ТЕХНОЛОГІЯ ЛІКАРСЬКИХ ПРЕПАРАТІВ

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Biotechnological research in the development of a functional product with a probiotic component

Aim. To develop the composition of a functional fermented product for therapeutic and preventive nutrition based on propionic acid bacteria enriched with carbohydrate-containing component – honey.

Materials and methods. The study objects were a lyophilized starter of propionic acid bacteria (*Propionibacterium freudenreichii subsp. shermanii)*, milk, honey and samples of a functional fermented product made on the basis of these components. We used physico-chemical methods (determination of titrated acidity; pH determination), microbiological methods (Koch cup method; Gram stain), technological methods (determination of the syneresis degree), and organoleptic methods (the sensory profile) in our study. The statistical processing of results was carried out using the Excel software for Windows.

Results and discussion. During the experimental studies it was found that the required amount of the sowing material (starter) to obtain a product of the proper quality should be 3.75 % of the amount of the raw material (propionic acid bacteria – 10^6 CFU/ ml). It was proven that the optimum parameters of the main biotechnological stage of the drink preparation process were: temperature – 30 °C, time – 12 h, conditions – anaerobic. It was shown that adding 5 % of honey improved the organoleptic, physico-chemical parameters of the product, increased its biological value and reduced the time of the technological process.

Conclusions. According to the results of the technological, physico-chemical, microbiological tests the composition of the fermented product based on propionic acid bacteria enriched with honey has been developed. The product developed meets the nutritional needs and is an additional source of biologically active substances; it will increase the immune status of the body and contribute to improving its overall health.

Key words: *functional food; propionic acid bacteria; honey; fermentation*

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Біотехнологічні дослідження під час розроблення функціонального продукту з пробіотичним компонентом

Метою роботи є розроблення складу функціонального ферментованого продукту для лікувально-профілактичного харчування на основі пропіоновокислих бактерій, збагаченого вуглеводовмісним компонентом – медом.

Матеріали та методи. Об'єктами дослідження були ліофілізована закваска пропіоновокислих бактерій (*Propionibacterium freudenreichii subsp. shermanii*), молоко, мед та зразки функціонального ферментованого продукту, виготовленого на основі цих компонентів. Фізико-хімічні методи: визначення титрованої кислотності; визначення pH. Мікробіологічні методи: чашковий метод Коха; забарвлення за Грамом. Технологічні методи: визначення ступеня синерезису. Органолептичні методи: сенсорний профіль. Статистичне обробляння результатів виконували за допомогою програмного забезпечення Excel для Windows.

Результати та їх обговорення. Під час експериментальних досліджень було доведено, що необхідна кількість посівного матеріалу (закваски) для отримання продукту належної якості має становити 3,75 % від кількості сировини (пропіоновокислі бактерії 10^6 КУО/мл). Оптимальними параметрами основної біотехнологічної стадії процесу приготування напою є: температура – 30 °С, час – 12 год, умови – анаеробні. Доведено, що додавання 5 % меду покращує органолептичні, фізико-хімічні показники продукту, підвищує його біологічну цінність та скорочує час технологічного процесу.

Висновки. На основі технологічних, фізико-хімічних, мікробіологічних випробувань розроблено склад ферментованого продукту на основі пропіоновокислих бактерій, збагачений медом. Розроблений продукт задовольняє потреби в харчовій цінності та є додатковим джерелом біологічно активних речовин, що підвищить імунний статус організму і сприятиме покращенню здоров'я.

Ключові слова: *функціональне харчування; пропіоновокислі бактерії; мед; ферментація*

Introduction. Recently, dietary supplements and functional products with probiotic components used to have a positive effect on the body are popular [1, 2].

The relationship of a macroorganism and its microflora is very close. The normal microflora affects the structure of the intestinal mucosa and its adsorption capacity, participates in the exchange of fatty acids, lipid metabolism, bile acids, water-salt and gas exchange. Microorganisms of the gastrointestinal tract carry out a number of enzymatic reactions, synthesize vitamin K, vitamins B, nicotine, folic and pantothenic acids. The insufficiency of representatives of the healthy microflora causes weakening of both cellular and humoral factors of the immunological defense. The normal microflora due to a pronounced antagonistic activity protects the body from the pathogenic microflora. The imbalance of human microbial ecology leads to serious illnesses both in the gastrointestinal tract in particular and in the body as a whole [3].

Among the numerous environmental factors, which largely determine the state of human health, nutrition as a means of maintaining the life and work capacity of a person occupies the main place. The interest in the healthy nutrition is not accidental since a proper nutrition helps to maintain the body in a tone and effectively counteract many diseases. The stability of the human body depends on the quality and quantity of food, the balance of nutrients. Improper nutrition leads to some negative consequences. Therefore, the rational nutrition corresponding to age, type of occupation, place of residence and health status is considered as an important factor in the prevention of most diseases [3, 4]. According to contemporary ideas about the needs of people in food ingredients, the need to use functional food products in the diet and to enrich them with sources of vitamins, antioxidants, etc., was identified [5]. The analysis of sources of scientific literature showed the promising use of propionic acid bacteria for the production of fermented beverages for nutrition [6, 7]. In recent years biotechnology is increasingly focused on the creation of functional products with the use of various microorganisms – producers of organic acids, representatives of the normal microflora of the gastrointestinal tract of living organisms. Their beneficial effect is related to their ability to synthesize specific biologically active components (organic acids, bacteriocin, vitamins, enzymes, etc.), and it contributes to improving the sanitary microbiological and organoleptic parameters of the finished product, as well as allows to intensify the production process. In this plan, along with lactic acid bacteria, more attention is paid to propionic acid bacteria. Their physiological and biochemical features, the availability of special requirements for living conditions, the active inhibition of mold fungi and other contaminating microorganisms are highly beneficial in the practical application of them as a starter [8].

Therefore, **the aim of this work** is to develop the composition of a functional fermented product for therapeutic and preventive nutrition based on propionic acid bacteria enriched with honey.

Materials and methods. The following study objects were selected for the development of the composition and technology of the functional fermented product: milk, honey and a lyophilized dried starter of propionic acid bacteria. Milk with a reduced content of fat (1.5 %) was used as the basis for a product of the preventive nutrition and as part of a nutrient medium for the cultivation of propionic acid bacteria. A lyophilized dried starter (*Propionibacterium freudenreichii subsp. shermanii*) was dissolved in the solution of sodium chloride and used as a seed material to extract the beverage. An additional carbohydrate component during the study was natural flower honey. The study objects were also samples of a functional product of various compositions obtained by fermentation of milk with propionic acid bacteria with the addition of honey.

We used physico-chemical methods (determination of titrated acidity; pH determination), microbiological methods (Koch cup method; Gram stain), technological methods (determination of the syneresis degree), and organoleptic methods (the sensory profile) in our study. The statistical processing of results was carried out using the Excel software for Windows. The statistical significance was $p \leq 0.05$.

The work on the development of the composition and technology of a functional fermented product was carried out in accordance with the rules of asepsis in the laminar box of the Department of Biotechnology of the NUPh.

Results and discussion. Fermentation is an important stage in the process of obtaining a functional fermented product. Fermentation parameters include the amount of the biological agent sowing material (the initial amount of the starter), temperature, the time of fermentation and the aeration mode. The fermentation temperature of 30 °C was chosen according to the literature. This is the optimal temperature for the highest physiological activity of *Propionibacterium freudenreichii subsp. shermanii*.

It is known that with an increase in the initial concentration of cells, the time for activating enzymes needed for them reduces, but more organic acids are formed in the process of cultivation, and it decreases the pH and thereby inhibits the cell mass growth. Therefore, it is important to choose the concentration of the initial seed material that would provide the optimum time for the technology and product quality.

Several experimental samples were prepared to determine other values of the fermentation parameters. To a volume of 200 ml of milk, an appropriate quantity (2.5 ml, 5 ml, 7.5 ml, and 10 ml) of the seed material with the concentration of viable cells of 10^6 CFU (colony forming units) /ml was added. After that, samples were placed in a thermostat under different conditions (aerobic and anaerobic); then the time of the clot formation and the amount of viable propionic bacteria were determined; and, after the completion of the fermentation, the amount of the separated serum, organoleptic characteristics, titrated acidity of the beverage were determined.

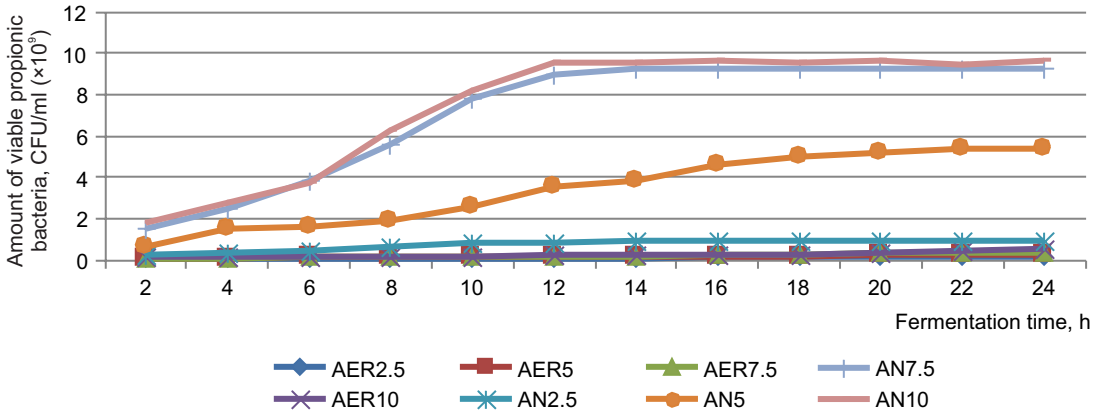

Fig. 1. Dynamics of accumulation of viable propionic bacteria in the samples, depending on the conditions of cultivation: AER2.5 – aerobic conditions of cultivation, 2.5 ml of the starter; AER5 – aerobic conditions of cultivation, 5.0 ml of the starter; AER7.5 – aerobic conditions of cultivation, 7.5 ml of the starter; AER10 – aerobic conditions of cultivation, 10.0 ml of the starter; AN2.5 – anaerobic conditions of cultivation, 2.5 ml of the starter; AN5 – anaerobic conditions of cultivation, 5.0 ml of the starter; AN7.5 – anaerobic conditions of cultivation, 7.5 ml of the starter; AN10 – anaerobic conditions of cultivation, 10.0 ml of the starter

The results of the test to determine the amount of viable propionic bacteria (Fig. 1) have shown that under the anaerobic conditions the concentration of propionic bacteria increases significantly in 2 h after the start of the fermentation; in 12 h the value reaches $9.2 \cdot 10^9$ CFU/ml (in the sample with the initial the amount of the sowing material 7.5 ml), and then it almost does not change. It should be also noted that when adding 2.5 ml and 5 ml of the starter at the beginning of the process, the amount of propionic bacteria in 24 h is only $0.5 \cdot 10^9$ CFU/ml and $5.4 \cdot 10^9$ CFU/ml, respectively, and with the addition of 10 ml – $9.6 \cdot 10^9$ CFU/ml. This proves the expediency of using the starter with the concentration of propionic bacteria – 10^6 CFU/ml in the amount of 7.5 ml (3.75 %).

It should be also noted that the aerobic conditions of cultivation did not meet the requirements of propionic bacteria – their amount practically did not change since the beginning of the fermentation, titrated acidity changed slightly, and the sour milk clot was not formed for 24 h; therefore, only the samples obtained under anaerobic fermentation conditions were further studied. The time of the sour-milk clot formation in all samples of the product obtained in the anaerobic conditions was in the range of 10 to 14 h and did not differ significantly depending on the amount of the starter added.

The results of determination of the titrated acidity and the amount of the separated serum (Fig. 2 and Fig. 3) showed that in the sample with 2.5 ml of the seed material

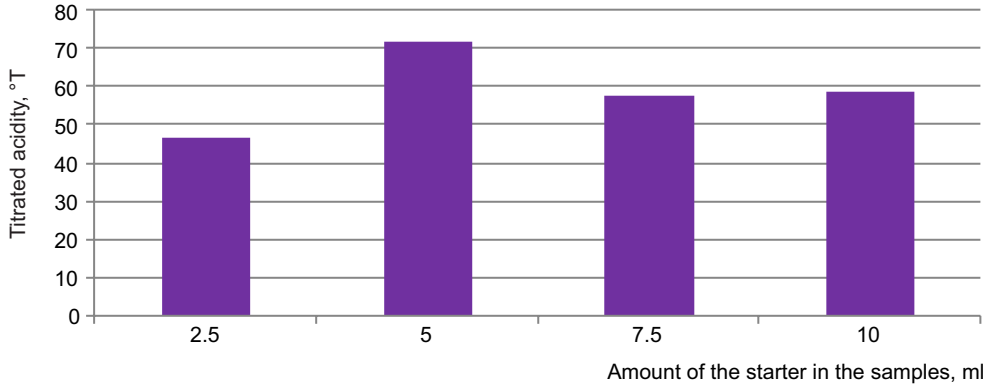

Fig. 2. The titrated acidity of the samples obtained under the anaerobic fermentation

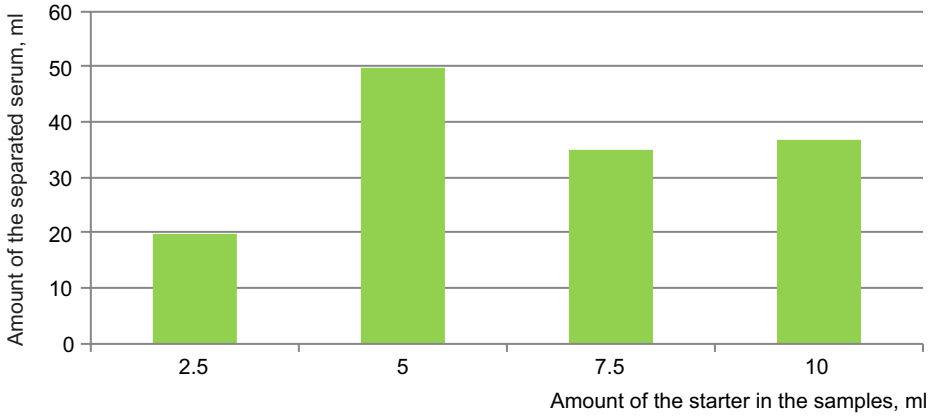

Fig. 3. The amount of the separated serum during the fermentation

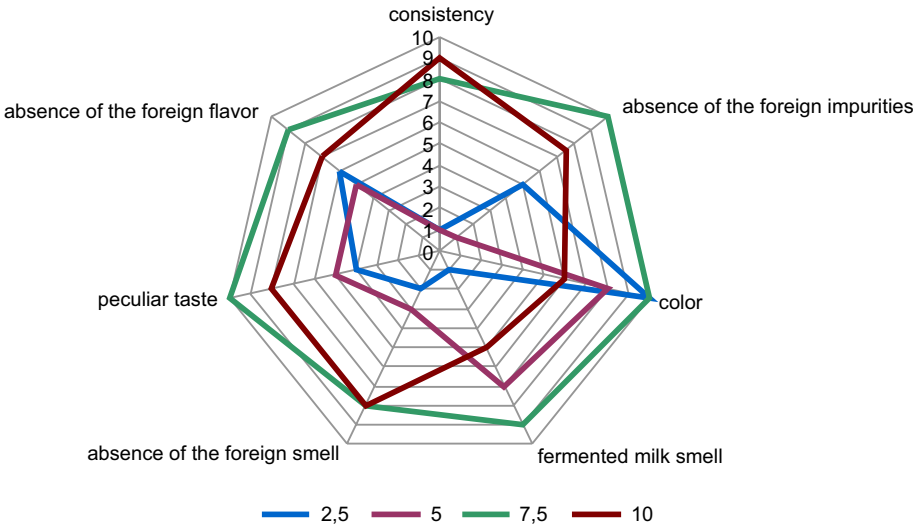

Fig. 4. The sensory profile of experimental samples with different content of the sowing material (the amount of the starter – 2.5 ml, 5 ml, 7.5 ml, 10 ml)

47 °T titrated acids and 20 ml of the serum (10 % from 200 ml of the total volume) were formed, in the sample with 5 ml – 72 °T and 50 ml (25 %) of the serum, respectively, being the highest result from the test specimens.

In the samples with 7.5 ml and 10 ml of the starter the titrated acidity values did not differ significantly and were within the statistical error of 58 °T and 59 °T, respectively. This pattern was also characteristic for determining the degree of syneresis (35 ml and 37 ml serum 17.5-18.0 %). The results obtained were proportional to the sensory profile of the samples under study.

The results of the determination of the sensory profile (Fig. 4) indicate that the sample with 2.5 ml of the starter has an unsatisfactory consistency (the sour milk clot is absent) and an unpleasant smell, which may indicate the development of a foreign microflora. The sample with 5 ml of the starter is characterized by satisfactory characteristics; it has a sour milk clot, but an unpleasant foreign smell and the taste of bitterness. The samples with 7.5 ml and 10 ml of the starter have a characteristic sour-milk smell and taste, but in the sample with a higher initial content of the starter (10 ml) the smell is too strong, and the taste is sour and acrid.

Therefore, we can conclude that the optimal fermentation parameters for obtaining of the functional product based on propionic acid bacteria are: the amount of the starter – 3.75 % (with the initial concentration of cells – 10^6 CFU/ml); fermentation temperature – 30 °C; fermentation time – 12 h; conditions – anaerobic.

It is known that propionic acid bacteria are used as nutrients of lactose, glucose and fructose. Thus, it was interesting to study the effect of the last two food components on the fermentation process and the qualitative characteristics of the product obtained with their addition. It is known that the natural leader in the content of these ingredients is honey, which contains fructose (up to 42 %) and glucose (up to 36 %). In addition, it is a valuable source of various biologically active components (vitamins, organic acids, flavanoids, phytoncides, etc.). Therefore, in order to increase the biological value of a fermented product at the next stage of experimental studies, the concentration of the additional component, honey, should be chosen. For these studies, we took the amounts of honey of 5 g, 10 g, and 15 g, which were chosen based on the literature data and the results of our own preliminary studies on the survival of propionic bacteria when adding these concentrations of honey [9, 10]. Three experimental samples were prepared containing 200 ml of milk, 3.75 % propionic bacteria and different amounts of honey. The samples were fermented for 12 h at 30 °C under the anaerobic conditions; after that the time of the clot formation, the amount of the secreted serum, titrated acidity and organoleptic characteristics of the product were determined.

The results of determining the titrated acidity and the amount of the separated serum (Fig. 5 and Fig. 6)

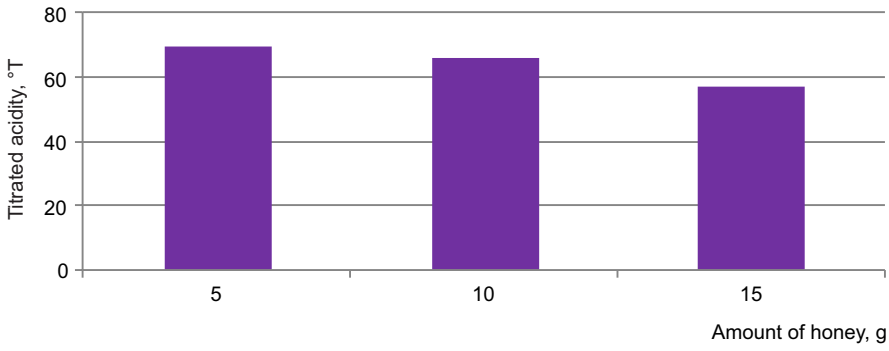

Fig. 5. The titrated acidity of samples of the fermented product with the different amount of honey

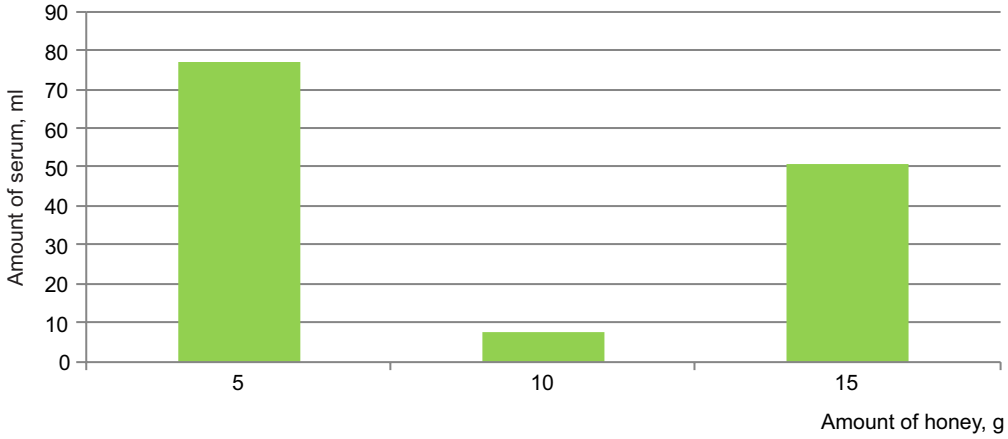

Fig. 6. The amount of the serum separated in the fermentation process of samples with the different amount of honey

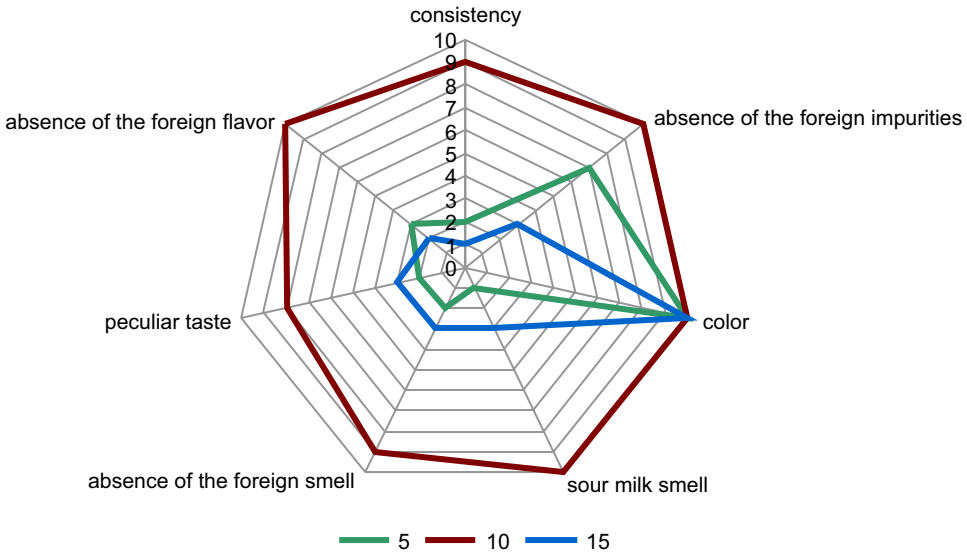

Fig. 7. The sensory profile of experimental samples of the fermented product with the different amount of honey (5 g, 10 g, 15 g)

showed that in the sample with 5 g of honey, 70 °T of titrated acids was formed and 77 ml of the serum (38.5 % from 200 ml of the total volume) was isolated, while in the sample with 15 g – 57 °T and 51 ml (25.5 %) of the serum, respectively.

In the sample containing 10 g of honey, the titrated acidity was intermediate and amounted to 66 °T, while the syneresis was almost absent – only 8 ml of the serum (4 %), and as indicated in determining of the sensory profile (Fig. 7), the consistency was homogeneous, and the taste of sour milk was pleasant.

Meanwhile, in a sample with less honey there was a loose flaky consistency and an unpleasant smell of mold, and in the sample with the maximum amount of honey (15 g), on the contrary, the clot was very dense, the consistency was inhomogeneous, there were dense foreign impurities, and the taste was unusual for sour-milk drinks – too sweet.

The results of determining the time of the sour-milk clot formation (Fig. 8) showed that the addition of an additional honey component could significantly reduce the time spent on conducting the fermentation process

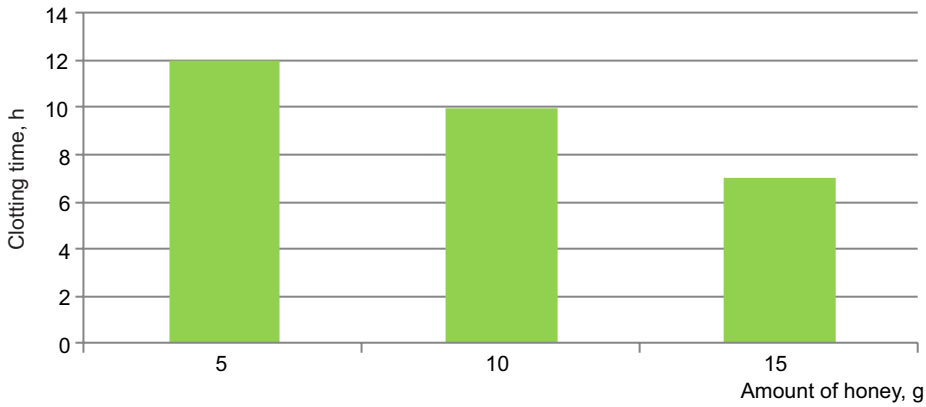

Fig. 8. The time to clot in samples with the different honey content

– by almost 5 h (with addition of 15 g), for 2 h (with addition of 10 g).

Thus, it is expedient to add 5 % of honey to the dairy raw material when producing a fermented product based on propionic acid bacteria.

Conclusions and prospects of further research. According to the results of the technological, physico-chemical, microbiological tests the composition of the fermented product based on propionic acid bacteria enriched with honey has been developed. During the experimental studies it has been found that the required amount of the sowing material (starter) to obtain a product of the proper quality should be 3.75 % of the amount of the raw material (propionic acid bacteria – 10^6 CFU/ml). It has been proven that the optimum parameters of the main biotechnological stage of the drink preparation process are: temperature – 30 °C, time – 12 h, conditions – anaerobic. It has been shown that adding 5 % of honey improves the organoleptic, physico-chemical parameters of the product, increases its biological value and reduces the time of the technological process. The product developed meets the nutritional needs and is an additional source of biologically active substances; it will increase the immune status of the body and contribute to improving its overall health.

Conflict of interests: authors have no conflict of interests to declare.

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