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RESEARCH CONCERNING THE PRODUCTION OF A PROBIOTIC DAIRY PRODUCT WITH ADDED MEDICINAL PLANT EXTRACTS

MOCANU GABRIEL – DĂNUȚ, ROTARU GABRIELA, BOTEZ ELISABETA,
VASILE AIDA, GÎTIN LILIANA, ANDRONOIU DOINA, NISTOR OANA

Bioingineering Department, Food Science and Engineering Faculty, „Dunărea de Jos" University
Danut.Mocanu@ugal.ro

VLĂSCEANU GABRIELA, DUNE ALINA

S.C. Hofigal Export Import S.A., Bucureşti

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The aim of this study was to evaluate the growth of probiotic bacteria in milk supplemented with medicinal plant extracts, in order to fabricate a probiotic dairy product named *CĂTINOLACT*. This dairy product was obtained from cow milk, sea-buckthorn (*Hippophae rhamnoides L.*) and liquorice (*Glycyrrhiza glabra L.*) extracts. The fermentation process was made at 42ºC, for 5 hours, using a lactic probiotic bacteria culture ABY 3 (*Bifidobacterium* species, *Streptococcus thermophilus*, *Lactobacillus acidophilus*, *Lactobacillus delbrueckii* subsp. *Bulgaricus*). During incubation and storage the following parameters were determined: the titratable acidity, the *p*H, the lactose content, the water holding capacity and the number of lactic bacteria. It was observed that the *CĂTINOLACT* product had been preserving its functional properties during storage ($1 \cdot 10^8 - 1 \cdot 10^9$ CFU/mL probiotic bacteria).

Keywords: probiotic product, sea-buckthorn extract, liquorice extract, *Lactobacillus acidophilus*, *Bifidobacterium* ssp.

1. Introduction

The functional foods are products which contribute to the maintenance of the optimal state of physical, psychical and mental health, through their active biological compounds when they are regularly consumed (Liu, 2003; Segal, 2007). As new effects are highlighted, concerning the favorable influences of the functional food over the prevention or even the treatment of some illnesses their production is being developed and diversified.

At the same time, the consumers' attention for the food products or for some of their constituents, which are able to provide a good state of health and to prevent the diseases, has increased.

The European Commission Concerted Action of Functional Food Science in Europe (FUFOSE) considers that a type of food is "functional" if it can be adequately proved that, besides the nutritional effects, it is favorably influencing one or more "target" functions in the organism. Therefore, it improves the general health state and/or decreases the risk of illnesses (Costin et al., 2007).

The use of medicinal plants for healing different human affections, dates back to the ancient times – the prehistoric ones – when man living in the middle of nature, fighting through various ways to ensure his existence, has noticed that some plants are good to eat, or heal diseases and some others are toxic.

The Global Health Organization recently announced that 75 – 80 % of the world's population treats themselves using natural remedies.

The sea-buckthorn fruit (*Hippophae rhamnoides L.*) is a general tonic which has strong antiscorbutic, bactericide, antidiarrheal, galactagogue, depurative, hypoglycemiant effects (Pârvu, 2003). Its active principles act as an adjuvant in the acute inflammatory affections of the intestine, stimulate the women's milk secretion during lactation, activate the physiological processes in the liver, decreasing the glucose concentration in the blood and inhibiting the proliferation of cancer (Pârvu, 2003, Gao et al., 2003; Manea et al., 2009).

The sea-buckthorn fruits have a high content of vitamin C (129.2 – 272.7 mg/100 g), vitamin E (16 mg/100 g), essential fatty oil (isorhamnetin), carotene (10 times more than the carrot), organic acids (1.5 – 4.1 %), tanning substances (1.8%) and mineral substances (calcium – 211.8 mg/100 g, phosphorus – 194.4 mg/100 g, magnesium – 186.1 mg/100 g, potassium – 165.1 mg/100 g, iron – 13.84 mg/100g and sodium – 2.8 mg/100g) (Shah et al., 2007; Zeb & Malook, 2009; Voicu et al., 2009).

The liquorice (*Glycyrrhiza glabra L.*) is a very popular medicinal plant which is rich in flavonoids (liquiritin, glabranine, glyzarin, fluoroglycine) with diuretic and antispasmodic activity (Pârvu, 2004). Furthermore, the triterpenic substances, wherefrom the glycyrrhizine – by itself or as derived compounds (glycyrrhizinic acid) – is the most important, are liquefying the tracheobronchial and pharyngeal secretions.

The content of glyceric and glabric acids in the liquorice influences the ionic equilibrium (Na+, K+) and has anti-inflammatory and antiulcerous activity. The steroid hormone from the liquorice is similar with the estradiol and presents estrogenic activity. According to the dose it can be: articular anti-inflammatory, laxative or purgative and useful in gastric ulcer, renal and bile calculi. It is also rich in amino-acids (aspartic acid, serine, proline, threonine, glycine, valine, alanine, isoleucine), carbohydrates (glucose – 0.6 ÷ 4.1 %, fructose – 0.3 ÷ 1.0 %, saccharose – 7.5 ÷ 20.3 %, sometimes maltose – 0.1 ÷ 0.6 %), vitamins from B group and mineral substances (Ca, Na, P, Fe, Mn, Zn, Cu, Mo) (Pârvu, 2004; Fu et al., 2004; Tenea et al., 2008).

The objective of this study was to evaluate the physico-chemical and microbiological properties of probiotic yoghurt with added medicinal plant extracts during incubation and storage period.

2. Materials and methods

2.1. Materials

The standardized cow milk with a fat content of 1.5 % was acquired from a collecting center in Galati, Romania. The following characteristics were determined with the Milk Lab. device: mineral substances – 0.72 %, nonfat dry matter – 9.08 %, lactose – 4.32 %, proteins – 3.52 % and acidity – 18 OT.

The sea-buckthorn and the liquorice extracts (medicinal plants provided by S.C. Hofigal Export Import S.A., Bucharest) were obtained as follows: the ratio between the vegetal material and the extraction solvent was 1:5, the extraction took place at room temperature for 2 hours. The aqueous extracts obtained were filtered using suitable filter paper for plant extracts provided by Sartorius Company, Romania with 0.065 kg/m2 retention capacity; the filtration time was of 30 s. Afterwards, the extracts were concentrated in a rotary evaporator Rotavapor Buchi at 50ºC, 0.2·105 Pa pressure and stored at 4ºC until utilization (Crăciunescu et al., 2005).

The lyophilized culture of lactic bacteria ABY 3 provided by the Ch.Hansen Company contains the following species: *Lactobacillus acidophilus*, *Lactobacillus delbrueckii ssp. bulgaricus*, *Streptococcus thermophilus* and *Bifidobacterium ssp.*

Three variants of the new probiotic product with medicinal plant extracts, named CĂTINOLACT, were fabricated and coded as follows:

A: milk + 5 % inoculum;

B: milk + 5 % inoculum + 6 % sea-buckthorn extract;

C: milk + 5 % inoculum + 6 % sea-buckthorn extract + 6 % liquorice extract.

Inoculum was obtained by incubation of 250 mL pasteurized milk inoculated with 0.02 % DVS culture, at 42ºC for 12 h.

To obtain the probiotic dairy product with added medicinal plant extracts the technological stages shown in Figure 1 were followed.

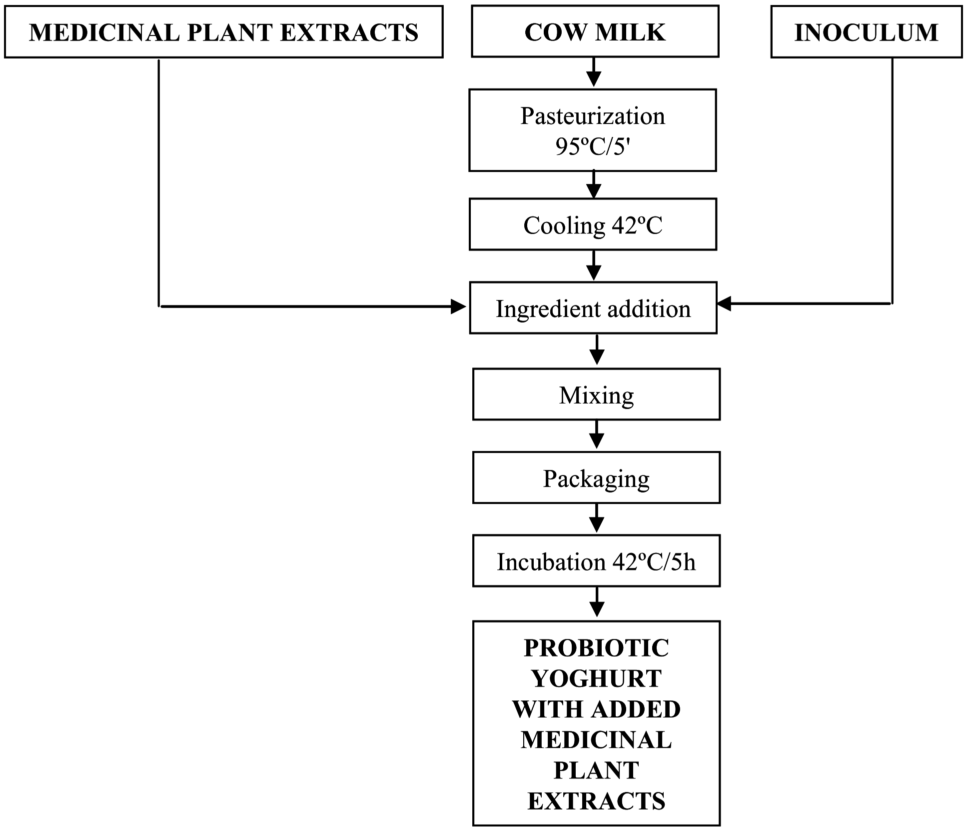


Figure 1. Techological flowchart for manufacturing the new product – Probiotic yoghurt with added medicinal plant extracts

2.2. The compositional analysis

The physico-chemical characteristics of the samples were evaluated as follows:

- ✓ The dry matter (D.M.) content through the oven drying method, at 102 …105°C temperature, according to the AOAC 925.23 standard Cap. 33.2.09;
- ✓ The fat content through the Gerber acid-butirometric test;
- ✓ The acidity was determined by titration with sodium hydroxide 0.1 N, following the correspondence: 1 mL NaOH 0.1N = 0.009008 g lactic acid;
- ✓ The lactose content through the DNS method (3,5-dinitrosalicylic acid) by reading the absorbance at 540 nm with a UV/VIS 6505 JENWAY spectrophotometer. The etalon curve was obtained using pure lactose solutions;
- ✓ The water holding capacity (WHC). A sample of about 20 g of yoghurt (Y) was centrifuged for 10 min at 2500 rpm and 20 ºC in a centrifuge. The whey expelled (WE, g) was removed and weighed. The WHC expressed in percentage was defined as WHC (%) = $\frac{(Y - WE)}{Y} \cdot 100$ (Pyo and Song, 2009);
- ✓ The *p*H with a pH-meter IQ-SCIENTIFIC type after calibration with *p*H = 4.7 and respectively, pH = 9, etalon solutions.

2.3. The evolution of the lactic bacteria number

The lactic bacteria number was established, every two hours during incubation and every two days during storage, through indirect counting using an automatic colony counter ACOLYTE.

2.4. The data analysis

The mono-factorial ANOVA method was used for the statistical data processing. The method was applied in order to observe if there are significant differences between the pH and the titratable acidity values of the three variants of the new probiotic product with medicinal plant extracts - CĂTINOLACT. The significant differences were determined for the $P < 0.05$ level of reliability (Salvador & Fiszman, 2004; Ekinci & Gurel, 2008; Guggisberg et al., 2009).

3. Results and discussion

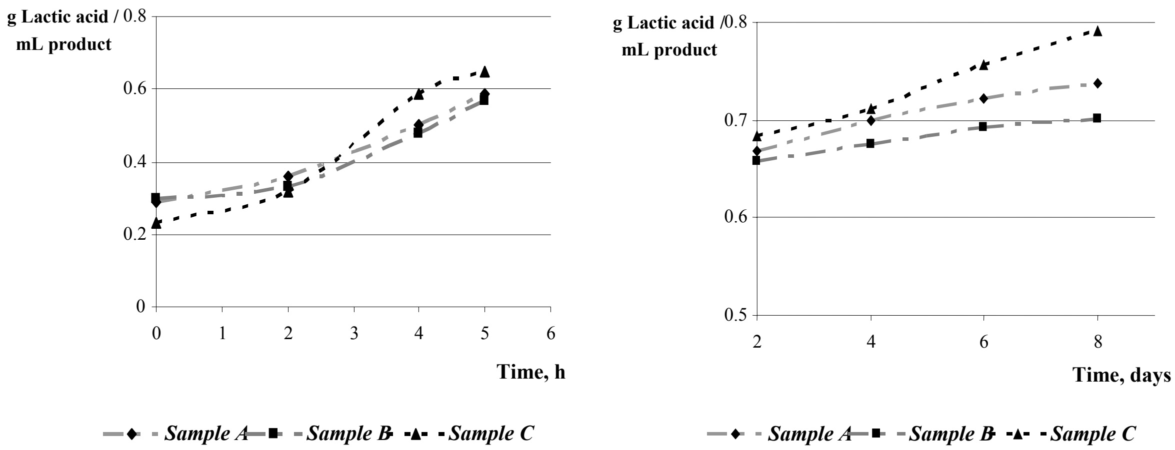
3.1. The physico-chemical characterization

The D.M. and the fats of the probiotic dairy product named CĂTINOLACT were determined, their values being shown in the table 1.

Table 1. The dry matter and the fats contents in the analyzed samples

Sample	*A*	*B*	*C*
D.M., %	12.6	12.2	11.8
Fat, %	1.5	0.9	0.6

The titratable acidity is a definitive parameter of the fermented dairy products. It was measured both during the incubation (figure 2a) and the storage (figure 2b).

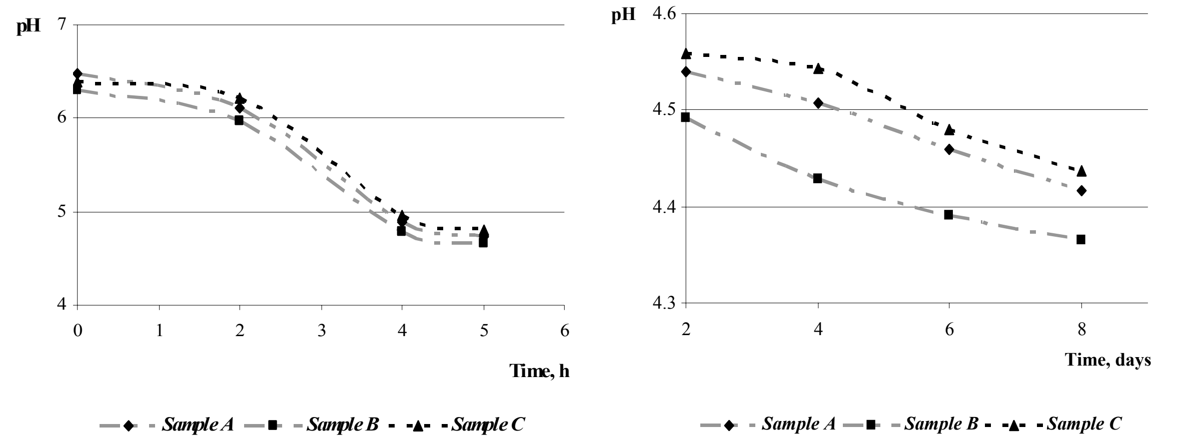


a) during incubation period b) during storage period

Figure 2. The titratable acidity variation

The titratable acidity increases slowly during the first two hours of the incubation period. Higher values are registered in the last hour of incubation for the sample C (0.648 g lactic acid/mL product), respectively for the sample A (0.585 g lactic acid/mL product). The increase is lower during the first storage day, but higher during the last days only for the sample C (0.792 g lactic acid/mL product) and A (0.738 g lactic acid/mL product).

A decrease of the pH (figure 3a) was observed during the incubation (from 6.473 to 4.727 for the sample A, from 6.297 to 4.665 for the sample B and from 6.386 to 4.806 for the sample C). Afterwards the pH values (figure 3b) were continuously decreasing by 0.1 units for all the three samples, until the end of the storage.



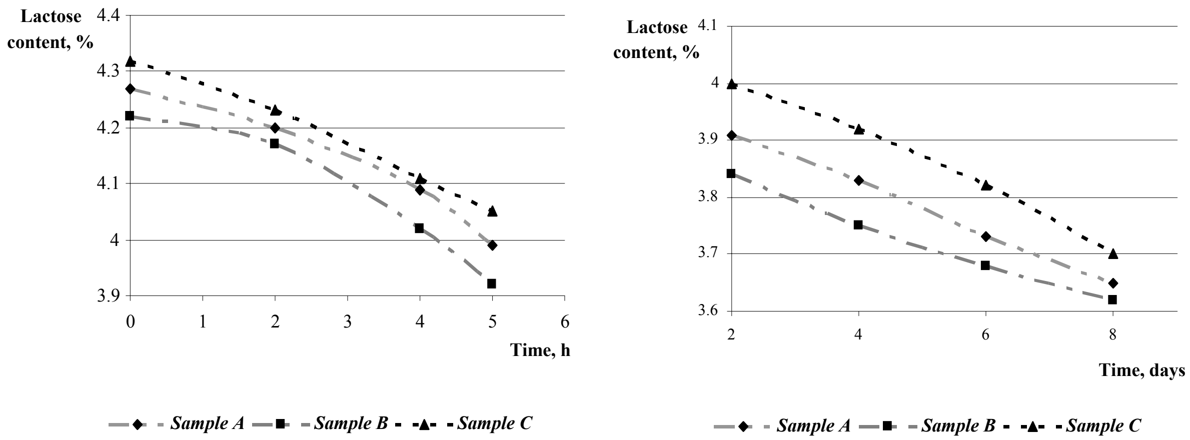
a) during incubation period b) during storage period

Figure 3. The pH variation

The pH evolution is correlated with the lactose fermentation intensity, but at the same time it is influenced by the buffer substances which are forming in the yogurt.

The lactose transformation process is highlighted through the pH decrease and implicitly through the acidity increase. The lactose degradation starts immediately after the DVS (Direct Vat Set) culture addition and continues during the incubation and the storage period.

During incubation control (figure 4a) the sample B has the lowest lactose level (3.92 %) and the sample C has the highest (4.32 %).



a) during incubation period b) during storage period

Figure 4. The lactose content variation

Because of the fermentation induced by the probiotic culture during the storage (figure 4b), the lactose decreases by 0.26 % in the sample A, by 0.22 % in the sample B and by 0.30 % in the sample C. Until the 8th day of storage, the lactose concentration supports decreases up to 3.65 % for the sample A, 3.62 % for the sample B and, respectively 3.70 % for the sample C.

The highest WHC on the first day of cold storage was obtained for sample A (82.2 %). On the 8th day of the storage period the WHC value for the sample A was 70.9 %. As for the samples in which there were added aqueous extracts of medicinal plants, the lowest value (56 %) of the water holding capacity was registered after 8 days of storage for the sample C (milk + inoculum + sea-buckthorn extract + liquorice extract). At the same time, the sample B had the water holding capacity of 65.2 % (figure 5).

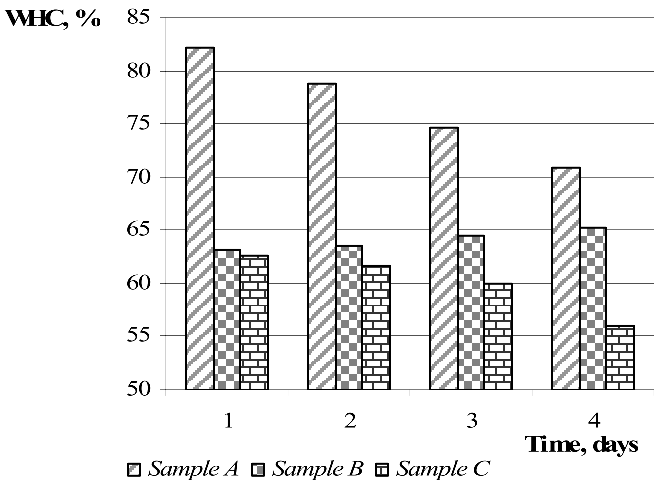
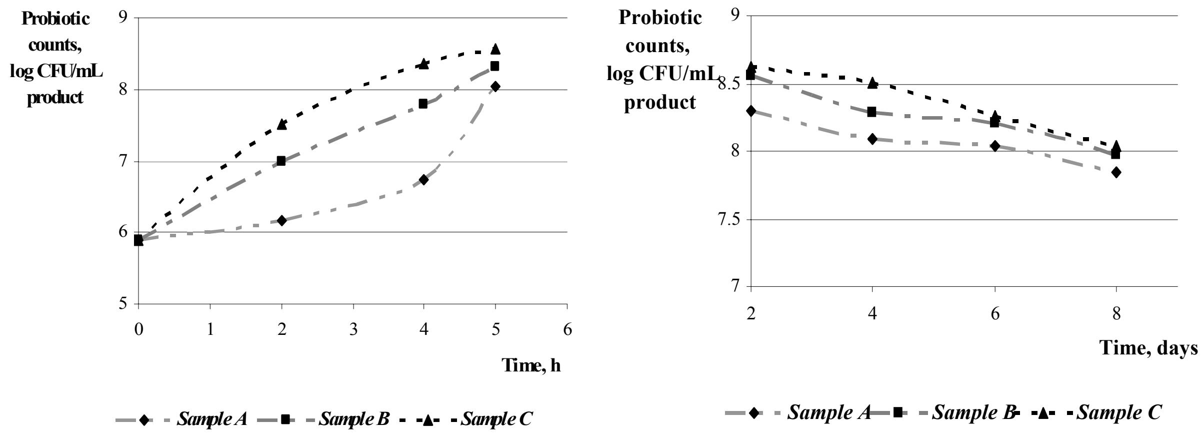


Figure 5. The water retention capacity of the yoghurt during the 8 day storage at 6ºC

3.2. The microbiological analysis

The evolution of the number of microorganisms was analyzed for each sample during incubation and storage period.



a) during incubation period b) during storage period

Figure 7. The microorganism's number

It can be observed from figure 7a that the number of lactic bacteria does not register a significant increase after the first hour of incubation period. The number of bacteria starts increasing after the second hour of incubation control. Thus, the highest number of lactic bacteria has been registered at the sample B ($2.5 \cdot 10^7$ CFU/mL product) and at the sample C ($3.2 \cdot 10^7$ CFU/mL product). In the figure 7b, one can observe that the lactic bacteria are still increasing on the second day of storage because of the high temperature of the samples (24ºC) at the beginning of the refrigeration.

The number of lactic bacteria is still high at the end of the 8 day storage. The highest registered values are encountered for sample B (0.9·108 CFU/mL product) and for sample C (1.1·108 CFU/mL product).

3.3. The statistical analysis

After processing the data obtained during the incubation period, one can observe that the pH and the titratable acidity are varying between the three samples ($P < 0.05$). Nevertheless, during storage, these parameters do not significantly vary in the case of all obtained technological variants ($P > 0.05$).

4. Conclusions

Some functional foods, which are therapeutically efficient for the human body, can be obtained by combining the milk and the medicinal plant extracts.

As a result of the lactose fermentation, the titratable acidity increased fast during the incubation period. At the end of the storage period, the highest value of titratable acidity was obtained for C sample (0.792 g lactic acid/mL product) and the lowest for the sample B (0.702 g lactic acid/mL product).

The pH of the CĂTINOLACT product decreased during incubation, being stabilized at storage.

The number of probiotic bacteria is maximal after the fifth hour of incubation (in the case of the sample C the value was $7.3 \cdot 10^8$ CFU/mL product). At the beginning of the storage period, the highest number of probiotic bacteria was registered at the sample B ($3.6 \cdot 10^8$ CFU/mL product) and C ($4.2 \cdot 10^8$ CFU/mL product).

At the end of the storage, the highest number of probiotic bacteria was encountered at the sample C ($1.1 \cdot 10^8$ CFU/mL product).

The research will continue by using other medicinal plants (dog rose - Rosa canina, artichoke - Cynara cardunculus) to diversify the assortments range and to obtain new products which combine the favorable effects of the probiotic bacteria over the human body with the curative virtues of the medicinal plants.

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