Chapter 11

Milk and Dairy Products: Vectors to Create Probiotic Products

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Additional information is available at the end of the chapter

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1. Introduction

The most important function of alimentation is represented by the assurance of human metabolic needs as well as wellbeing and satisfaction induced by sensorial characteristics of food. In the same time, by modulating some target functions of the body, the food components might have benefic psychological and physiological effects, beside the nutritional ones, already accepted.

In fact, food must contribute to health improving/protection and sustain systems of defence against different aggressions. We are situated at a new frontier of nutrition, in which the foods are evaluated by their biological potential and by their ability to reduce the risk of developing certain diseases. We can talk today about the fact that food for health represent an expanding field: *probiotic functional food*.

In essence, probiotic functional food are products that, by their biological active compounds and consumed in current diets, contribute to optimal human physical and psihycal health.

The appearance and development of functional probiotic food are the response of production field to the results of cellular and molecular biology field research, which demonstrates the implication of food components in proper functioning cellules and subcelular structures. The importance of these studies is essential in contemporaneous context in which the environment assaults by many ways the human body, fully stressing it's protection, adaption and equilibrium maintenance systems. By their specific action, the food components might contribute to the maintain the normal parameters of cellular edificium and of the human body equilibrium.

Nowadays we are assisting to an intensification of research in food – alimentation – health relationship field. The ideea that food might increase/defend health due to active biological components from it's composition conquers more and more acceptability in the scientific community and there are many publication in this field. Unlike the last years, the customers from many countries become more and more interested in health beneficial determined by alimentation, including probiotic functional food. In Romania, even before the adherence to UE, there were registered studies concerning manufacturing of probiotic functional foods, especially in dairy industry and explaining the induced benefits for health.

In this trend of food science are included some of the studies developed over the years by researchers from Galati Food Science and Engineering Faculty.

2. Probiotics: What are they?

2.1. Definitions

The name probiotic comes from the Greek *„pro bios“* which means *„for life“*. The history of probiotics began with the history of man; cheese and fermented milk were well known to the Greeks and Romans, who recommended their consumption, especially for children and convalescents. Probiotics are defined as the living microorganisms administered in a sufficient number to survive in the intestinal ecosystem. They must have a positive effect on the host [1].

The term „probiotic“ was first used by [2] in 1965 to describe the „substances secreted by one microorganism that stimulate the growth of another“. A powerful evolution of this definition was coined by [3] in 1974, who proposed that probiotics are „organisms and substances which contribute to intestinal microbial balance“ [4]. In more modern definitions, the concept of an action on the gut microflora, and even that of live microorganisms disappeared [5] in 1998 defined probiotics as the „food which contains live bacteria beneficial to health“, whereas [6] in 2001 defined them as „microbial cell preparations or components of microbial cells that have a beneficial effect on the health and well-being“.

Some modern definitions include more precisely a preventive or therapeutic action of probiotics. [7] in 1997 for example, defined probiotics as „microorganisms which, when ingested, may have a positive effect in the prevention and treatment of a specific pathologic condition“. Finally, since probiotics have been found to be effective in the treatment of some gastrointestinal diseases [6], they can be considered to be therapeutic agents. It is clear that a number of definitions of the term „probiotic“ have been used over the years but the one derived by the Food and Agriculture Organization of the United Nations/World Health Organization [8] and endorsed by the International Scientific Association for Probiotics and Prebiotics [9] best exemplifies the breadth and scope of probiotics as they are known today:“live microorganisms which, when administered in adequate amounts, confer a health benefit on the host“.

This definition retains historical elements of the use of living organisms for health purposes but does not restrict the application of the term only to oral probiotics with intestinal outcomes [10]. Despite these numerous theoretical definitions, however, the practical question arises whether a given microorganism can be considered to be a probiotic or not.

Some strict criteria have been proposed. [11] in 1992, for example, proposed the following parameters to select a probiotic: total safety for the host, resistance to gastric acidity and pancreatic secretions, adhesion to epithelial cells, antimicrobial activity, inhibition of adhesion of pathogenic bacteria, evaluation of resistance to antibiotics, tolerance to food additives and stability in the food matrix.

The probiotics in use today have not been selected on the basis of all these criteria, but the most commonly used probiotics are the strains of lactic acid bacteria such as *Lactobacillus, Bifidobacterium* and *Streptococcus* (*S. thermophilus*); the first two are known to resist gastric acid, bile salts and pancreatic enzymes, to adhere to colonic mucosa and readily colonize the intestinal tract [4, 12].

2.2. Properties of lactic acid bacteria

The lactic acid bacteria are generally defined as a cluster of lactic acid-producing, low %G+C, non-spore-forming, Gram-positive rods and cocci that share many biochemical, physiological, and genetic properties. They are distinguished from other Gram positive bacteria that also produce lactic acid (e.g., *Bacillus*, *Listeria*, and *Bifidobacterium*) by virtue of numerous phenotypic and genotypic differences. According to current taxonomy, the lactic acid bacteria group consists of twelve genera (table 1). All are in the phylum *Firmicutes*, Order, *Lactobacillales*. Based on 16S rRNA sequencing and other molecular techniques, the lactic acid bacteria can be grouped into a broad phylogenetic cluster, positioned not far from other low G +C Gram positive bacteria.

Five sub-clusters are evident from this tree, including: (1) a *Streptococcus-Lactococcus* branch (Family *Streptococcaceae*), (2) a *Lactobacillus* branch (Family *Lactobacillaceae*), (3) a separate *Lactobacillus-Pediococcus* branch (Family *Lactobacillaceae*); (4) an *Oenococcus-Leuconostoc-Weisella* branch (Family *Leuconostocaceae*), and (5) a *Carnobacterium-Aerococcus-Enterococcus-Tetragenococcus-Vagococcus* branch (Families *Carnobacteriaceae, Aerococcaceae*, and *Enterococcaceae*).

Seven of the twelve genera of lactic acid bacteria, *Lactobacillus*, *Lactococcus*, *Leuconostoc*, *Oenococcus*, *Pediococcus*, *Streptococcus*, and *Tetragenococcus*, are used directly in food fermentations. Although *Enterococcus* sp. Are often found in fermented foods (e.g., cheese, sausage, fermented vegetables), except for a few occasions, they are not added directly. In fact, their presence is often undesirable, in part, because they are sometimes used as indicators of fecal contamination and also because some strains may harbor mobile antibioticresistance genes.

Importantly, some strains of *Enterococcus* are capable of causing infections in humans. Likewise, *Carnobacterium* are also undesirable, mainly because they are considered as spoilage organisms in fermented meat products. Finally, species of *Aerococcus, Vagococcus*, and *Weisella* are not widely found in foods, and their overall significance in food is unclear.

2.3. Probiotics as functional foods

In the last decades consumer demands in the field of food production has changed considerably. Consumers more and more believe that foods contribute directly to their health [13, 14]. Today foods are not intended to only satisfy hunger and to provide necessary nutrients for humans but also to prevent nutrition-related diseases and improve physical and mental well-being of the consumers [15, 16].

Genus	Cell morphology	Fermentation route	Growth at		Growth in NaCl at		Growth at pH		Lactic acid isomer
			10ºC	45ºC	6.5%	18%	4.4	9.6	
Lactobacillus	rods	homo/hetero[4]	±[5]	±	±	-	±	-	D, L, DL[6]
Lactococcus	cocci	homo	+	-	-	-	±	-	L
Leuconostoc	cocci	hetero	+	-	±	-	±	-	D
Oenococcus	cocci	hetero	+	+	±	-	±	-	D
Pediococcus	cocci (tetrads)	homo	±	±	±	-	+	-	D, L, DL
Streptococcus	cocci	homo	-	+	-	-	-	-	L
Tetragenococcus	cocci (tetrads)	homo	+	-	+	+	-	+	L
Aerococcus	cocci (tetrads)	homo	+	-	+	-	-	+	L
Carnobacterium	rods	hetero	+	-	-	-	-	-	L
Enterococcus	cocci	homo	+	+	+	-	+	+	L
Vagococcus	cocci	homo	+	-	-	-	±	-	L
Weisella	coccoid	hetero	+	-	±	-	±	-	D, L, DL

[1]Adapted from [17]
[2]Adapted from [18]
[3]Refers to the general properties of the genus; some exceptions may exist
[4]Species of *Lactobacillus* may be homofermentative, heterofermentative, or both
[5]This phenotype is variable, depending on the species
[6]Some species produce D-, L-, or a mixture of D- and L-lactic acid.

Table 1. Genera of lactic acid bacteria and their properties [1, 2, 3]

In this regard, functional foods play an outstanding role. The increasing demand on such foods can be explained by the increasing cost of healthcare, the steady increase in life expectancy, and the desire of older people for improved quality of their later years [19, 15, 20].

The term ''functional food'' itself was first used in Japan, in the 1980s, for food products fortified with special constituents that possess advantageous physiological effects [21, 22]. Functional foods may improve the general conditions of the body (e.g. pre- and probiotics), decrease the risk of some diseases (e.g. cholesterol-lowering products), and could even be used for curing some illnesses.

The European Commission's Concerted Action on Functional Food Science in Europe (FuFoSE), coordinated by International Life Science Institute (ILSI) Europe defined functional food as follows: ''a food product can only be considered functional if together with the basic nutritional impact it has beneficial effects on one or more functions of the human organism thus either improving the general and physical conditions or/and decreasing the risk of the evolution of diseases. The amount of intake and form of the functional food should be as it is normally expected for dietary purposes. Therefore, it could not be in the form of pill or capsule just as normal food form'' [23].

European legislation however, does not consider functional foods as specific food categories, but rather a concept [22, 24]. Therefore, the rules to be applied are numerous and depend on the nature of the foodstuff. Functional foods have been developed in virtually all food categories. From a product point of view, the functional property can be included in numerous different ways as it can be seen in table 2.

Type of functional food	Definition	Example
Fortified product	A food fortified with additional nutrients	Fruit juices fortified with vitamin C
Enriched products	A food with added new nutrients or components not normally found in a particular food	Margarine with plant sterol ester, probiotics, prebiotics
Altered products	A food from which a deleterious component has been removed, reduced or replaced with another substance with beneficial effects	Fibers as fat releasers in meat or ice cream products
Enhanced commodities	A food in which one of the components has been naturally enhanced through special growing conditions, new feed composition, genetic manipulation, or otherwise	Eggs with increased omega-3 content achieved by altered chicken feed

Table 2. Prominent types of functional food [20, 25, 26]

It should be emphasized however, that this is just one of the possible classifications. According to alternative classification, some functional products are (1) ‘‘add good to your life’’, e.g. improve the regular stomach and colon functions (pre- and probiotics) or ‘‘improve children’s life’’ by supporting their learning capability and behaviour. It is difficult, however to find good biomarkers for cognitive, behavioural and psychological, functions. Other group (2) of functional food is designed for reducing an existing health risk problem such as high cholesterol or high blood pressure. A third group (3) consists of those products, which ‘‘makes your life easier’’ (e.g. lactose-free, gluten-free products) [27].

These products have been mainly launched in the dairy-, confectionery-, soft-drinks-, bakery- and baby-food market [16, 20, 26].

3. Health benefits of probiotics

Since Metchnikoff’s era, a number of health benefits have been contributed to products containing probiotic organisms. While some of these benefits have been well documented and established, others have shown a promising potential in animal models, with human studies required to substantiate these claims. More importantly, health benefits imparted by probiotic bacteria are very strain specific; therefore, there is no universal strain that would provide all proposed benefits, not even strains of the same species. Moreover, not all the strains of the same species are effective against defined health conditions. Some of these strain specific health effects are presented in figure 1.

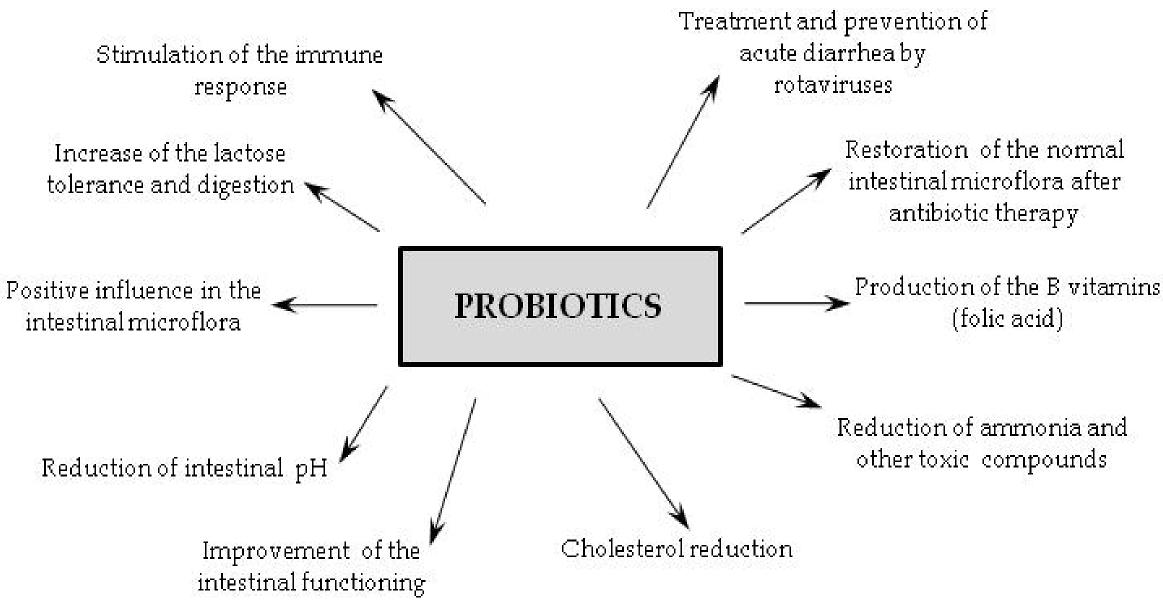

Figure 1. Probiotic beneficial effects on human health [28, 29]

4. Probiotic dairy products

Foods that affect specific functions or systems in the human body, providing health benefits beyond energy and nutrients—functional foods—have experienced rapid market growth in recent years. This growth is fueled by technological innovations, development of new products, and the increasing number of health-conscious consumers interested in products that improve life quality. Since the global market of functional foods is increasing annually, food product development is a key research priority and a challenge for both the industry and science sectors. Probiotics show considerable promise for the expansion of the dairy industry, especially in such specific sectors as yogurts, cheeses, beverages, ice creams, and other desserts. This book chapter presents an overview of functional foods and strategies for their development, with particular attention to probiotic dairy products.

4.1. Types of probiotic dairy product

The most common probiotic dairy products worldwide are various types of yogurt, other fermented dairy product, various lactic acid bacteria drinks and mixture of probiotic (fermented) milks and fruit juice. Probiotic cheese, both fresh and ripened, have also been launched recently. In table 3 are listed some dairy functional food products that have been developed recently in Faculty of Food Science and Engineering.

4.1.1. Fermented milks and beverages

Fermented milks and beverages make up an important contribution to the human diet in many countries because fermentation is an inexpensive technology, which preserves the food, improves its nutritional value and enhances its sensory properties.

Type of dairy functional food products	Description/Name
Fermented dairy products	Drink yogurts with La-5 and carrot juice/ BIOCOV
	Yogurt with La-5 and biomass of *Spirulina platensis*/YLaSP
	Yogurt with BB 12 and biomass of *Spirulina platensis*/YBbSP
	Yogurt with ABY 3/ABT 5 and medicinal plant extracts/ AFINOLACT
	Yogurt with ABY 3/ABT 5 and medicinal plant extracts/ CATINOLACT
	Yogurt with ABY 3/ABT 5 and medicinal plant extracts/ ROSALACT
Cheeses	Dessert based on fresh cheese and some fruit pulp
	Appetizer – type fresh cheese
	Probiotic Telemea cheese

Table 3. Some examples of dairy probiotic products developed

[30, 31] in 2011, proposed the realization of a probiotic dairy drink with added carrot juice. This probiotic product was obtained using goat milk (fat = 3.63%, proteins = 3.05%, lactose = 4.55%, dry matter = 12.05% and density = 1.030 $g \cdot mL^{-1}$) which has been pasteurized at a temperature of 72ºC, for 20 minutes, a probiotic culture type Nutrish containing *Lactobacillus acidophilus* La-5 and carrot juice (dry matter = 9.35%, pH = 6.23, titratable acidity = 0.14 malic acid/100g, ash = 0.7%). After pasteurization, milk was quickly cooled to inoculation temperature at 37°C. The incubation of obtained fermented dairy drink was made at 37°C for 5 hours.

The addition of carrot juice (at a percentage of 10%) had a positive effect on physical – chemical and microbiological parameters of fermented dairy drink. Combining goat milk with carrot juice can get some food with potential therapeutic role.

As a result of the lactose fermentation, the titratable acidity increased fast during the incubation period. At the end of the storage period (after 5 days), the highest value of titratable acidity was 61 ºT. The pH of the obtained new product decreased during incubation period, and will stabilize during storage period, pH = 5.1 after 5 days of storage. The evolution of the number of microorganisms was analyzed for each sample during incubation and storage period. It was observed that the fermented dairy drink with added carrot juice product had been preserving its functional properties during storage (over 10^8 $cfu \cdot mL^{-1}$ probiotic bacteria).

The products were analyzed in terms of fluid flow thus establishing their rheological behavior. The literature shows that the rheological properties of fermented dairy products depend on the development of lactic bacteria as a consequence of metabolic changes leading physicochemical substrate in milk.

In figure 2 is presented the variation of shearing stress (τ, Pa) according to the shearing rate ($\dot{\gamma}$, s^{-1}). There was determined that samples have a rheological behavior similar with the one of the non-Newtonian fluids, time independent, therefore a pseudoplastic behavior. Specific for a fluid with this type of behavior is the flow resistance decrease as a result of the fluid shearing rate increase.

For all samples, it was noted that for low values of shear rate, tangential shear stress variation depending on shear rate was increasing (regression coefficient R^2 values varies from 0.962 and 0.995).

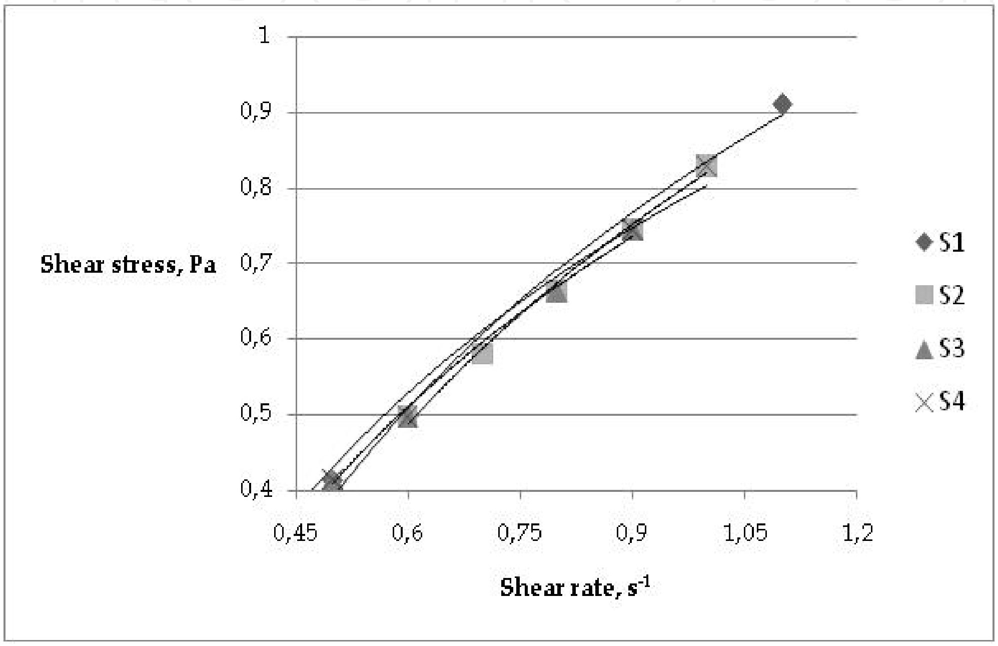

Figure 2. The shearing stress variation according to the shearing rate

To obtain yoghurt with *Spirulina platensis* biomass was used pasteurized cow milk (non fat dry matter = 9.08%, fat = 1.5%, proteins = 3.52%, lactose = 4.32%, mineral salts = 0.72%). Pasteurization of milk is achieved by maintaining standardized milk at 95 °C for 5 minutes. After pasteurization, milk was cooled to inoculation temperature at 42°C.

The inoculation of milk for obtaining these fermented dairy products is with a probiotic culture containing *Lactobacillus acidophilus* La-5 respectively *Bifidobacterium lactis* BB 12, at this time was added and biomass of *Spirulina platensis* (0.5 – 1% according to [32]).

After inoculation follows the distribution and packaging and incubation was made at 42°C for 6 hours in the thermostats set at the optimal temperature for the development of these bacteria. Meanwhile yoghurt gel gets a specific consistency. Cooling and storage of obtained yoghurts is performed at 6 °C for 15 days. In this storage period, coagulum is more compact, the flavor and taste become more pleasant. As a result of the lactose fermentation, the titratable acidity increased. This is slightly higher for the samples with La 5 from those with BB12.

All products with Spirulina platensis biomass have titratable acidity higher than control sample (1.1 times higher for samples with BB 12 and 1.2 times higher for samples with La 5).

The evolution of pH is correlated with lactose fermentation intensity and increased with titratable acidity, but in the same time it is influenced by the buffer substances that are found in *Spirulina platensis* biomass or formed during the manufacture of yoghurt. The pH of fermented dairy products fall between the values 4.11 and 4.53, values considered normal for such products.

The addition of *Spirulina platensis* biomass (figure 3) has positively influenced the number of viable probiotic microorganisms.

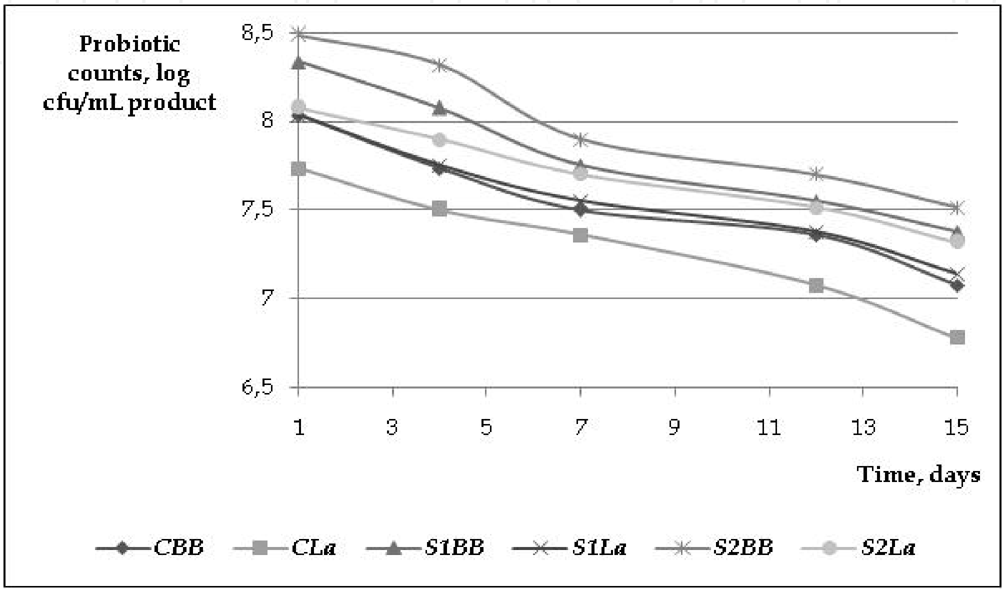

Figure 3. Viable counts variation during storage period

At the end of the storage period (after 15th days) the number of probiotic lactic bacteria for both, control samples and samples with *Spirulina platensis* biomass is still high, which shows that the product with *Spirulina platensis* biomass has been preserving its functional properties during storage period.

4.1.2. Cheeses

Perhaps no other fermented food starts with such a simple raw material and ends up with products having such an incredible diversity of color, flavor, texture, and appearance as does cheese. It is even more remarkable that milk, pale in color and bland in flavor, can be transformed into literally hundreds of different types of flavorful, colorful cheeses by manipulating just a few critical steps.

Just what happened to cause the milk to become transformed into a product with such a decidedly different appearance, texture, and flavor? To answer that question, it is first necessary to compare the composition of the starting material, milk, to that of the product, the finished cheese (figure 4).

In an attempt to diversify the range of probiotic dairy products, there has been made a series of research on the introduction of probiotic bacteria in cheese. According to [33], cheese is an

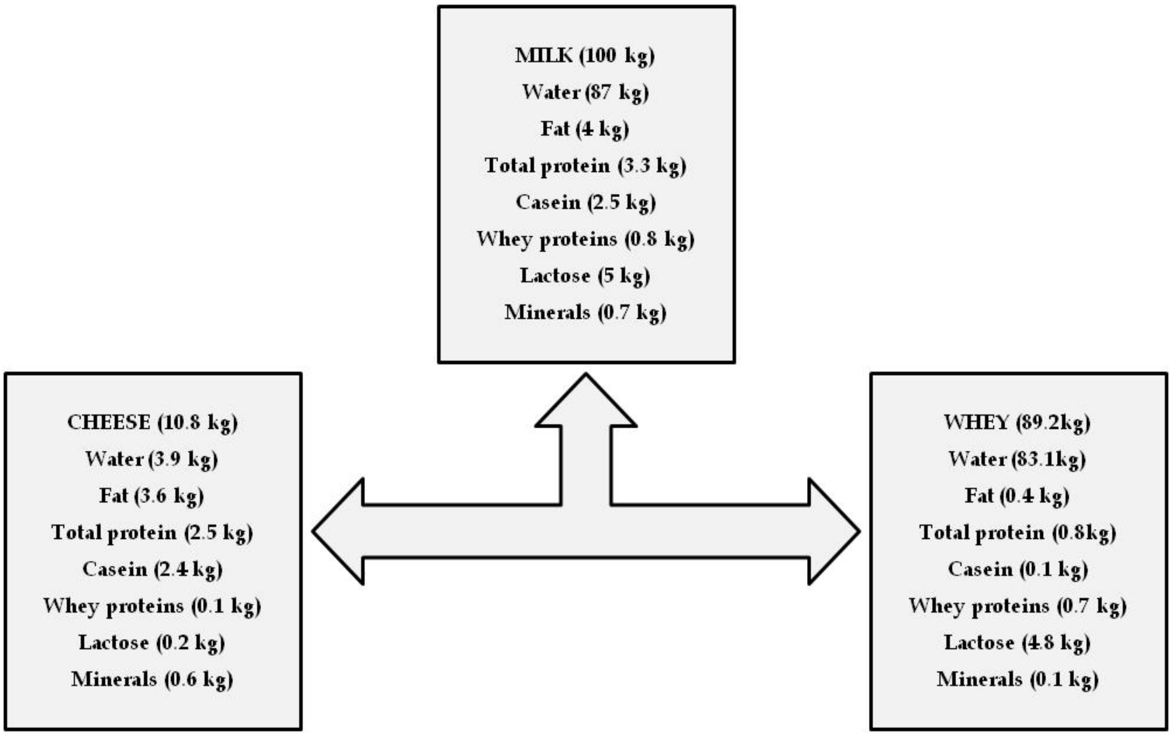

Figure 4. Partition of milk into cheese and whey (adapted from [18]).

interesting way of supplying probiotic bacteria due to the chemical composition of the raw milk that encourages their growth, metabolism and viability and also due to their relatively low acidity compared to other food products. The most of research has been focused on fresh cheese, but there are published some results on probiotic brined or ripened cheese, too.

Fresh cheese, mixt coagulated, is the most suitable cheese to carry probiotic bacteria, due to the high composition of nutrients, low acidity and low salt content. In 2009, [34] used probiotic fresh cheese and peach pulp in order to obtain a dessert, according to figure 5. Probiotic bacteria, *Lactobacillus acidophilus* La 5, was introduced in the fresh cheese as an agent of milk maturation, during coagulation stage. The product was rich in nutritive components (proteins: 10.9...11.3%; fat: 9.1...10.4% and minerals: 2...2.3%) and has a pseudoplastic rheological behaviour. This influenced the sensorial properties of the product, which achieved a creamy texture including in its structure the minced peach pulp and fat globules from the cream.

The research of the above mentioned authors continued, in the attempt to obtain a similar product using goat milk [35]. The amount of nutrients increased, comparing to the previous product (proteins: 12.4...12.5%; fat: 10.1...12.2% and minerals: 2.1...2.4%) but the rheological behaviour was not affected. Although there was expected a reserved attitude of the consumer because of the unpleasant flavour of goat milk, this was not observed.

In 2010 a new probiotic product based on fresh cheese was obtained, by mixing fresh cheese with caraway, cream and salt. The probiotic bacteria (*Bifidobacterium lactis* BB 12) were introduced in cheese at milk maturation stage. In figure 6 it can be observed that the caraway favourised the development of probiotic bacteria.

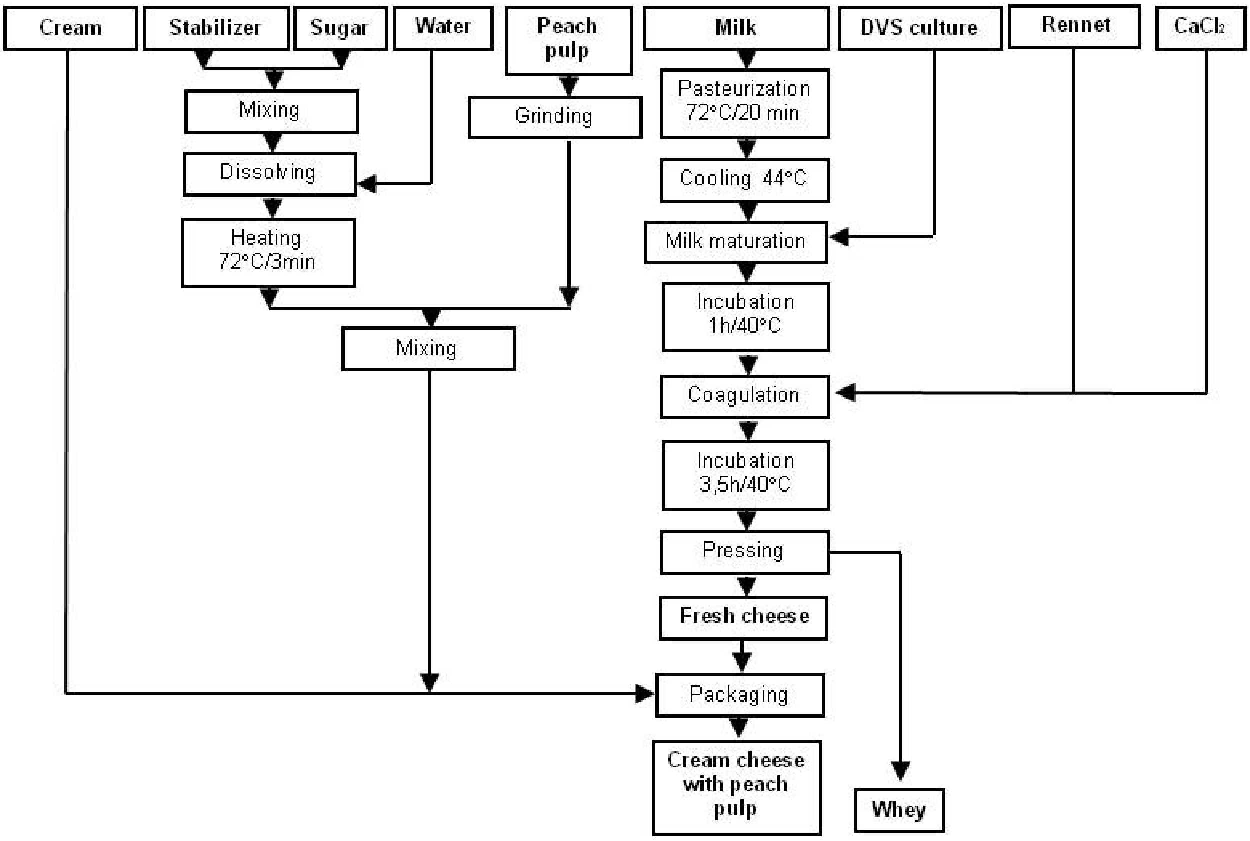

Figure 5. Technological flowchart for manufacturing the new product – Dessert based on fresh cheese and peach pulp

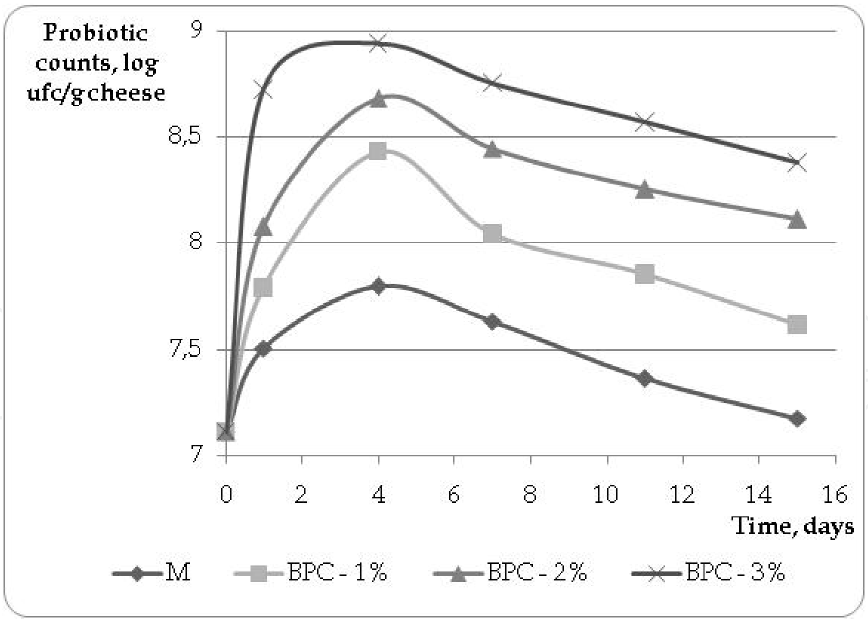

Figure 6. Evolution of bacteria during storage period

[36] and [37] studied the viability of probiotic bacteria *Bifidobacterium lactis, Lactobacillus acidophilus* and *Strepococcus thermophilus* in Telemea cheese during ripening and storage time. Telemea is a cheese variety originated in Romania, from where its manufacture spread

to other Balkan countries and Turkey [38]. The specific of this variety of cheese is ripening in brine. Evolution of probiotic bacteria during different stages of manufacturing process is presented in table 4. Conclusion of the study is that Telemea cheese can be considered a probiotic product, even if the high salt concentration disadvantages probiotic bacteria growth, as long as the number of viable cells remains above 10^7 cfu·g^{-1}.

Stage	*Bifidobacterium lactis*	*Lactobacillus acidophilus*	*Lactobacillus bulgaricus*	*Streptococcus thermophilus*
Inoculated milk	2.71	2.50	2.90	8
Milk, 10 minutes after renneting	2.68	3.21	4	12
Coagulum, before pressing	2.60	7.49	9.40	24.9
Pressed coagulum	2.40	7.29	9.50	24.3
Salted coagulum	2.20	7.20	9.60	22.1
Cheese ripened for 5 days	1.84	6.90	8.90	15
Cheese ripened for 10 days	1.75	6.36	8.40	14.2
Cheese ripened for 15 days	1.65	4.67	7.30	13.4
Cheese ripened for 20 days	1.57	4.21	6.70	12.6
Cheese ripened for 25 days	1.48	3.48	5.30	11.3
Cheese ripened for 30 days	1.41	2.90	4.20	9.1
Cheese ripened for 35 days	0.99	2.31	3.90	7.2
Cheese ripened for 40 days	0.60	2.21	3.50	6

Table 4. Evolution of probiotic bacteria during manufacturing of Telemea cheese (10^7 cfu·g^{-1})

There are registered many other studies about probiotic cheese and methods of manufacturing probiotic cheese. Most of them introduce probiotic bacteria in the milk maturation stage, but there are reports about introducing them after pressing [39] or immobilized on fruit pieces [40].

5. Improvement of benefical effect of probiotic dairy products through the use of bioactive compounds from plants

By sensorial analysis of several combinations milk-medicinal plants, as well as by physical and chemical analysis, there were selected the following medicinal plants: bilberry, sea-buckthorn, rosehip, liquorice, plants rich in active principles considered important for their pharmacological profile.

The research presented in this subchapter was realised on 14 variants of probiotic products (encoded according to table 5), manufactured from cow milk and medicinal plant extracts (bilberry, seabuckthorn, rosehip and liquorice), fermented by two types of probiotic cultures: ABY 3 (*Bifidobacterium lactis*, *Lactobacillus acidophilus*, *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus*) and ABT 5 (*Bifidobacterium lactis*, *Lactobacillus acidophilus* and *Streptococcus thermophilus*).

Crt. No.	Product	Code	Culture	Description
1.	Control	M – 3	ABY 3	Milk + 0.02% DVS culture
2.	Afinolact	A – 3		Milk + 0.02% DVS culture + 6% bilberry extract
3.		LD+A – 3		Milk + 0.02% DVS culture + 6% bilberry extract + 6% liquorice extract
4.	Cătinolact	C – 3		Milk + 0.02% DVS culture + 6% seabuckthorn extract
5.		LD+C – 3		Milk + 0.02% DVS culture + 6% seabuckthorn extract + 6% liquorice extract
6.	Rosalact	Mă– 3		Milk + 0.02% DVS culture + 6% rosehip extract
7.		LD+Mă – 3		Milk + 0.02% DVS culture + 6% rosehip extract + 6% liquorice extract
8.	Control	M – 5	ABT 5	Milk + 0.02% DVS culture
9.	Afinolact	A – 5		Milk + 0.02% DVS culture + 6% bilberry extract
10.		LD+A – 5		Milk + 0.02% DVS culture + 6% bilberry extract + 6% liquorice extract
11.	Cătinolact	C – 5		Milk + 0.02% DVS culture + 6% seabuckthorn extract
12.		LD+C – 5		Milk + 0.02% DVS culture + 6% seabuckthorn extract + 6% liquorice extract
13.	Rosalact	Mă – 5		Milk + 0.02% DVS culture + 6% rosehip extract
14.		LD+Mă – 5		Milk + 0.02% DVS culture + 6% rosehip extract + 6% liquorice extract

Table 5. Experimental variants

The addition of aqueous medicinal plants extract positively influenced the number of viable probiotic microorganisms. At the end of storage period the number of probiotic lactic bacteria is high for both control samples and samples with medicinal plants, meaning that the products maintain their functional character [31, 41-46].

For the obtain products there was demonstrated the cytoprotective character, by studing the total antioxidant capacity, the total content of polyphenols, the superoxiddismutasic (SOD) activity, the minerals content, the ascorbic acid and anthocyaninis.

The results of the study reveal that probiotic dairy products with added medicinal plants contain a high level of total polyphenols and a high total antioxidant capacity. All these products are an excellent source of minerals with high biodisponibility in human diet. The addition of medicinal plants extract improved the SOD activity.

5.1. Identification of bioactive compounds from plants

Medicinal plants are extremely valuable biological currants. The valorification of this potential represents a never-ending source of raw materials for pharmaceutics and food industry. World Health Organisation has recently announced that 75-80% of world's population is treated with natural remedies.

The plants do not cure all the diseases but they might be extremely helpful in rational treating of some diseases and are not to dangerous. The plants have favourable effect to human body and unfavourable effect to some pathogen agents due to certain substances from their composition. In every plant species there must be known that substance or substances which assure them the therapeutic effect (the active principles).

In order to test the chemical composition of studied plants (bilberry, sea-buckthorn, rosehip and liquorice) there were determined by chemical analysis: ascorbic acid (for seabuckthorn and rosehip), glycyrrhizic acid (for liquorice) and anthocyaninis (for bilberry). The concentrations of the active principles in medicinal plants samples are reported in table 6.

Medicinal plants / Active principles	Bilberry (*Vaccinium myrtillus L.*)	Sea-buckthorn (*Hippophaë rhamnoides L.*)	Liquorice (*Glycyrrhiza glabra L.*)	Rosehip (*Rosa canina L.*)
Anthocyanins expressed as cyanidin-3-glucoside chloride	0.38 ± 0.06* (0.32÷0.47)	-	-	-
Ascorbic acid	-	0.8 ± 0.09* (0.66÷0.89)	-	1.24 ± 0.06* (1.18÷1.32)
Glycyrrhizic acid	-	-	5.03 ± 0.32* (4.6÷5.32)	-

The values were expressed in mean ± standard errors of regression and values in parenthesis indicate minimum and maximum level recorded.

Table 6. Active principles in medicinal plants

Regarding the active principles content there was demonstrated that all the analysed medicinal plants respect the values presented in European Pharmacopee, V edition: minimum 0.3% cyanidin–3–glucozide chloride in bilberry, the analysed probes having a maximum content of 0.47%. The ascorbic acid in seabuckthorn must be minimum in 0.5% and in rosehip minimum 1%. The analysed samples registered values of 0.66÷0.98% for seabuckthorn and 1,18% for rosehip. The glycyrrhizic acid, the main active principle in liquorice, must be minimum 4% (according to European Pharmacopee) and the determined values varied between 4.6 and 5.38% [31, 41-46].

5.2. The action of bioactive compounds from plants on probiotic bacteria metabolism

To have a probiotic effect, the strains of probiotic bacteria must be present in the product enough number. It is generally considered that the daily dose of probiotic strains must be between $1 \cdot 10^8$ and $1 \cdot 10^9$ cells. A portion of 100 g, the probiotic product should contain between 10^6 and 10^7 cfu·mL^{-1} product. The addition of aqueous medicinal plant extracts has positively influenced the number of viable probiotic microorganisms due to the presence of fermentable sugars and some growth factors (mineral salts, non-protein nitrogen). At the end of incubation period (after 5 hours), for the samples with medicinal plant extracts the lowest number of microorganisms has established for the samples: Mă–3 ($7.8 \cdot 10^8$ cfu·mL^{-1} probiotic bacteria) or A–5 and C–5 ($1.8 \cdot 10^9$ cfu·mL^{-1} probiotic bacteria) instead the higher number of probiotic bacteria was registered for LD+Mă–3 ($4.5 \cdot 10^9$ cfu·mL^{-1} probiotic bacteria) and LD+Mă–5 ($5.4 \cdot 10^9$ cfu·mL^{-1} probiotic bacteria).

After 8th days of storage period (table 7) the higher number of viable microorganisms was found in the sample with ABT 5 culture (LD+A–5: $2.6 \cdot 10^8$ cfu·mL^{-1} probiotic bacteria), and lowest number of probiotic bacteria cells was recorded for sample with ABY 3 culture (C–3: $0.9 \cdot 10^8$ cfu·mL^{-1} probiotic bacteria). The storage in refrigerated conditions causes a reduction of the number of probiotic bacteria up to 4.37 times in the products obtained with ABY 3 culture and 3.62 times for those with ABT 5.

Microbiological characteristics			
Sample code	cfu·mL^{-1} product	Sample code	cfu·mL^{-1} product
M – 3	$3 \cdot 10^7$	M – 5	$8 \cdot 10^7$
A – 3	$1 \cdot 10^8$	A – 5	$1.4 \cdot 10^8$
LD+A – 3	$1.8 \cdot 10^8$	LD+A – 5	$2.6 \cdot 10^8$
C – 3	$9 \cdot 10^7$	C – 5	$1.1 \cdot 10^8$
LD+C – 3	$1.9 \cdot 10^8$	LD+C – 5	$1.8 \cdot 10^8$
Mă – 3	$1.2 \cdot 10^8$	Mă – 5	$1.3 \cdot 10^8$
LD+Mă – 3	$2.2 \cdot 10^8$	LD+Mă – 5	$2.1 \cdot 10^8$

Table 7. Microbiological characteristics of fermented dairy products wit ABY 3 or ABT 5 after 8th days of storage

At the end of storage period, the number of probiotic lactic acid bacteria for both control samples and for samples with medicinal plant extracts is still high ($1 \cdot 10^7 \div 1 \cdot 10^8$ cfu·mL^{-1} probiotic bacteria), which shows that the products has been preserving its functional properties during storage period. Both cultures can be used in the production of probiotic products [31, 41-46].

Besides the cytoprotective effect conferred by the presence of probiotic bacteria, research has shown that products with added medicinal plants have a increased cytoprotective nature and because the content of biologically active compounds. Experimental results showed that the probiotic fermented dairy product with added medicinal plant extracts have a high content of total polyphenols with beneficial effects on human health, which help to prevent various diseases, such as cardiovascular disease, diabetes [47, 48] and consequently a higher total antioxidant capacity.

The higher amount of total polyphenols (table 8) was determined for samples: LD+Mă–3 (280.78 μg·mL^{-1}) or LD+Mă–5 (285.56 μg·mL^{-1}).

Total polyphenols expressed as catechin, μg·mL^{-1}			
Sample code	ABY 3	Sample code	ABT 5
M – 3	62.086	M – 5	82.086
A – 3	99.91	A – 5	106
LD+A – 3	152.95	LD+A – 5	158.6
C – 3	72.086	C – 5	87.73
LD+C – 3	135.56	LD+C – 5	147.3
Mă – 3	262.95	Mă – 5	239.47
LD+Mă – 3	280.78	LD+Mă – 5	285.56

Table 8. The total polyphenols content for samples with ABY 3 or ABT 5 culture

Compared with control samples (not containing medicinal plant extracts) total antioxidant capacity (Table 9) increased by 3.25-9.94 times in products made with ABY 3 culture and 2.1-8.3 times the ABT 5 products.

TEAC, mM·L^{-1}			
Sample code	ABY 3	Sample code	ABT 5
M – 3	0.16	M – 5	0.2
A – 3	0.57	A – 5	0.43
LD+A – 3	0.70	LD+A – 5	0.73
C – 3	0.52	C – 5	0.48
LD+C – 3	0.81	LD+C – 5	0.62
Mă – 3	1.19	Mă – 5	1.27
LD+Mă – 3	1.59	LD+Mă – 5	1.66

Table 9. Total antioxidant capacity for sample with ABY 3 or ABT 5 culture

For products manufactured with ABY 3 culture and those with ABT 5 was observed that the total antioxidant capacity and total polyphenols content is higher for mixtures with liquorice extract, from the rest of the samples tested, except for samples Mă–3 şi Mă–5.

In addition to being an excellent source of protein, probiotic dairy products based on milk and medicinal plant extracts are a good source of minerals, calcium, potassium, phosphorus, magnesium, zinc. The minerals in these products are from raw milk, and medicinal plant extracts. Because the extracts of bilberry, sea-buckthorn, rosehip and liquorice have different mineral content, the products made have a different content in some microelements.

Distribution of broad in probiotic dairy products based on milk and medicinal plant extracts depends on the content of plant extracts and reactions/associations that occur during the technological process. The results of measurements are presented in Tables 10 and 11.

Crt. No.	Sample name	Microelements content, mg/100g product									
		Ca	Mg	Na	K	Mn	Fe	Zn	Cu	Pb	Cd
1.	M – 3	130	6	36	130	-	0.1	0.4	ND	ND	ND
2.	A – 3	135	7	39	115	0.1	0.15	0.45	ND	ND	ND
3.	C – 3	135	7.5	37.5	125	0.1	0.2	0.5	ND	ND	ND
4.	Mă – 3	137.5	9	41	135	0.1	0.27	0.5	ND	ND	ND
5.	LD+A – 3	140	7.8	47.5	135	0.1	0.21	0.5	ND	ND	ND
6.	LD+C – 3	141	7.8	40	140	0.1	0.25	0.5	ND	ND	ND
7.	LD+Mă - 3	139.5	9.3	46	150	0.1	0.3	0.5	ND	ND	ND

Table 10. Mineral concentration of fermented dairy products with ABY 3 culture

Crt. No.	Sample name	Microelements content, mg/100g product									
		Ca	Mg	Na	K	Mn	Fe	Zn	Cu	Pb	Cd
1.	M – 5	130	6	36	130	-	0.1	0.4	ND	ND	ND
2.	A – 5	135	7	34.5	110	0.1	0.14	0.45	ND	ND	ND
3.	C – 5	140	7.8	32.5	115	0.1	0.21	0.5	ND	ND	ND
4.	Mă – 5	140	8	35	120	0.1	0.25	0.5	ND	ND	ND
5.	LD+A – 5	150	7.5	39.5	140	0.1	0.17	0.5	ND	ND	ND
6.	LD+C – 5	145	8	38	135	0.1	0.23	0.5	ND	ND	ND
7.	LD+Mă–5	147	9	41	140	0.1	0.3	0.5	ND	ND	ND

Table 11. Mineral concentration of fermented dairy products with ABY 5 culture

Minerals in fermented dairy products based on milk and medicinal plant extract fulfill in human body the following functions:

- Are composed of hard tissue: Ca and Mg contribute in a major portion at the formation of the skeleton and teeth. Ca is also one of the most sensitive elements that regulate cellular functions. Is the regulator of enzymes involved in carbohydrate, lipid and protein metabolism, is also involved in important physiological processes such as muscle contraction, blood coagulation, apoptosis and necrosis;

- Are components of soft tissue: Fe and K in the form of organic compounds contribute to muscles, organs and blood. Fe are component of hemoglobin involved in oxygen transport, the of myoglobin, the body's oxygen tank. Fe is considered a major potential prooxidant metals from the human body;
- Are regulators of biological functions: as solubilized salts in body fluids contribute to sensitivity of nervous stimulus, maintain muscle elasticity, adjustment of pH digestive fluids and other secretions, maintaining of osmotic pressure.

Fermented dairy product based on milk and medicinal plant extracts had a higher superoxiddismutase activity. The relationship between the iron and SOD activity is presented in figures 7 and 8.

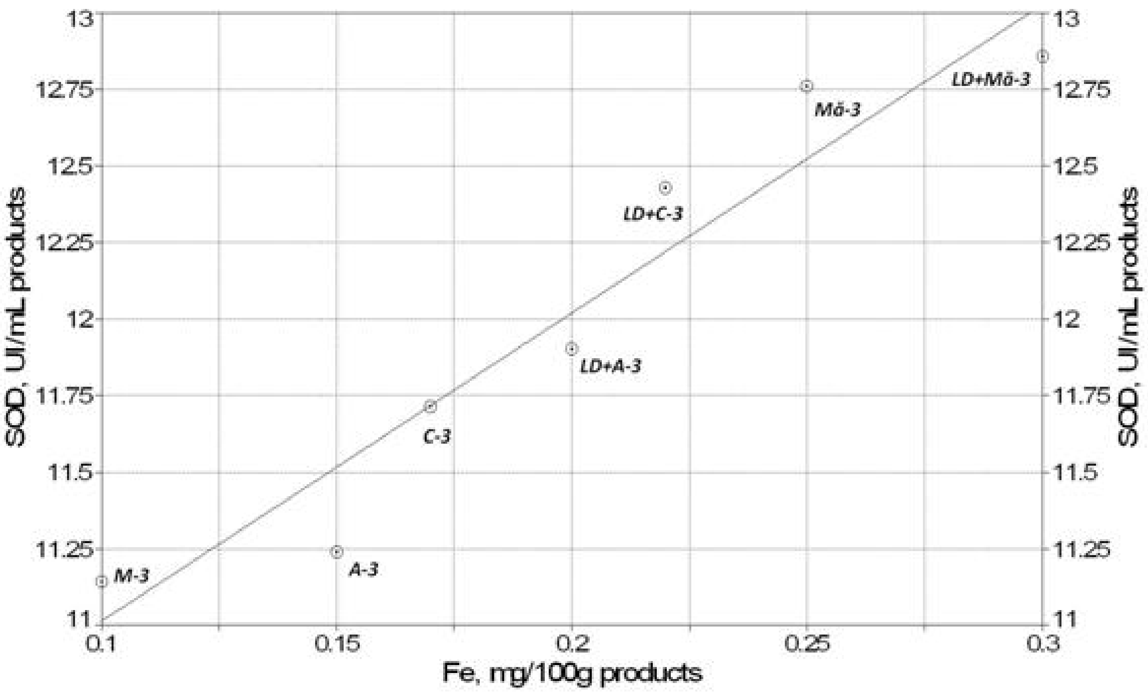

Figure 7. The relationship between SOD activity and iron content of products obtained with ABY 3 culture

For all samples of fermented dairy products with medicinal plant extracts is an increase in SOD activity compared with the control sample. Measured activity is total SOD-like activity (which contributes enzyme as such and superoxiddismutase-like activity of polyphenols and iron or zinc). SOD activity ranged from 11.142 to 12.857 IU·mL^{-1} product; it was maximum for the sample LD+Mă–3. Samples obtained with ABY 3 culture had a higher SOD activity than samples with ABT 5.

5.3. Probiotic dairy products with added plant extracts

To obtain the probiotic dairy products with medicinal plant extracts was used standardized cow milk to 1.5% fat. The technological process for production of fermented dairy products

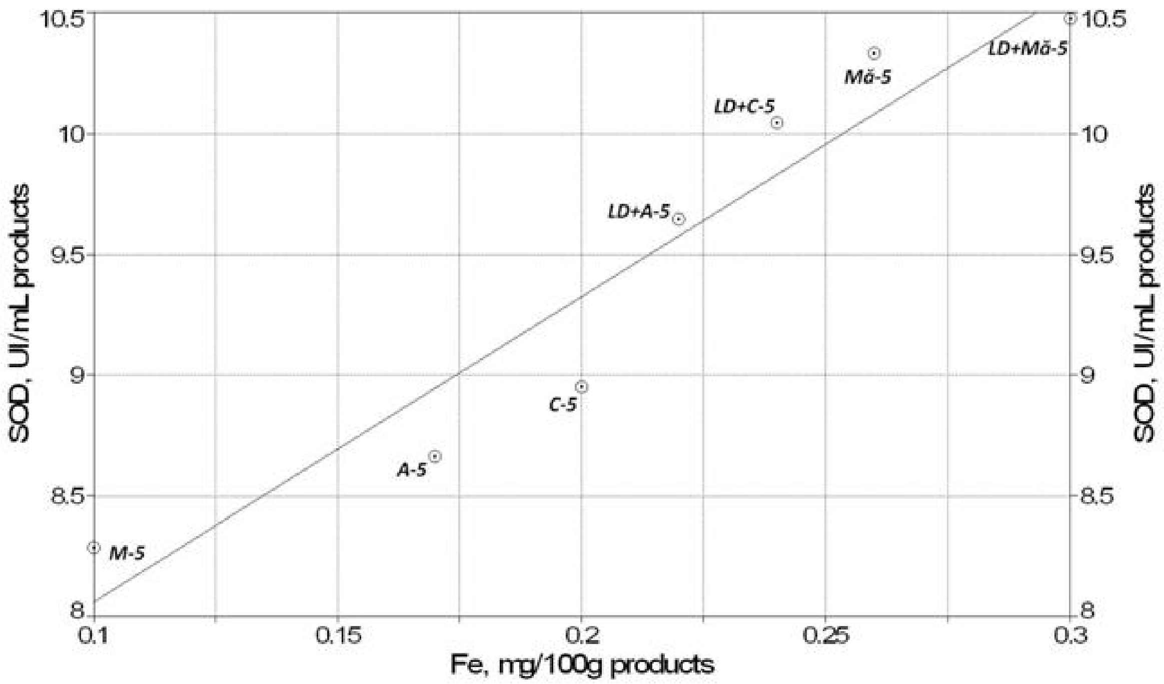

Figure 8. The relationship between SOD activity and iron content of products obtained with ABY 5 culture

Characteristics \ Sample	Bilberries extract	Sea-buckthorn extract	Rosehip extract	Liquorice extract
Dry matter, g/100g	4.84	4.84	5.29	4
Ash insoluble in hydrochloric acid, g/100g	0.74	0.83	0.67	0.81
Total carbohydrate, g/100g	4.69	0.2	7.19	7.29
Total proteins, g/100g	0.21	0.41	0.62	2.15
Calcium, mg/100g product	13.2	4.4	34.5	30
Magnesium, mg/100g product	15.4	4.4	13.8	60
Sodium, mg/100g product	6.6	4.4	8.05	9
Potassium, mg/100g product	132	110	300	230
Manganese, mg/100g product	2.2	2.2	1.38	0.4
Iron, mg/100g product	1.1	2.2	0.46	0.6
Zinc, mg/100g product	0.66	0.44	0.69	0.6
Copper, mg/100g product	0.22	0.22	0.23	0.6
Lead, mg/100g product	ND	ND	ND	ND
Cadmium, mg/100g product	ND	ND	ND	ND
Caffeic Acid, g/100g	1.47	1.69	1.63	0.54
Cyanidin-3-glucoside chloride, g/100g	0.55	-	-	-
Ascorbic acid, g/100g	-	0.26	0.18	-
Glycyrrhizic acid, g/100g	-	-	-	1.96

Table 12. Characteristics of concentrated medicinal plant extracts

with medicinal plant extracts is presented in figure 8. The pasteurization of milk is achieved by maintaining standardized milk at 95 °C for 5 minutes. After pasteurization, milk is cooled to a temperature of 42 °C. Milk inoculation for these probiotic dairy products was made with two Probio-Tec probiotic cultures type: ABY 3 respectively ABT 5, at this time were added and aqueous extracts of medicinal plants (bilberries, sea-buckthorn, rosehip and liquorice) that have a number of characteristics presented in table 12.

After inoculation follows the distribution and packaging and incubation was made at 42°C for 6 hours in the thermostats set at the optimal temperature for the development of these bacteria. Meanwhile yoghurt gel gets a specific consistency. Cooling and storage of obtained yoghurts is performed at 6 °C for 8 days. In this storage period, coagulum is more compact, the flavor and taste become more pleasant.

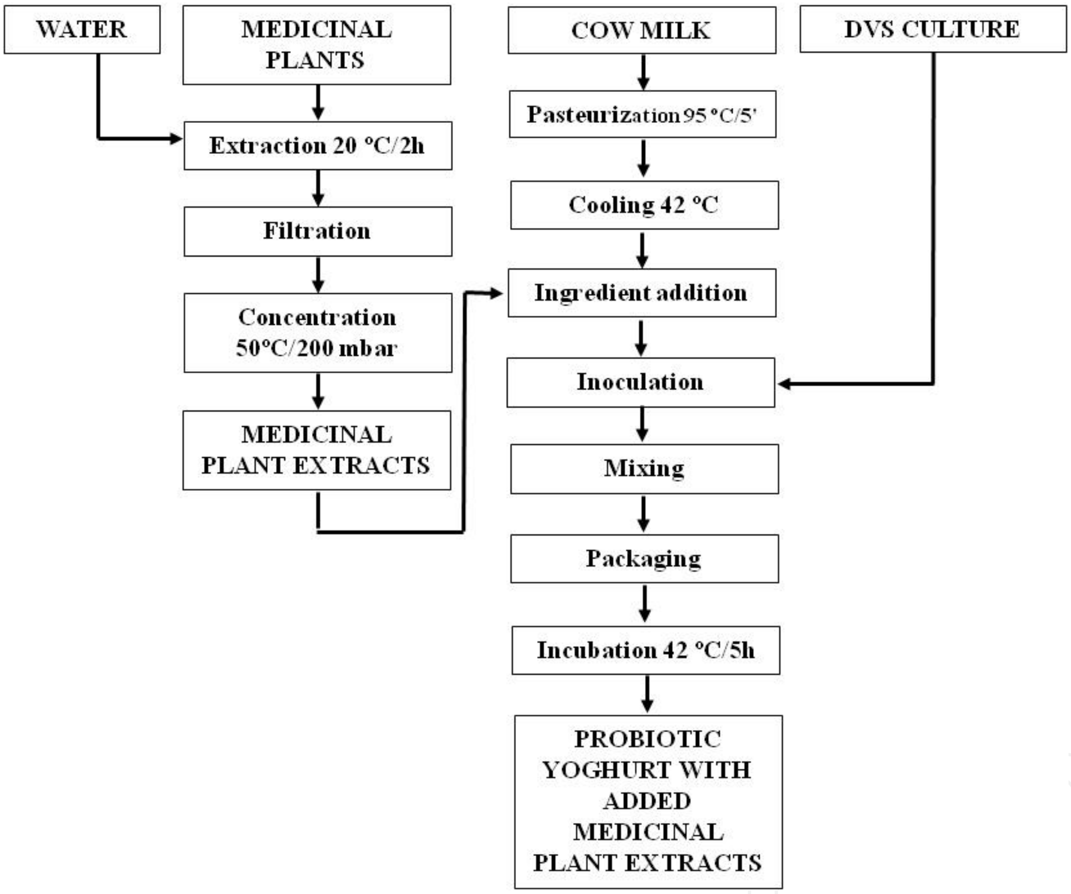

Figure 9. Technological flowchart for manufacturing the new product – Probiotic yoghurt with added medicinal plant extracts

The characteristics of fermented dairy products studied, in terms of chemical properties are:

- Total dry matter have values between 12.05% and 12.5% (lowest for products that contain liquorice extract), exceeding the minimum specified in Romanian standard for fermented dairy products (12%);
- The fat content of the samples vary between 0.6% and 1.3% lowest in products with liquorice compared with other, because smaller proportions of milk of these products;

- Lactic fermentation is faster for the samples with added plant extracts because of monosaccharides content (glucose, fructose, arabinose, xylose) and oligosaccharides (sucrose, raffinose, maltose, xiloglucan) from medicinal plants, which are fermented faster than lactose;
- Titratable acidity at the end of incubation period is between 67ºT and 78ºT, with higher values for products liquorice extracts. After 8th days of storage period, the titratable acidity is between 84ºT and 97ºT;
- The pH of products after incubation period varies between 5.035 and 5.287. After 8th days of storage it reaches values of 4.225-4.553, lowest value was obtained for the products with ABT 5 culture;
- ABT 5 probiotic culture which contains *Bifidobacterium lactis*, *Lactobacillus acidophilus* and *Strepococcus thermophilus* is more active than ABY 3 consists of *Bifidobacterium lactis*, *Lactobacillus acidophilus*, *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Strepococcus thermophilus*;
- The established technological flowchart leads to obtaining some appropriate products in terms of physical-chemical characterization.

6. Conclusions

The researchers team of the Faculty of Food Science and Engineering, with many researchers in the scientific world, were concerned to investigate the possibility of obtaining probiotic products based on milk. Use milk as a vehicle for creating probiotic product was a constant concern of the staff of the Faculty of Food Science and Engineering in recent years. Probiotic character and functional role of probiotic products was obtained by adding fruit and vegetable juices, medicinal plant extracts, *Spirulina platensis* biomass, etc. We plan to continue research in this direction by investigating other products that may stimulate growth of probiotic bacteria.

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