

Probiotic yogurt with medicinal plants extract: Physical – chemical, microbiological and rheological characteristics

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Abstract

The objective of this research was to investigate the growth of probiotic bacteria in milk supplemented with medicinal plant extracts, in order to fabricate a novel probiotic dairy product.

The fermented dairy product was obtained from cow milk, bilberry (*Vaccinium myrtillus* L.) and liquorice (*Glycyrrhiza glabra* L.) extracts. The fermentation process was made at 40 °C, for 5 hours, using a fast fermentation starter culture (ABT-5) as source of *Lactobacillus acidophilus* (A), bifidobacteria (B) and *Streptococcus thermophilus* (T).

During incubation and storage the following parameters were determined: the titratable acidity, the pH, the lactose content, the syneresis, the water holding capacity, the dynamic viscosity and the number of lactic bacteria. The final product was stored at 5 ± 1°C for 8 days.

It was observed that the product had been preserving its functional properties during storage and the rheological measurements showed that the product is a non-Newtonian fluid with time-independent characteristics.

Keywords: novel probiotic dairy product, bilberry extract (*Vaccinium myrtillus* L.), liquorice extract (*Glycyrrhiza glabra* L.), *Lactobacillus acidophilus*, *Streptococcus thermophilus*, flowing proprieties.

1. Introduction

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 [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 [10, 19].

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 [8, 19, 20].

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 [3].

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 **bilberry** fruit (*Vaccinium myrtillus L.*) contains \approx 1–15% of 15 different anthocyanins including those in the cyanidin, peonidin, delphinidin, petunidin, and malvidin classes [2], tannins (0.30÷0.43 %), phenolic and organic acids (benzoic acid, citric acid, lactic acid, malic acid, oxalic acid, succinic acid, tartaric acid), carbohydrates (7÷13 %). Is the most important source of ascorbic acid (12÷20 mg %), axerophthol (3÷5 mg %), nicotinic acid (0,2 mg %), aneurin (0,02 mg %), lactoflavin (0,02 mg %), tocopherol and minerals: potassium (50 mg %), calcium (10 mg %), phosphorus (8 mg %), sulfur (8 mg %), magnesium (6 mg %), chlorine (5 mg %), manganese (3 mg %), iron (1 mg %) and other biologically active compounds [15, 17].

The therapeutic properties of bilberry are attributed to the presence of anthocyanosides, a class of water-soluble chemicals (anthocyanin glucosides) belonging to a larger class of substances known as plant bioflavonoids. Pharmacologically, anthocyanosides are thought to have a stabilizing effect on collagen, prevent capillary fragility, and improve microcirculation. They are also thought to have antioxidant activity. Aside from its purported role in improving night vision, bilberry has been used to help in the treatment of glaucoma, cataracts, retinopathy, diabetes mellitus, and arthritis.

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 [16]. 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) [6,11-13,16,21].

2. Materials and methods

Materials. Cow milk was acquired from a collecting center in the County of Galati. By the help of a Milk Lab device, the following characteristics were determined: mineral substances – 0.72%, nonfat dry matter – 9.08 %, lactose – 4.35 %, proteins – 3.52 %, fats – 1.5% and titratable acidity – 19 °T.

- The bilberry 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 50 °C, 0.2·10⁵ Pa pressure and stored at 5 °C until utilization [4].

- The lyophilized culture of lactic bacteria ABT 5 provided by the Chr. Hansen Company contains the following species: *Lactobacillus acidophilus*, *Streptococcus thermophilus* and *Bifidobacterium ssp.*

Three variants of the new probiotic product with medicinal plant extracts were fabricated and coded as follows: M – 5: milk + 5 % inoculum; A – 5: milk + 5 % inoculum + 6 % bilberry extract; LD+A – 5: milk + 5 % inoculum + 6 % bilberry + 6 % liquorice extract.

Inoculum was obtained by incubation of 250 mL pasteurized milk inoculated with 0.2 U/L, corresponding to 2 % (v/v) DVS culture, at 40 °C for 12 hours. To obtain the probiotic dairy product with added medicinal plant extracts the technological stages shown in Figure 1 were followed.

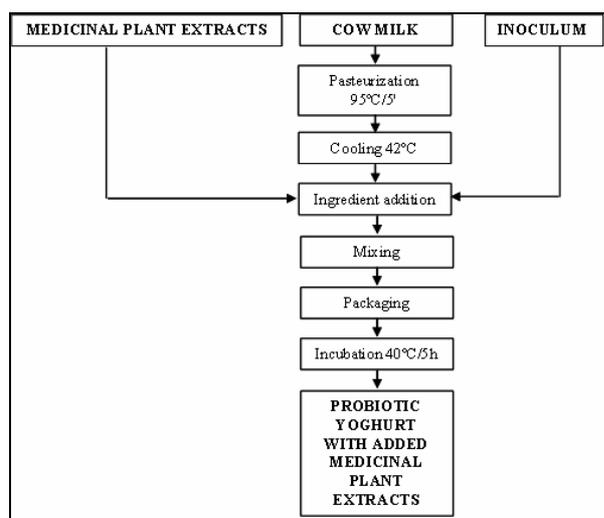


Figure 1. Technological flowchart for manufacturing the new product – Probiotic yoghurt with added medicinal plant extracts

Physicochemical analyses. Titratable acidity (T.A.) was determined as % lactic acid by titrating with 0.1 N NaOH; using phenolphthalein as an indicator. The pH of the control and probiotic yogurt with added medicinal plant extracts was measured by using a digital IQ-SCIENTIFIC pH-meter.

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, fat content by the Gerber method.

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 syneresis. Approximately 10 g of the stirred yogurt was transferred in a conical tube using a 5 mL pipette and left at 4 °C for 2 h for stabilization. The stirred samples were then centrifuged at 2500 rpm for 15 min at 10 °C. The separated whey was weighed. The syneresis was expressed as the percentage weight of the whey separated from the gel over the initial weight of the gel [1].

The water holding capacity (WHC). A sample of about 5 g of yoghurt (Y) was centrifuged for 10 min at 2500 rpm and 20 °C in a centrifuge.

The whey expelled (WE, g) was removed and weighed. The WHC expressed in percentage was defined as $WHC (\%) = \frac{(Y - WE)}{Y} \cdot 100$ [18]. All measurements were performed in duplicate.

The evolution of the lactic bacteria number. Total population of viable microorganisms was counted on regular MRS medium (pH = 5.5). All plates were incubated anaerobically at 40 °C for 48 h.

The lactic bacteria number was established, from two to two hours during incubation period and from two to two days during storage period, through indirect counting using an automatic colony counter ACOLYTE. All the experiments was in duplicate and the results were expressed as cfu/mL.

Rheological measurements. The dynamic viscosity and the torque, of the probiotic dairy product with medicinal plants extracts, were measured at 9 °C using a rotary viscometer Brookfield DV – E, equipped with a LV 2 spindles [9].

The data analysis. Analysis of variance (ANOVA) was applied to the entire dataset, 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. The means were separated by use of the least significant difference (LSD) test. Significant differences were determined at $P = 0.05$ [5,7].

3. Results and discussion

Physico-chemical characterization. The D.M. (dry matter) and the fats of the probiotic dairy product named *AFINOLACT* were determined, their values being shown in the table 1.

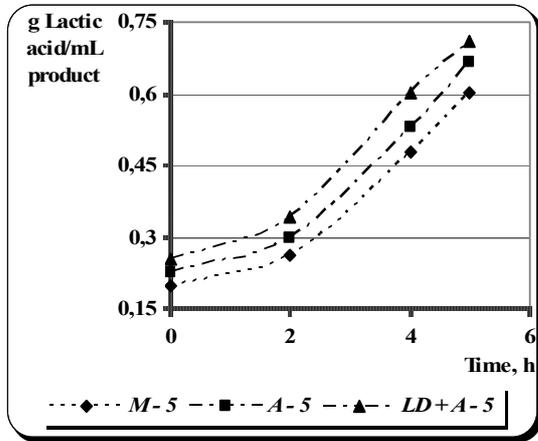
Table 1. The dry matter and the fats contents in the analyzed samples

Sample	M – 5	A – 5	LD+A – 5
D.M., %	12.7	12.4	12.05
Fat, %	1.5	1.2	0.6

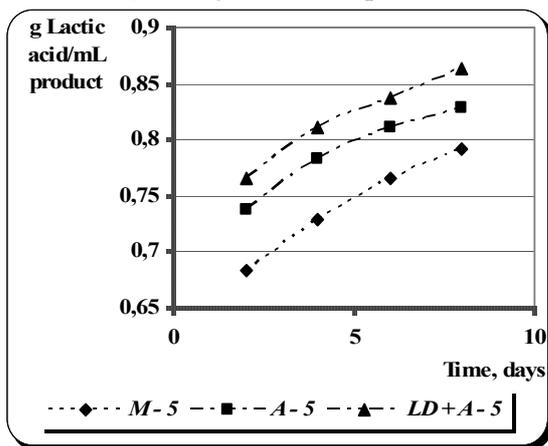
From examination of the following data is noted:

- the dry matter, for sample with medicinal plant extracts, is higher for sample A–5, exceeding the minimum value provided in Romanian standard for fermented dairy product (12 %);
- the fat content is lower in all samples with liquorice (sample LD+A–5) compared to other samples, because the proportion of milk is decreasing.

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



a) during incubation period



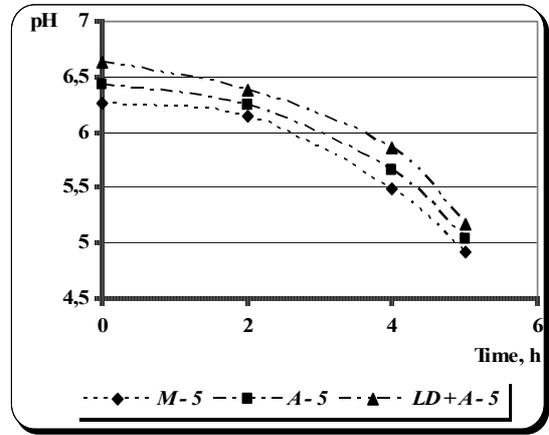
b) during storage period

Figure 2. The titratable acidity variation

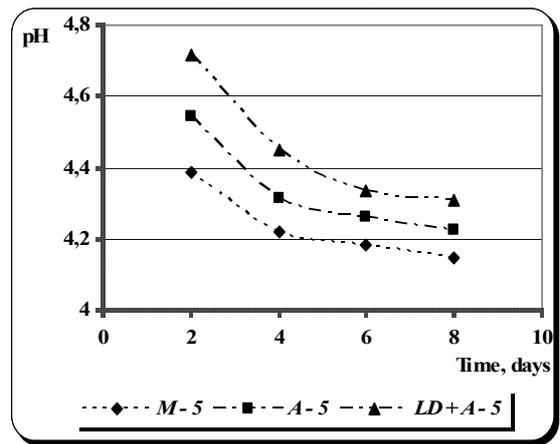
During incubation period, the titratable acidity for blank sample and for sample with added medicinal plant extracts, increase slowly during the first two hours the higher values are registered for the sample A-5 (0.297 g lactic acid /mL product) respectively for the sample LD+A-5 (0.342 g lactic acid/mL product).

At the end of incubation period the lowest values for titratable acidity was determined for sample M-5 (0.603 g lactic acid /mL product), and higher values for sample LD+A-5 (0.711 g lactic acid /mL product). After 4 days of storage at 5 °C the titratable acidity continues to rise.

At the end of 8 days of storage, higher values of titratable acidity were recorded, for sample A-5 (0.828 g lactic acid / mL product), and sample LD+A-5 (0.864 g lactic acid / mL product).



a) during incubation period



b) during storage period

Figure 3. The pH variation

A decrease of the pH (figure 3a) was observed during the incubation period. Afterwards the pH values (figure 3b) were continuously decreasing for all the three samples, until the end of the storage period.

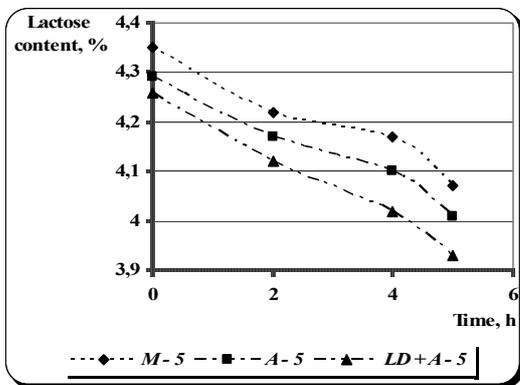
The pH of milk was 6.729. By adding the DVS (Direct Vat Set) culture and medicinal plant extracts the pH decrease to 6.262 for sample M-5; 6.433 for sample A-5 and 6.537 for LD+A-5 sample.

After 2 hours of incubation at 40°C, the pH in sample M-5 recorded a decrease of 0.1 ÷ 0.2 units and 0.3 units for samples with added medicinal plant extracts.

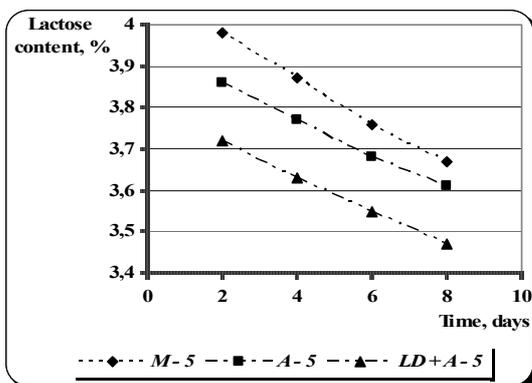
During storage period the pH values continue to decrease, at the end of this period the highest value of this parameter was measured for sample LD+A-5 (4.307), while the sample M-5 has the lowest pH values (4.144).

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

The lactose transformation process is highlighted through the pH decrease and implicitly through the titratable acidity increase. The lactose degradation starts immediately after the DVS culture addition and continues during incubation (figure 4a) and storage period (figure 4b).



a) during incubation period



b) during storage period

Figure 4. The lactose content variation

During incubation period (after 2 hours) the higher values highest value of lactose content in the case of samples with medicinal plant extracts, was recorded for sample A-5 (4.17%). After 5 hours of incubation the lowest values of this parameter were recorded for sample A-5 (4.01%, and LD+A-5 (3.93%).

During the storage period, lactic acid bacteria have an optimal developed and their fermentative metabolism have consumed a significant percentage of the initial lactose.

After 4 days of storage the lowest value, in terms of lactose content was determined for sample LD+A-5 (3.63%) and highest amount of lactose was recorded for sample M-5 (3.87%). In the 8th day of storage, the lowest amount of lactose is recorded for the sample LD+A-5 (3.47%). At the end of storage period the higher lactose content was measured for sample M-5 (3.67%).

The quantity of whey removed from fermented dairy product with ABT 5 culture, varies between 4 and 54% (figure 5).

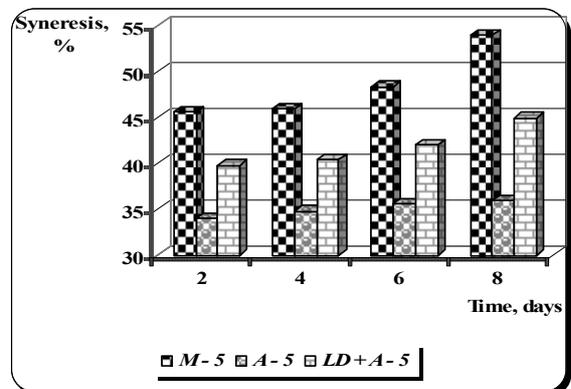


Figure 5. The syneresis of sample ABT 5 culture during storage period

The lowest amount of whey removed at 6 days of storage has been established for: A-5 (35.6%) and the higher amount of whey removed was determined for sample M-5 (46%). At the end of storage period the highest amount of whey removed was recorded by sample M-5 (54%) and the lowest amount of whey was determined for sample A-5 (36%).

The water holding capacity varies between 85.9% and 48.3% (figure 6). At 4 days of storage the highest water holding capacity of the sample were registered for sample LD+A-5 (80.8%) and 65.2% for sample A-5.

For the samples with medicinal plant extracts, the highest value of water holding capacity after 8 days of storage, was recorded for the sample LD+A-5 (milk+inoculum+blueberry extract+liquorice extract), the value being 74.4% compared with the sample M-5 (48.3%) stored in the same period.

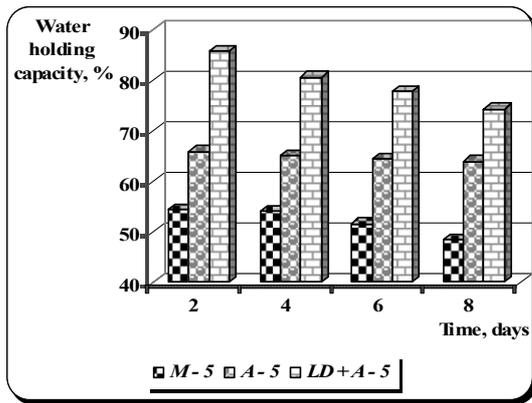
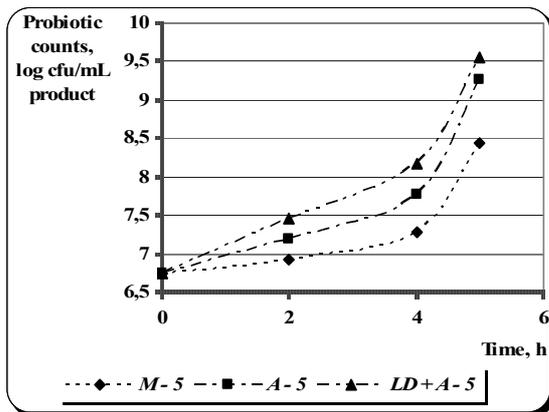
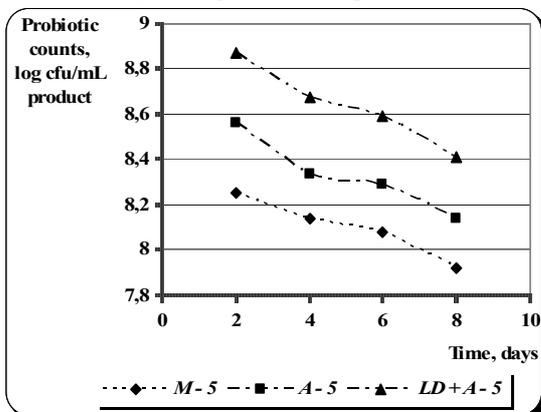


Figure 5. The water capacity capacity of the yoghurt during the 8 day storage at 6 °C

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



a) during incubation period



b) during storage period

Figure 7. The microorganisms number

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 LD+A-5 ($3.3 \cdot 10^7$ cfu/mL product) and the lowest number of probiotic bacteria, was recorded for the sample A-5 ($1.5 \cdot 10^7$ cfu/mL product).

At the end of incubation period, the maximum number of cfu/mL product was established for sample LD+A-5 ($3.6 \cdot 10^9$ cfu/mL product) and the lowest number of cfu/mL product was established for sample M-5 ($2.7 \cdot 10^8$ cfu/mL product).

From figure 7b, we can observe that from the 2nd day of storage period there is a decrease in lactic acid bacteria. At the end of storage, it is found that products containing medicinal plant extracts have a higher number of viable microorganisms in comparison with the control sample, which shows that retains the functionality during the storage period.

The number of lactic bacteria is still high at the end of the 8 storage days. The highest registered values are encountered at the sample LD+A-5 ($2.6 \cdot 10^8$ CFU/mL product).

The rheological analysis. The rheological behavior of the probiotic dairy products fabricated with medicinal plants is presented in figure 8 (the shearing stress variation according to the shearing rate) and figure 9 (the dynamic viscosity variation according to the shearing stress).

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 0.9585 for sample LD+A-5 and 0.9979 for sample M-5).

Shear stress variation depending on the shear rate had shown a development, especially at higher shear rate values 0.05 s^{-1} .

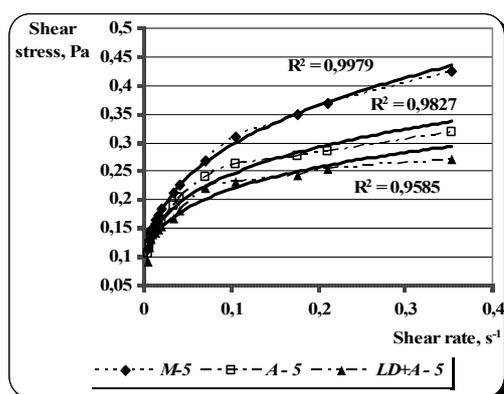


Figure 8. The shearing stress variation according to the shearing rate.

The dynamic viscosity of samples containing medicinal plant extracts at lowest values of shear rate then 0.05 s^{-1} varied from $5.31 \text{ Pa}\cdot\text{s}$ for sample M-5 at $4.261 \text{ Pa}\cdot\text{s}$ for sample LD+A-5. At values of shear rate of 0.35 s^{-1} the dynamic viscosity varied between $0.769 \text{ Pa}\cdot\text{s}$ for sample LD+A-5 to $1.203 \text{ Pa}\cdot\text{s}$ for sample M-5.

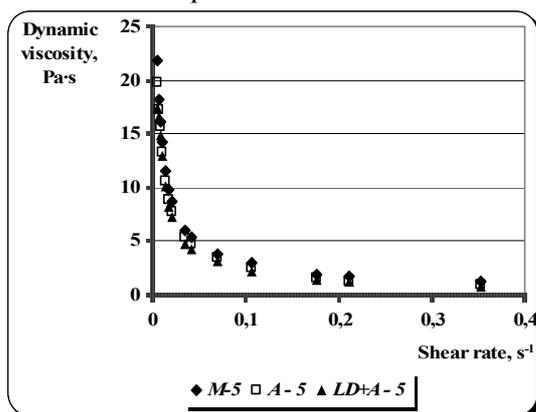


Figura 9. Variația vâscozității dinamice în funcție de viteza de forfecare

After processing the data obtained during 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 aliments, which are therapeutically efficient for the human body, can be obtained by combining the milk and the medicinal plants 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 the sample LD+A-5 ($0.864 \text{ g lactic acid/mL product}$) and the lowest for the sample M-5 ($0.792 \text{ g lactic acid/mL product}$).

The pH of the *AFINOLACT* product decreased during incubation, being stabilized at storage. For the sample with medicinal plant extracts, water holding capacity after 8 days of storage is reduced with 13.39% .

Syneresis is influenced by the product composition, *Sinereza* este influențată de compoziția produsului, especially the mineral content, titratable acidity and DVS culture type. The syneresis is more intense for sample with higher titratable acidity. At the end of the storage, the highest number of probiotic bacteria was encountered at the sample LD+A-5 ($2.6 \cdot 10^8 \text{ cfu/mL product}$).

The rheological analysis showed that the addition of medicinal plants does not modify significantly the flowing proprieties of the probiotic dairy products. According to the rheological criteria the products obtained during this study were categorized as non-Newtonian fluids, time independent and with a pseudoplastic behavior.

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