of milk. (Guetouache Mourad, 2014)

Also, cow's milk contains microorganisms with an important role both in the functioning of the digestive tract of the human body, but they are also used in obtaining the cultures of selected lactic bacteria used in the manufacture of dairy products.

From research results it is evident that milk is a favorable fermentation medium for the production of menaquinone MK-7 using Bacillus subtilis. Whole milk has proven to be the most effective medium. Thus, it is possible to obtain a new functional dairy product with a high MK-7 content, which can act in the prevention of health problems such as: osteoporosis. and cardiovascular disease (Rachel Southee and others, 2016).

Fungal spoilage limits the shelf life of fermented dairy products. Lactiplantibacillus plantarum strains inhibited the growth of mycelial fungi. The inhibitory effects of lactic acid bacteria against yeasts were also determined in yogurt. Yeasts of the genus Candida, Saccharomyces and Torulla delbrueckii cause fermentations with CO2 release that bubble dairy products and alter taste and aroma. Yeast inhibition by lactic acid bacteria was strain-specific and unrelated to activity against mycelial molds.

Yeasts and mold mycelia grow in refrigerated fermented dairy products at low pH.

Lactic bacteria metabolites with antifungal activity include acetic acid, propionic acid, reuterin, diacetyl, cyclic dipeptides, and fatty hydroxy acids. Several of these compounds, including acetic acid, propionic acid, and diacetyl are also flavor substances that beneficially (Nuanyi Liang and others, 2022).

Lactic acid bacteria (LAB) are a broad group of gram-positive bacteria that have been actively used worldwide in food production due to their nutritional, technological, bioprotective and flavor-related characteristics, which define them as powerful biological tools to be used in fermentations. food applications (Guillermo Eduardo and others, 2022).

Lactic bacteria can inhibit foodborne pathogens and extend the shelf life of foods by replacing chemical preservatives. Exploring the biodiversity of lactic acid bacteria in different food sources, their isolation, identification and characterization is a new approach for the formation of a variety of functional fermented dairy products. Future perspectives on the study of lactic acid bacteria may be related to the expansion of our knowledge in the fields of genetics and genetic engineering. Genetic science can help improve existing strains and develop new strains with specific characteristics (Hafize Fidan et al., 2022).

Different species of lactic acid bacteria are the most important option in the production of fermented foods, because they are considered food grade as their products can be used directly in food, and they are used in a cheap and wide range of nutrient substrates. They can contribute to lowering the glycemic load of nutrition through their production of organic acid, alcohol, polysaccharides and vitamins (Ceren Mutlu et al., 2022).

The intestinal microflora regulates the physiological functions of the organism, such as energy metabolism and immunity. Lactic acid bacteria, including Lactobacillus plantarum, have a specific polyunsaturated fatty acid saturation metabolism that generates several fatty acid species, such as hydroxy fatty acids, fatty oxyacids, conjugated fatty acids, and trans fatty acids. Two bacterial metabolites of γ-linolenic acid (GLA), 13-hydroxy-cis-6,cis-9-octadecadienoic acid (γHYD) and 13-oxo-cis-6,cis-9-octadecadienoic acid (γKetoD).). We demonstrate that both γHYD and γKetoD enhanced fatty acid β-oxidation and reduced intracellular triglyceride accumulation. These findings suggest that γHYD and γKetoD, which could be generated by intestinal lactic acid bacteria can improve lipid metabolism in the human intestine (Makoto Noguchi et al., 2022).

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Lactobacillus are sources of bioactive compounds with diverse functions and activity. Lactobacillus metabolites, including short-chain fatty acids, exopolysaccharides, and bacteriocins, have promising anticancer potential. Research on interactions between bioactive metabolites of lactic acid bacteria and immune mechanisms has demonstrated that these substances could exert a strong immunomodulatory effect, which would explain their vast therapeutic potential. The anticancer activity of lactic acid bacteria has been confirmed both in vitro and in animal models against cancer cells from various malignancies. LAB inhibit tumor growth through various mechanisms, including antiproliferative activity, apoptosis induction, cell cycle arrest, as well as antimutagenic, antiangiogenic and anti-inflammatory effects (Katarzyna Garbacz, 2022)

Some of the best-known functional foods

in Europe are dairy products which contain probiotic cultures of Lactobacillus and Bifidobacterium. (Gillian E. Gardiner, et. al, 2002)

The probiotics and pebiotics functional

foods are developed for their capacity to modify the activity in the gastrointestinal tract as the number of bacteria in human tract can differ. (S. Salminen, et. al. 1998)

(S. Salminen, 1998) confirm that

prebiotics can stimulate the growth of beneficial bacteria in human gut. Also, the pathogen or virulence growth may be repressed with prebiotics. The best-known prebiotics are fructooligosaccharides and galactooligosaccharides. Their daily intake may stimulate the growth of bifidobacteria and reduce the number of other populations, such as bacteroides and clostridia.

Probiotics that are often used are

Lactobacillus and Bifidobacterium. These can be consumed with fermented dairy products or fermented vegetables. It was shown that the consumption of fermented products may improve the gut health, the immune system and also can prevent against cancer. (S. Salminen, et. al. 1998)

MATERIAL AND METHOD

This study was made in order to observe the properties of fresh cow's milk and its behavior when inoculated with acidic dairy products for 24 h.

The raw milk was collected from cows in a particular family farm in Bihor County, Nojorid village. The cows' breed is Bălțata Românească.

For this study were used the next materials:

- 1.5 liters of boiled milk
- 500 ml raw milk
- 25 ml of yogurt, kefir, sane
- NaOH
- Isoamyl alcohol
- H_2SO_4
- Burette
- Microscope
- Methyl blue

The applied methods in this study were:

- Determination of titratable acidity with Torner Method;
- Determination of the fat percentage by the butyrometric method;
- Determination of total dry matter by drying in the oven;
- Determination of unfat matter;
- Inoculation with 25 ml of acidic dairy products (yogurt, kefir, sane) from the market

Microscopic analysis of smears.

RESULTS AND DISCUSSIONS

The raw milk's composition is

represented in the Figure 1.

This shows the high quality of the milk both in terms of dry matter concentration and fat percentage. The fat level is higher in this milk than in usual milk from Bălțata Românească breed, as is said in (Vasile Cighi, 2021) the fat percent in the milk from this breed is 3,8%. This can be justified by animals feeding which was rich in nutritive compounds. This fact made the percentage of fat in the milk analyzed to be closer to that of the Jersey breed which has a fat percent of 5,2 (Harjinder Singh, 2002).

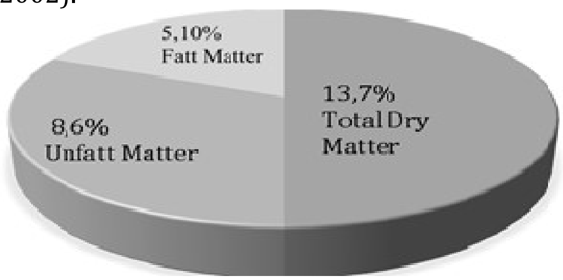

Figure 1 **The Raw Milk's Chemical Composition**

The raw milk's acidity analysis shows that it increased due to lactose fermentation followed by lactic acid formation under the action of lactic bacteria from the spontaneous microflora of milk (figure 2). As a result, the acidity in fermented milk increased about 3 times.

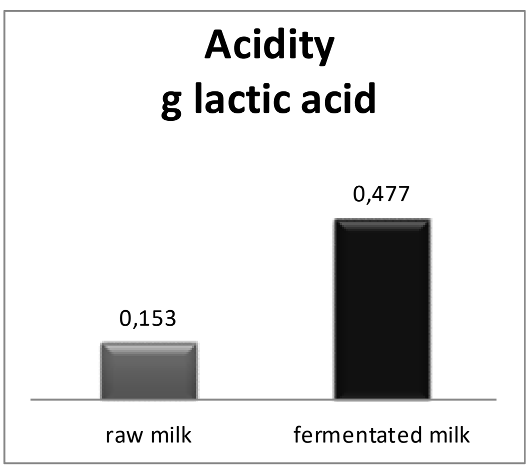

Figure 2 **The raw Milk's acidity Analysis**

The raw milk was pasteurized at high temperature, then cooled at specific temperature for the microorganisms' development in the home-made products. When it reached the specific temperature for the development of each microorganism, it was inoculated.

The evolving of the acidity in raw milk, commercial products and home-made products are presented in table 1.

Table 1

The Evolving of Acidity in the Milk Products Analysed

Product	A	
	°T	g lactic acid
Raw Milk	17	0,153
Fermented Milk	53	0,477
Commercial Yogurt	108	0,972
Home-Made Yogurt	69	0,621
Commercial Sane	107	0,963
Home-Made Sane	99	0,891
Commercial Kefir	99	0,891
Home-Made Kefir	88	0,792

The microscopic analysis of fermented milk shows that in its composition had developed a large number of lactic bacteria (Lactobaccilus lactis and Streptoccocus Lactis) and yeast which results in a rich microflora.

These aspects are shown in the Figure 3.

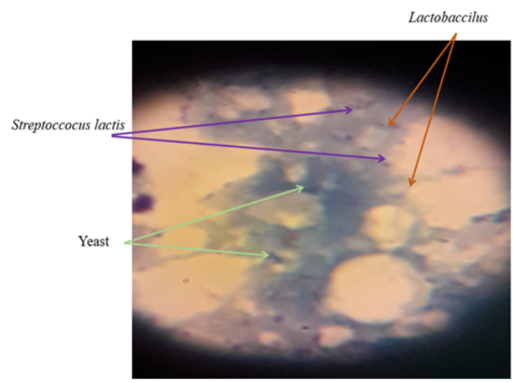

Figure 3 **The Microflora developed in the fermented Milk**

The specific microbiologic culture for yogurt develops at 42-44°C. Because the milk inoculated with culture for yogurt was kept at room temperature, its acidity has a lower level than the one from commerce.

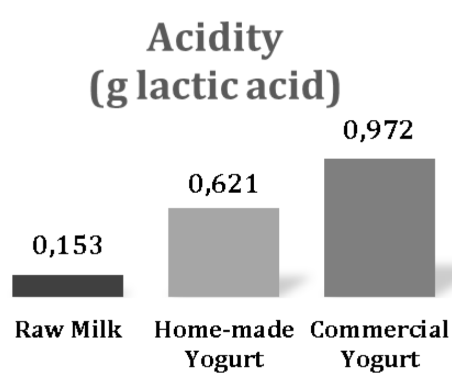

Figure 4 **The Differences between the Acidity of raw Milk, home-made Yogurt and commercial Yogurt**

As a result, the acidity in home-made yogurt increased fourfold compared with the acidity of raw milk. But, the acidity of commercial yogurt has a higher level due to the technological processes applied in obtaining both yogurts.

Also, the home-made yogurt was analyzed microscopically. Under the microscope it was observed that the home-made yogurt contains diversified microflora, rich in Streptoccocus lactis, Diploccocus, Lactobaccilus lactis. This is revealed in the Figure 5.

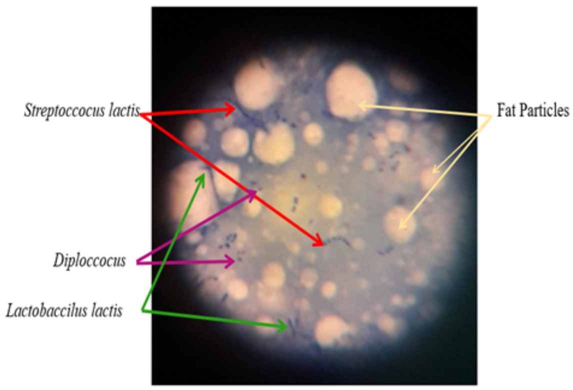

Figure 5 **The Microflora developed in the home-made Yogurt**

Sane was obtained threw reinoculation whith a product from comerce. The microbian culture from sana has a slow action and as a result the inoculated product has a lower acidity than the one from the market.
The differences between raw milk, home-made sane and commercial sane are shown in the figure 6.

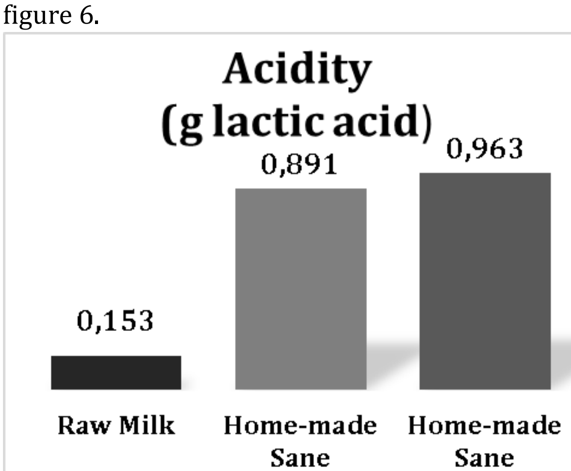

Figure 6 **The Differences between the Acidity of raw Milk, home-made Sane and commercial Sane**

The acidity of home-made sane increased almost six times in comparison with the acidity of raw milk, but is lower than the acidity of commercial sane due to the technological process, as in te case of yogurt.
higher acidity than the home-made one due to the technological processing methods.

Home-made sane was analyzed under the microscope and it revealed the presence of Streptoccocus Lactis in the microflora, which confers a creamy structure and a defined taste in the final product. The microscopic analysis is shown in figure 7.

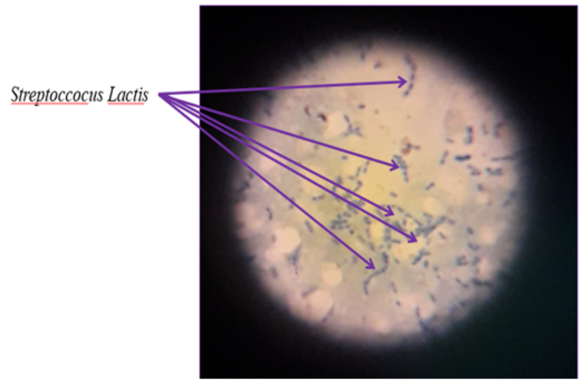

Figure 7 **The Microflora developed in the home-made Sane**

Both, alcoholic and lactic fermentation take place in the process of obtaining kefir. Lactic bacteria develop in symbiosis with yeasts. As a result, the technological process of obtaining kefir can occur at room temperature.

The acidity of home-made kefir is lower than the acidity of commercial kefir, but the clot in the home-made one is creamier and firmer.
The differences between raw milk, home-made kefir and commercial kefir are shown in the figure 8.

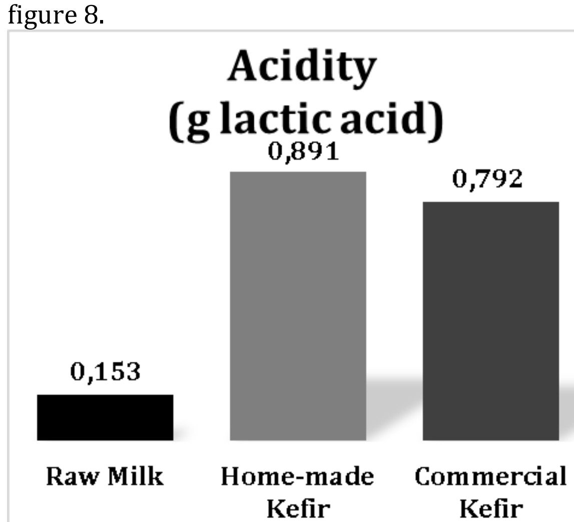

Figure 8 **The Differences between the acidity of raw milk, home-made Kefir and commercial Kefir**

The acidity of home-made kefir is higher than the acidity of raw milk used due to the fermentation which resulted in lactic acid. Also, it was observed that commercial kefir has a

Moreover, the microscopical analysis of home-made kefir was realized. It shown that the product obtained through inoculation has a rich microflora which confirms that the raw milk used has a high level of nutritive substances. The microorganism developed are lactic bacteria (Streptococcus lactis) and yeast (Torula Efiri). As a result, the taste of home-made kefir is effervescent. The home-made kefir's microflora is presented in Figure 9.

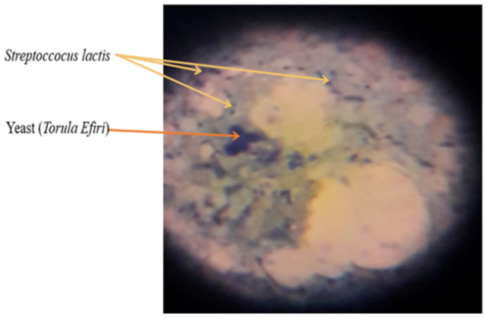

Figure 9 **The Microflora developed in the home-made Kefir**

CONCLUSIONS

The microflora in our products is reach so it demonstrates that the milk used contains a high level of nutritive substances that help the microorganisms developement.

So, our milk used as raw material is reach in prebiotics and probiotics.

It can be concluded that acidified milk products contain a large number of microorganisms which may help our digestive system. We can choose them from many types of products and consume them as we like. So, these acidified products contain essential amino acids which represent food for intestinal microflora.

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