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Characterization of probiotic yoghurt obtained with medicinal plant extracts and modelling of bacteria cell growth during its production

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Abstract

In order to obtain a probiotic dairy product, ROSALACT (manufactured from cow`s milk, rosehip extract and liquorice extract), the growth of probiotic bacteria in a aqueous medicinal plant extracts medium was evaluated in this study.

The fermentation process was performed at 42 °C, for about 5 hours, using a probiotic freeze dried culture's ABY 3 and ABT 5, supplied by Chr. Hansen laboratories, Denmark. Titratable acidity, pH values, lactose content and cfu/mL values were measured during incubation and storage period.

Data's for the cell growth obtained during the incubation period was modelled by two sigmoidal functions, Gompertz and Logistic models, to evaluate similarities between the models. For all the samples, the Gompertz and Logistic equations were statistically sufficient to describe of the bacterial growth curve. For all modelated results by two sigmoidal functions, the correlation coefficient values were estimated between 0.9529 and 0.9999.

Keywords: probiotic product, rosehip extract, liquorice extract, incubation period, Gompertz and Logistic model.

1. Introduction

The probiotic bacteria are defined as "live microorganisms which, when administered in adequate amounts confer a health on the host" [7,8]. Food products which contain probiotics can be categorized as functional aliments and together with the prebiotics they represent the largest segment of the functional food market in Europe, Japan and Australia [23].

Functional foods, defined as products and/or components with beneficial effects on health, are considered as major phenomena in the science and production of food in the started millennium. In 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 [3]. Using plants in the treatment of diseases has become a tradition, "*nature pharmacy*" is a source for therapy. Valuing this biological potential is a inexhaustible source of raw materials for pharmaceuticals and food industry. In the last two or three decades to achieve the outlined idea of foods rich in biologically active substances, called "*food-drug*", which are intended metabolic diseases prevention [16]. 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.

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Furthermore, the triterpenic substances, wherefrom the glycerizine – by itself or as derived compounds (glycirizinic 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 calculosis. 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) [9,15,17,20].

The **rosehip** fruit (*Rosa canina* L.) is an excellent source of total phenols [13], vitamin C (300–4000 mg/100g) [6], carotenoids (497.6 mg/kg) [12], carbohydrates: glucose, sucrose and other sugars by 18–28% [22], organic acids: malic acid (9.8 %), citric acid (3 %), gallic acid (0.5 %) [18] and mineral substances [1,2,19,21].

Active principles from rosehip fruit stimulate the processes to eliminate toxins from the body; liqeufy the bile driving to it elimination; prevent renal calculosis forming; attenuates and healing intestinal inflammation; dilate arteries providing a better blood flow; promotes the elimination intestinal worms.

High content of vitamin C and other substances ensure the normal working of the endocrine glands, brain, heart, liver, spleen. Also involved in favorable tissue breathing [15,17].

2. Materials and Methods

2.1. Materials. Cow milk which was acquired from a collecting center from the Galati County. With the Milk Lab device there were determined the following characteristics: mineral substances – 0.72 %, nonfat dry matter – 9.08 %, lactose – 4.27 %, proteins – 3.52 %, fats – 1.5 % and titratable acidity – 18°T;

The rosehip fruits and the liquorice powder were acquired from S.C. Hofigal Export Import S.A., Bucharest.

The aqueous extracts (rosehip – *MA* and liquorice – *LD*) were obtained as follows: 100 g vegetal material was subjected to the water extraction at 20°C for two hours. After filtration through filter paper type 3 m, with a 65 g/m^2 retention capacity, for 30 s, concentration under vacuum was performed in a rotary evaporator Buchi (extraction parameters were: temperature t = 50°C and pressure p = 0,8 bar) after being stored at 4°C until usage [4,5];

The lyophilized cultures of lactic acid bacteria ABY 3 and ABT 5 provided by the Chr.Hansen Co. contains the following species: *Lactobacillus acidophilus, Lactobacillus delbrueckii* ssp. *bulgaricus, Streptococcus thermophilus* and *Bifidobacterium* ssp.

Were obtained six variants of the novel probiotic product, named *ROSALACT*, with medicinal plants extracts, variants encoded according to the type of lyophilized culture used.

2.2. Methods

2.2.1. Compositional analysis. The *p*H values were determined using an IQ-SCIENTIFIC *p*H meter. Titratable acidity was measured in 10 mL sample and 20 mL distilled water with 0.1 N NaOH using phenolphthalein as indicator. The results were expressed as g lactic acid/mL product.

The lactose content was determined by DNS method (3,5-dinitrosalicylic acid) using UV/VIS 6505 JENWAY spectrophotometer for absorbance λ = 540 nm. The etalon curve was drawn using standard solution of pure lactose.

*2.2.2. Microbiological analysis.*Total population of viable microorganisms was counted on regular MRS medium (*p*H = 5.5). All plates were incubated anaerobically at 42 °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.

2.2.3. Modelling of lactic acid bacterial cell growth. In this study, the Gompertz and Logistic models have been applied to analyze the effect of different aqueous extract that we use for the obtaining of *ROSALACT* novel probiotic dairy product. To model the results were fitted to duplicate sets of growth data using CurveExpert software (Version 1.3.).

According to [10], the better results are obtained by a fitting procedure with the Gompertz model when we compared that model with the Logistic model. The Gompertz equation [3,11,14,24,25] is:

$$y = a \cdot e^{-e^{b-c\cdot t}} \quad (1)$$

and the Logistic equation is:

$$y = \frac{a}{1 + b \cdot e^{-c\cdot t}} \quad (2)$$

where: y = log (N/N_0), N = cfu/mL; N_0 = cfu/mL when t = 0; t = time, h; a, b and c are model parameters.

3. Results and discussion

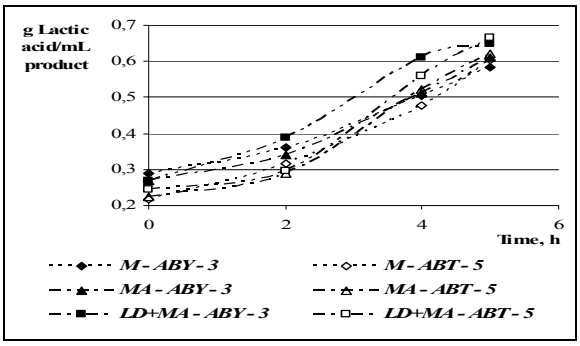
3.1. Physico-chemical characterization. The titratable acidity is a definitive parameter of the fermented dairy products. It was measured both during the incubation period (Figure 1a) and the storage period (Figure 1b).

The titratable acidity increases slowly during the first two hours of incubation period. Higher values are registered for the blank sample *M–ABY–3* (0.36 g lactic acid/mL product) and *LD+MA–ABY–3* sample (0.387 g lactic acid/mL product). In the last hour of incubation period, the higher values of titratable acidity were registered for *LD+MA–ABT–5* sample (0.666 g lactic acid/mL product) and *LD+MA–ABY–3* sample (0.648g lactic acid/mL product).

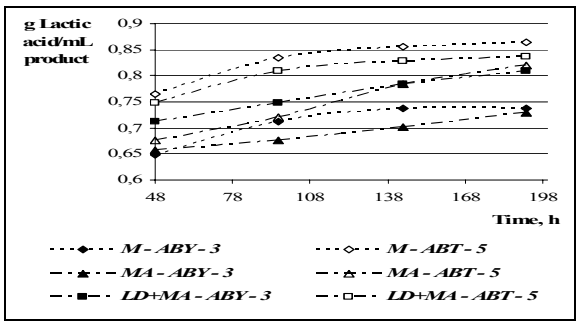
In the second day of storage, titratable acidity increase slowly, maximum value being achieved for the sample with ABT 5 culture (0.765 g lactic acid/mL product). At the end of the 8th days of storage, higher values of this parameter are registered for blank sample *M–ABT–5* (0.864 g lactic acid/mL product) and for *LD+MA–ABT–5* sample (0.837 g lactic acid/mL product).

A decrease of the *p*H values was observed during the incubation period (Figure 2a), later during storage period by the end of this period (Figure 2b). *p*H values were continuously decreasing with 0.1 units for all samples with ABT 5 culture and with 0.2 units for all samples with ABY 3 culture.

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

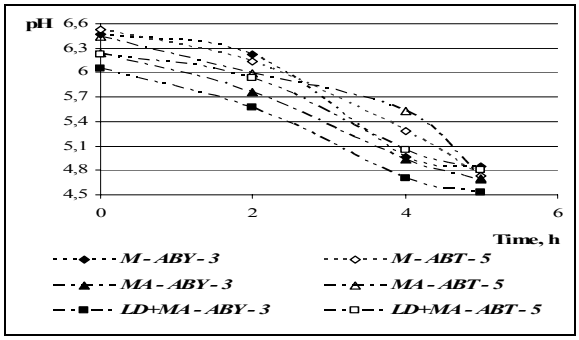


a) during incubation period

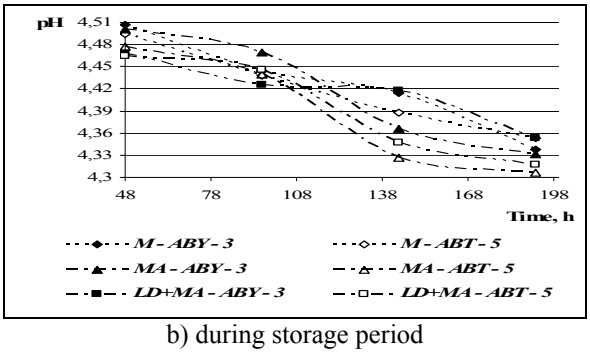


b) during storage period

Figure 1. Titratable acidity variation



a) during incubation period

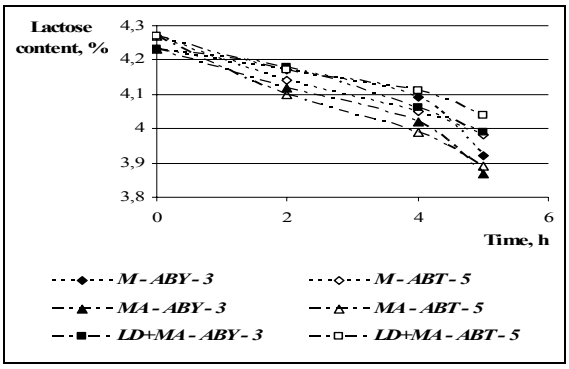


b) during storage period

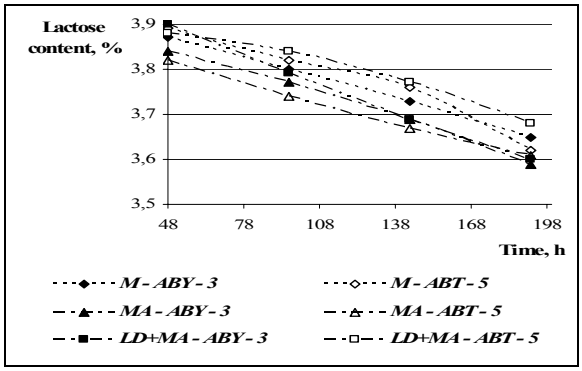
Figure 2. *p*H values variation for analyzed samples

The lactose transformation process is highlighted through the *p*H decrease and implicitly through the titratable acidity increase.

The lactose degradation starts immediately after the DVS (Direct Vat Set) culture addition and continues during incubation and storage period. During incubation period (Figure 3a) the sample *MA–ABY–3* has the lowest lactose level (3.87 %) and the sample *MA–ABT–5* has the highest (4.27 %).



a) during incubation period



b) during storage period

Figure 3. Lactose content variation for *ROSALACT* product

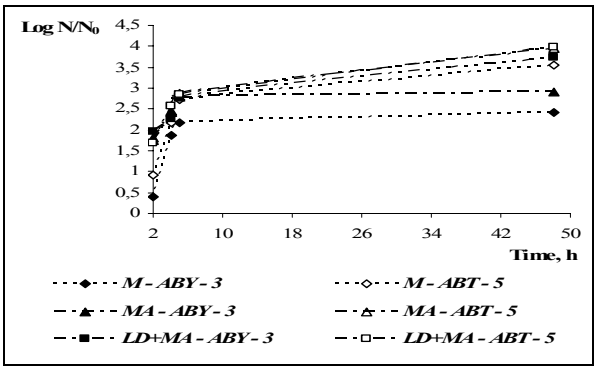
*3.2. Microbial analysis.*The evolution of the number of microorganisms was analyzed for each sample during incubation and storage period.

It can be observed from Figure 4a that the number of lactic bacteria does not register a significant increase after the first hour of incubation period.

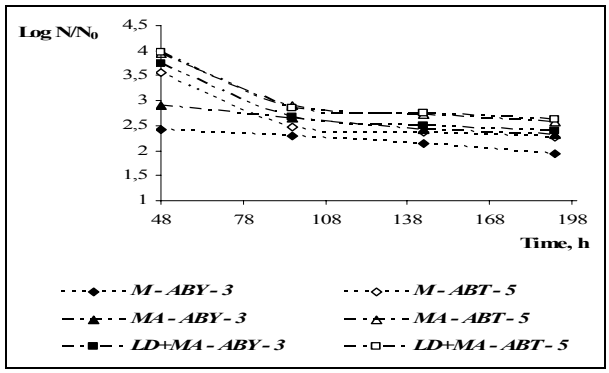
The number of lactic acid bacteria starts increasing after the second hour of incubation, the highest number of lactic acid bacteria has been registered at the *LD+MA–ABY–3* sample ($4.8 \cdot 10^8$ cfu/mL product) and *LD+MA–ABT–5* sample ($4.2 \cdot 10^8$ cfu/mL product).

The lowest number of Cfu/mL has been for blank samples, $1.2 \cdot 10^8$ cfu/mL product, for sample *M–ABY–3*, respectively $3.1 \cdot 10^8$ cfu/mL product for sample *M–ABT–5*.

From the Figure 4b we observed that the lactic acid bacteria are still increasing in the second day of storage because of the high temperature of the samples (15 ºC) at the beginning of the refrigeration period (2...4ºC).



a) during incubation period



b) during storage period

Figure 4. Viable counts variation

The number of lactic acid bacteria is still high at the end of the 8th storage days, the highest registered values are encountered at the *LD+MA–ABT–5* sample ($2.5 \cdot 10^8$ cfu/mL product) and at the *MA–ABT–5* sample ($2.3 \cdot 10^8$ cfu/mL product), which shows that the *ROSALACT* product has been preserving its functional properties during storage period ($1 \cdot 10^8 – 1 \cdot 10^9$ cfu/mL probiotic bacteria).

3.3. Statistical analysis. After processing the data (obtained during incubation period) the differences between the two models, Gompertz and Logistic, were still very small.

Table 1. Gompertz relations for the incubation period

Sample	*Equation*	*Correlation coefficient, R^2*	*Confidence limits*	*Standard Error, S*
M–ABY– 3	$\log\frac{N}{N_0} = 2.4144548 \cdot e^{-e^{2.5346898\ -\ 0.97541616}}$	$R^2 = 0.9999$	-1.4980117…..-0.82613661 4.5310018…..12.404837 1.9108014…..4.5310018	0.0169
M–ABT– 5	$\log\frac{N}{N_0} = 3.564242 \cdot e^{-e^{1.3561173\ -\ 0.52318233}}$	$R^2 = 0.9997$	-0.27966524…..-0.23284314 0.73553745…..2.5476323 0.26244605…..0.73553745	0.0396
MA–ABY– 3	$\log\frac{N}{N_0} = 2.9223514 \cdot e^{-e^{0.45308647\ -\ 0.5533882}}$	$R^2 = 0.9881$	-1.2234495…..-0.94819126 4.286437…..10.162177 2.1914216…..4.286473	0.1293
MA–ABT– 5	$\log\frac{N}{N_0} = 3.9539524 \cdot e^{-e^{0.41739375\ -\ 0.30043402 \cdot t}}$	$R^2 = 0.9981$	-0.614125511…..0.01511762 0.48176312…..1.8082478 0.16879275…..0.48176312	0.0981
LD+MA–ABY– 3	$\log\frac{N}{N_0} = 3.7550116 \cdot e^{-e^{0.46402764\ -\ 0.2208891}}$	$R^2 = 0.9876$	-0.121054…..0.14886539 0.55311566…..2.1921572 0.18686105…..0.55311516	0.2131
LD+MA–ABT– 5	$\log\frac{N}{N_0} = 3.9777591 \cdot e^{-e^{0.49465034\ -\ 0.32490558}}$	$R^2 = 0.9995$	-0.15292883…..-0.01935958 0.4874594…..1,895186 0.17394501…..0.4874594	0.4784

Table 2. Logistic model for the incubation period

Sample	*Equation*	*Correlation coefficient, R^2*	*Confidence limits*	*Standard Error, S*
M –ABY– 3	$\log\frac{N}{N_0} = \frac{2.3912422}{1 + 79.666972 \cdot e^{-1.4031576 \cdot t}}$	$R^2 = 0.9991$	-123.88068…..-0.87381927 901.48489…..242910.68 3,8373438…..901.48489	0.0662
M–ABT– 5	$\log\frac{N}{N_0} = \frac{3.557307}{1 + 12.541119 \cdot e^{-0.74253977 \cdot t}}$	$R^2 = 0.9999$	-2.1381128…..-0.24555554 22.349079…..1106.5073 0.53563858…..22.349079	0.0215
MA–ABY– 3	$\log\frac{N}{N_0} = \frac{2.9227863}{1 + 2.3736859 \cdot e^{-0.67315497 \cdot t}}$	$R^2 = 0.9896$	-2.5229665…..-0.94873081 11.884346…..72.922877 2.4704959…..11.884346	0.1211
MA–ABT– 5	$\log\frac{N}{N_0} = \frac{4.9156819}{1 + 7.9689593 \cdot e^{-0.5115416 \cdot t}}$	$R^2 = 0.9812$	-0.0695588…..1.2046401 3.4587294…..120.5515 0.12655377…..3.4587294	0.5225
LD+MA–ABT– 3	$\log\frac{N}{N_0} = \frac{3.6556787}{1 + 5.5480216 \cdot e^{-0.61092398 \cdot t}}$	$R^2 = 0.9529$	-0.19698738…..0.61421424 3.2859974…..72.339016 0.24662634…..3.2859974	0.5921
LD+MA–ABT– 5	$\log\frac{N}{N_0} = \frac{3.8890713}{1 + 7.3138768 \cdot e^{-0.66114618 \cdot t}}$	$R^2 = 0.9768$	-0.19167269…..-0.37005508 4.86863……139.66001 0.25169062……..4.86863	0.4499

Table 1 and 2 show the mathematical equations, correlation coefficient value (R^2), the confidence limits and standard error (S) for both models, in all analyzed samples.

The adopted models has following values of correlation coefficient:according with Gompertz relation the lowest value for R^2 was recorded for *LD+MA–ABY–3* sample (R^2 = 0.9876), and the the most highest value was for blank sample *M–ABY–3*

(R^2 = 0.9999), respectively for Logistic model the highest value for R^2 was determined for blank sample *M–ABT–5* (R^2 = 0.9999), and the lowest value for *LD+MA–ABY–3* sample (R^2 = 0.9529)

The lowest values of standard error, for all analyzed samples, shows that the Gompertz and Logistic equations were statistically sufficient to describe the growth curve of lactic acid bacteria during incubation period.

4. Conclusions

Some functional aliments, which are therapeutically efficient and less harmful 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 is growing fast during the incubation period.

The highest titratable acidity value was obtained for the *M–ABT–5* sample (0.864 g lactic acid/mL product) and the lowest for the *MA–ABY–3* sample (0.729 g lactic acid/mL product) at the end of the storage period. The *p*H of the *ROSALACT* product has decreased during incubation, being stabilized at storage.

At the end of the storage, the highest number of probiotic bacteria was encountered at the *LD+MA–ABT–5* sample ($2.5 \cdot 10^8$ cfu/mL product). For all analyzed samples, shows that the Gompertz and Logistic models were sufficient to describe the growth curve of lactic acid bacteria during incubation period.

The research will continue by using other medicinal plants (rosemary – *Rosmarinus officinalis* L., sage – *Salvia officinalis* L., artichoke – *Cynara cardunculus* L.) 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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