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# PHYSICO-CHEMICAL AND MICROBIOLOGICAL QUALITY INDICATORS OF FERMENTED WALNUT MILK-BASED DRINK

*The article theoretically substantiates and proves the method of obtaining a fermented drink made from a mixture of nut and cow's milk. Psyllium was used as a consistency stabilizer and a component that enriches the product with dietary fiber. The optimal ratio of nut and cow's milk was determined as 60:40 volume percent, respectively, and the concentration of psyllium was 1.5%. A product with improved organoleptic properties and increased biological value was obtained. The physicochemical and microbiological indicators were analyzed and the nutritional value of the recommended drink was calculated. The developed fermented drink with the addition of plant raw materials (nut milk and psyllium) will contribute to expanding the range of products with a reduced lactose content.*

***Keywords:*** *nut milk; fermented drink; psyllium; plant-based raw materials; physicochemical indicators; nutritional value. Table: 5. Fig.: 3. References: 22.*

**Urgency of the research.** Current trends in food consumption show an increase in consumer demand for ecological and functional products. The number of followers of vegetarian nutrition, which includes the use of alternative sources of nutrients, is also growing. Fermented combined dairy products are beneficial for human health due to the presence of both cow's milk components and fermentation products and, especially, live microflora, which has probiotic properties.

**Target setting.** Traditional fermented drinks based on cow's milk (yogurts) have a thick consistency and a corresponding pleasant taste. And if it is easy to get a pleasant taste of a product based on plant milk, especially such as coconut and almond, obtaining a dense consistency or an unbreakable clot is problematic and difficult without the use of thickeners or structure-forming agents. Therefore, the authors consider it advisable, firstly: to use not only plant types of milk, but also their mixtures with cow's milk, and secondly: to use such stabilizers, which, in turn, will also increase the biological value of the resulting product.

**Actual scientific researches and issues analysis**. Walnut is a promising raw material for the production of vegetable drinks thanks to its rich composition. It contains significant amount of proteins, unsaturated fatty acids (especially omega-3), vitamins (group B, E), minerals (calcium, magnesium, iron, zinc) and antioxidants such as polyphenols. These components contribute to improvement of general health, in particular support the work of cardiovascular and nervous systems, as well as strengthen the immunity. General oils content in the nuclei oscillates from 60.9% to 73.1%, and the crude protein content is from 13.5% to 20.2%. The main fatty acids in walnut oil are oleic, linoleic, linolenic and palmitic acids, while linoleum acid content fluctuates from 48.9% to 58.2%, and oleic acid from 20.0% to 25.3% [1].

In studies [2, 3] amino acid composition was analyzed and amino acid score of protein of walnut and drink based on was calculated. Comparison of the amino acid composition of the studied products with the reference protein made it possible to calculate the utility coefficient, which is 0.58 for the drink protein and 0.62 for the nut protein, which reflects the level of protein digestibility. Biological value of walnut drink, determined by the coefficient of differences of amino acid composition, is 62.96 %, which is high indicator for plant protein [2; 3].

It is known that along with the taste, nutritional value and benefits of yogurt, the appearance and consistency of the product are important for consumers [4]. Milk-protein gel is formed as a result of the vital activity of lactic acid bacteria, which ferment milk sugar to lactic acid and other derivatives. As a result of the increase in the concentration of Hydrogen ions, casein coagulation occurs with the formation of a spatial structural system, which determines the structural and mechanical properties that characterize the further behavior of the product under conditions of deformations that arise during the technological process [5]. For mixed yogurt, the destruction of the structure occurs immediately after fermentation. Mixed yogurt can be considered as a dispersion of gel particles in whey, but re-formation of bonds occurs , which soon create a weak gel structure [6]. In yogurt based on a mixture of plant and animal milk, due to the reduction in the amount of lactose, there is a problem of liquid consistency and the use of additives. Thus, in the work [7], the technology of using traditional thickeners is investigated: agar-agar, gelatin. Experiments were also conducted using corn starch, chia seeds and flax. Using the mentioned thickeners, they were first dissolved in a small volume of room temperature plant-based milk and withstood for a certain time. The remaining milk was heated to 60 °C, then the milk with thickener was added, the mixture was heated to 60 °C, and then cooled to 40 °C. For the fermentation of the product, the pure sourdough was added to prepared semi-finished product. Yogurt starter – mixture is presented by Bulgarian rods and thermophilic streptococcus or dry probiotic in powder form or capsules [7]. The obtained samples had better structural and mechanical properties than the control samples without structure-forming agents.

Also, the influence of food additives on the organoleptic, structural-mechanical and physico-chemical properties of yogurt was studied in [8]. Pumpkin powder, which consists of 60% dietary fiber and 15% pectin substances was used. It was added to the ready-made curd in an amount of 2 to 10%. It was found out that pumpkin powder, as a source of dietary fiber, pectin substances, vitamins, macro- and microelements, also allows to improve the structure of yogurt significantly. At optimal concentration of 1.5–3.5%, it has a positive effect on organoleptic and structural- mechanical product properties. With the increase of additive concentrations emergence of too pronounced smell and taste of pumpkin, as well as excessive density consistency were observed [8].

In the article [9], the appropriateness of using starch molasses with different degree of saccharification in the composition yogurts was scientifically justified. The dependence of conditional yogurt viscosity and ability of its clots to hold moisture on the degree of saccharification of molasses was detected. It has been proven that caramel-type molasses, which contains dextrins, has the highest ability to structure. Insignificant deceleration in process fermentation in the presence of higher sugars in low and medium dextrose molasses equivalent was recorded, while molasses with high degree saccharification almost has no effect on activity lactic acid bacteria.

Among various starch products the most promising for use in fermented milk drinks are dry glucose syrups and maltodextrins that do not require additional water introduction and are convenient for storage and use. It has been established that high contents of higher sugars in maltodextrins lengthens, and the increase of monosaccharides in syrups and molasses accelerates process fermentation of dairy mixtures in the production of yogurt and bioyogurt with a fat content of 0.05-1.0 % [10].

In the article [11] based on experimental data a recipe and technology of yogurt for the elderly people with the addition dietary fiber and substitute sugars — fructose has been developed. Yogurt was made by the tank method with a fat content of 2.5%. For fermentation direct application of bacterial preparation FD DVS ABY-3 was used. Yogurt production was carried out in two stages: in the first stage, fructose was added in various doses for sweetening, and in the second stage, oat flakes were added to the chilled yogurt. [11].

The authors [12] investigated the antioxidant properties of plants such as Chinese lemongrass, blood-red hawthorn, rose hips, sea buckthorn, three-parted string, and sage in fermented milk drinks. Pectin was used as a stabilizer.

Non-traditional herbal ingredients that can be used as fillers for yogurts are poppy and sesame seeds. Adding them in a ratio of 1:1 in an amount of 5% of general yogurt mass allows to maintain the structure, consistency and viscosity of the product without forming serum [13].

As a stabilizer of the consistency of fermented beverages, it is also possible to use plantain seeds [14]. Dietary fibers obtained from the seeds of plantain oval and flea are designated by the term " psyllium ", and in foreign literature the term " isvagul " is also used. It contains up to 85% soluble fiber, vitamins of group B1, B2 and B3, trace elements: Zn, Mg, Ca, Co, Cr and others, as well as polyunsaturated fatty acids. In terms of its properties, the husk of plantain seeds differs from many dietary fibers and, due to the presence of fiber, is able to exhibit gel-forming properties [15].

Energy value of psyllium is 42-46 kcal, as it almost doesn't contain easily digestible carbohydrates. The chemical composition is also represented by uronic acids, tannins, flavonoids, carotenoids, polysaccharides, saponins, mucilages, ascorbic acid, organic acids. Psyllium has almost no smell and taste of its own [15].

Experimental studies indicate the presence of hypoglycemic and hypolipidemic properties in psyllium, therefore its consumption provides prevention of the development of atherosclerosis and other diseases of the cardiovascular system [16].

The possibility of using psyllium together with pectin in the formulation of ice cream based on almond and hemp milk was proven in [17], where attractive organoleptic and technological characteristics were noted.

The addition of psyllium husk improves the body, consistency, appearance and mouthfeel and slows down the syneresis of cow's milk yoghurt [18]. It has been observed that with the increase of fibre levels, the sour taste sensation increases. The increase of acid with increasing psyllium husk content is explained by the prebiotic effect, which may have supported the growth of *L. bulgaricus* and *S. thermophilus*, leading to greater acid production. Overall, in terms of sensory characteristics, yoghurt enriched with 0.5% psyllium husk had the highest overall acceptability [18].

**Uninvestigated parts of general matters defining**

Literature review showed that a large number of studies are being conducted to find effective consistency stabilizers in the technology of fermented beverages, however, beverages based on a mixture of nut and cow's milk have not been studied, and the search for plant components that would simultaneously improve consistency and be a source of dietary fiber and other biologically active substances has not been conducted.

**The research objective** is the development of a fermented drink based on nut milk enriched with psyllium seed husk and the assessment of its quality according to physicochemical, organoleptic and microbiological indicators.

**The statement of basic materials.** The main raw material used in the production of fermented drinks, enriched with psyllium is walnut milk. Walnuts that comply with DSTU (The State Standarts of Ukraine) 8900:2019 "Walnuts. Technical conditions" were used for the production of plant milk. According to the standard, they have undamaged, light kernels. The consistency is crispy. The taste and smell are inherent, without bitterness, moderately oily.

Additional raw material – cow's milk TM "Ferma", which corresponds with the requirements of DSTU 2661: 2010 " Drinking cow 's milk. General technical conditions ". The mass proportion of fat is 2.5 %, protein – 2.8%.

Fermentation was carried out using dry bacterial starter "Yogurt Vivo ", manufactured in accordance with TU (Technical conditions) 15.5-3060300036-001:2009 "Bacterial starters". The starter contains: *Streptococcus thermophilus, Lactobacillus delbrueckii ssp, Bulgaricus, Lactobacillus acidophilus, Bifidobacterium lactis*. The starter culture provides a number of lactic acid microorganisms of at least 10 $^{7.}$ It inhibits the growth of pathogenic and conditionally pathogenic bacteria in the human body.

The additive used is psyllium husk of psyllium seeds (TM SoloSvit). It complies with TU No. 10.8-42063780-001: 2018. Its water absorption capacity was assessed using the gravimetric (weight) method.

According to quality indicators (organoleptic, physicochemical and microbiological), the finished product - yogurt must comply with DSTU 4343:2004 "Yogurts. General technical conditions". The finished product was evaluated according to the following organoleptic indicators: consistency, appearance, taste, color, smell. Acidity was determined by titration, density - by the areometric method, pH - by the potentiometric method, determination of the mass fraction of protein - by the formalin titration method; bacteria of the *Escherichia coli* group were determined by sowing on liquid Endo medium, the presence of yeast was determined by sowing on liquid medium Sabouraud [19].

The research conducted included:

1. Production and analysis of nut milk and a number of its mixtures with cow's milk.
2. Fermentation of the resulting mixtures with different dosages of psyllium.

Nut milk was made by grinding nut kernels in an aqueous medium. From previously soaked nuts for 12 hours at a temperature of 20–25 °C the shells were separated from the walnut kernels, then the kernels were ground in water (at the rate of 100 g of nuts per 500 ml of water) using a blender for 180 seconds at a speed of 10,000 rpm. Then the mixture was infused for 30 minutes and ground again for 60 seconds at a speed of 12,000 rpm. The cake was separated using a filter.

The study of the quality indicators of fermented drinks with a combined composition was carried out for mixtures of 50:50 and 60:40 volume percentages of nut and cow's milk. The control was a drink based on cow's milk. The content of plantain seed husk was 1.0%, 1.5%, 2.0% of the mass of all mixtures and the control.

The milk was heated to a temperature of 40 °C , the “Yogurt Vivo ” starter was added and fermented at a temperature of 40 °C for 4 hours. Psyllium (pharmacy) was added in its pure form, without prior preparation, 30 minutes after the start of fermentation.

Analysis of nut milk, cow's milk and their mixtures was carried out using the Milkotester milk analyzer Master ECO by a number of indicators: fat, dry non-fat milk residue, density, freezing point, protein, lactose, mineral salts, added water. Analysis of all samples were carried out at a temperature of 30°C. Results of the analysis are presented in Fig. 1.

Analysis of the mixtures showed that cow's milk has a higher content of dry non-fat residue, mineral salts, higher density, lower freezing point compared to nut milk. Nut milk has a higher fat content. The mixtures have intermediate results in terms of indicators.

Plant-based milk drinks contain less milk sugar, which forms a milk-protein gel as a result of the activity of lactic acid bacteria, so the technology of the fermented nut milk drink required the use of a plant additive that improves the consistency and at the same time enriches the finished drink with substances beneficial to health.

To determine the feasibility of using Psyllium as a stabilizer of the resulting drink the study of its moisture absorption capacity has been carried out.

A weight of psyllium was soaked in water, nut milk, and cow's milk and left to stand for 0-40 minutes. 1 gram of psyllium was placed in a container with small holes and immersed in another container of water. Every 5 minutes, the container with the weight was removed and weighed.


*a* *b*

*c* *d*

*Fig. 1. Photo of the results of the analysis of cow 's milk (a), nut milk (b), a 50:50% mixture (c), a mixture of 60% nut milk and 40% cow 's milk (d):*
*Ж – fat; Б – protein; С – dry dehydrated milk residue; Л – lactose;*
*П – density; М – mineral salts; Т – freezing point; В – added water*

Source: developed by the authors.

Influence of all liquids on the process of swelling was determined by the magnitude of the limiting degree swelling. Degree swelling was defined as the ratio between the mass of absorbed liquid and initial mass of seeds. The measurement results are shown in Fig. 2.

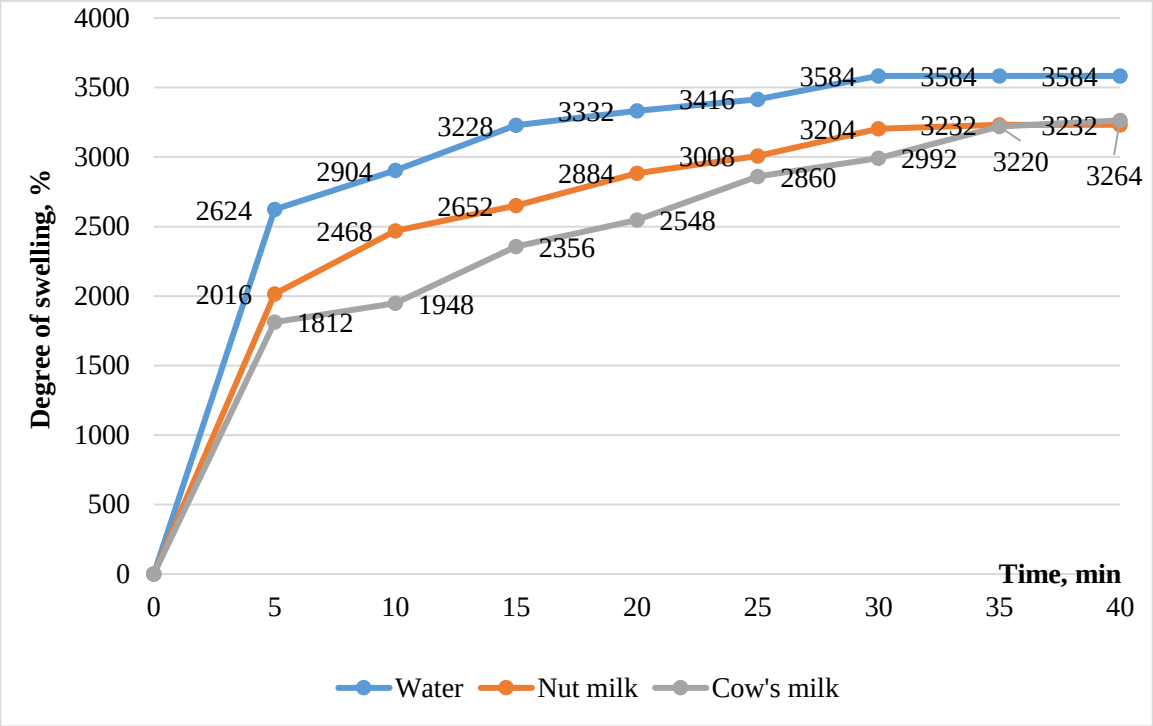


*Fig. 2. Dependence of moisture absorption capacity on time*

Source: developed by the authors.

The obtained data indicate intensive water absorption by psyllium during the first five minutes, then the speed of the process slows down, as evidenced by a less intensive mass growth. The highest degree of swelling was observed in water. In nut and cow's milk, the process of water absorption occurs more slowly, which is associated with the presence of electrolytes, in particular calcium salts and other minerals. They are able to form hydrated shells, retaining part of the low-molecular liquid and preventing its interaction with the macromolecules of high-molecular substances of the seed shell. The swelling process is the least effective in cow's milk, since it has a higher viscosity.

The results of studies of physicochemical and organoleptic quality indicators of fermented beverage samples are presented in Tables 1, 2 (for a ratio of nut milk to cow's milk of 50:50) and 3 (for a ratio of nut milk to cow's milk of 60:40), in which, respectively:

sample 1 – cow's milk yogurt;
sample 2 – fermented milk mixture drink without psyllium;
sample 3 – fermented drink of a mixture of milk and 1% psyllium;
sample 4 – fermented drink from a mixture of milk and 1.5% psyllium;
sample 5 – fermented drink from a mixture of milk and 2% psyllium.

*Table 1 – Physicochemical properties of samples at a 50:50 nut-to-cow milk ratio*

| Indicator | Sample No. | | | | |
|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 |
| Density, kg / m $^3$ | 1136 | 1013 | 1030 | 1070 | 1180 |
| Titrated acidity, ° T | 74.5 | 50.5 | 46.5 | 54.5 | 20.0 |
| Active acidity, pH units | 4, 4 7 | 4.55 | 4.43 | 4.47 | 4.48 |
| Mass fraction of protein, % | 3.5 | 2.5 | 2.4 | 2.4 | 2.4 |
| Mass fraction of moisture, % | 87.4 | 88.0 | 88.8 | 88.4 | 88.9 |
| Mass fraction of dry matter% | 12.6 | 12.0 | 11.2 | 11.6 | 11.1 |

Source: developed by the authors.

*Table 2 – Organoleptic characteristics of samples at a 50:50 nut-to-cow milk rati* *

| Indicator | Sample No. | | | | |
|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 |
| Consistence | Homogeneous, with a broken clot | Liquid, homogeneous | Thick, with psyllium inclusions | Thick, homogeneous, with psyllium flakes | Very viscous, jelly- like |
| Color | White | Light gray | Light pink | Light pink | Gray with a pink tint |
| Scent | Pure fermented milk | Light nutty scent | Light nutty scent | Light nutty scent | Faint nutty smell |
| Taste | Pure fermented milk | Pleasant taste, slightly earthy | Pleasant taste, slightly earthy | Pleasant taste, without an earthy aftertaste | Pleasant taste, without an earthy aftertaste |

* Organoleptic indicators of samples with a ratio of nut and cow's milk of 60:40 practically did not differ from the 50:50 ratio.

Source: developed by the authors.

*Table 3 – Physicochemical properties of samples at a 60:40 nut-to-cow milk ratio*

| Indicator | Sample No. | | | | |
|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 |
| Density, kg / m $^3$ | 1136 | 1010 | 1020 | 1066 | 1150 |
| Titrated acidity, ° T | 74.5 | 57.5 | 56.3 | 53.5 | 32.0 |
| Active acidity, pH | 4,47 | 4.55 | 4.39 | 4.40 | 4.46 |
| Mass fraction of protein, % | 3.5 | 2.4 | 2.3 | 2.3 | 2.3 |
| Mass fraction of moisture, % | 87.4 | 89.1 | 89.2 | 89.5 | 89.9 |
| Mass fraction of dry matter, % | 12.6 | 10.9 | 10.8 | 10.5 | 10.1 |

Source: developed by the authors.

Analysis of the data obtained showed that the addition of psyllium contributes to an increase in the density of the product by 17-67 kg/m $^3$ depending on its concentration for 50:50 mixtures and by 10-50 kg/m $^3$ – for mixtures 60:40. The highest value of titrated acidity was in the control sample, and with the increasing psyllium content, acidity decreases by 30%. Active acidity decreases slightly.

The mass fraction of protein compared to the control sample is lower, but almost does not change with the increase of psyllium content. A slight increase in the mass fraction of moisture (2 %) is observed with the increase of psyllium content, which may be due to its strong moisture absorption capacity.

According to organoleptic evaluation, samples with 2% psyllium have a jelly-like consistency that is not typical for a drink, samples without added psyllium and with 1% have an earthy taste. The color and smell of all samples are similar, their intensity slightly decreases with the increasing content of the additive.

Based on the conducted physicochemical and organoleptic studies of the quality indicators of the manufactured samples, a fermented drink prepared from 60% nut milk and 40% cow's milk with the addition of 1.5% psyllium seed husk was selected as the optimal one.

Shelf life studies were conducted by measuring the titrated acidity of the following samples: control (yogurt made from cow's milk), fermented beverages with a volume ratio of 50:50 and 60:40 walnut and cow's milk with the addition of 1.5% psyllium husk on the first, fourth, seventh and tenth days. The samples were stored in refrigerated conditions at a temperature of 5°C for 10 days. The results are shown in Fig. 3.

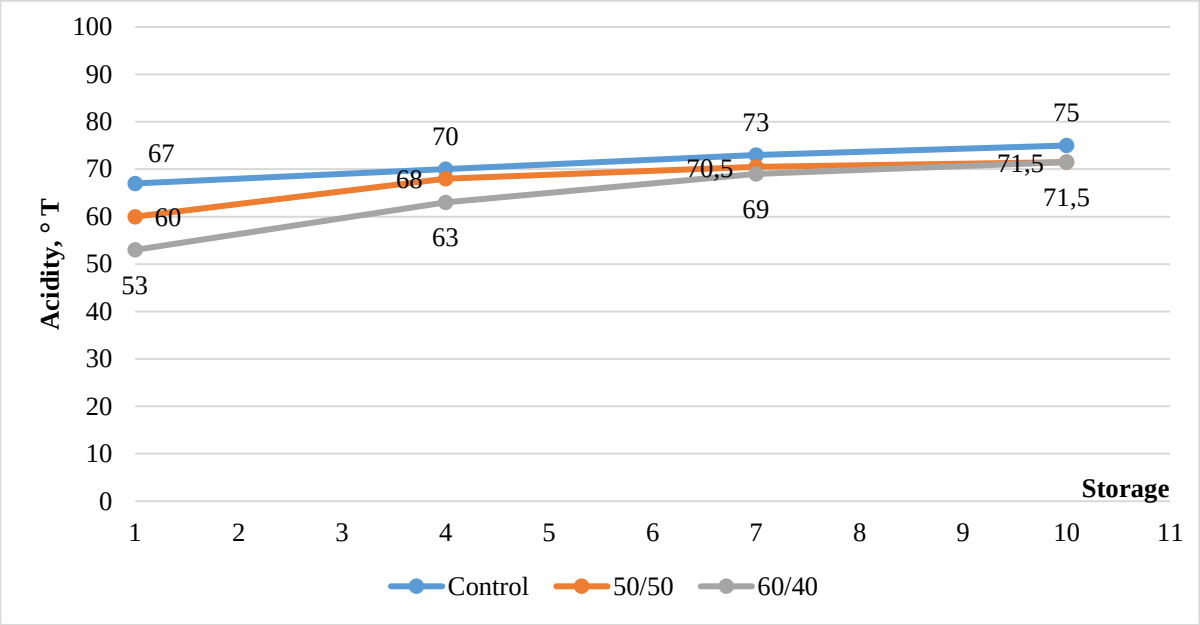


*Fig. 3.* Dynamics of titrated acidity during storage

Source: developed by the authors.

The obtained data indicate both lower initial acidity values of samples with psyllium and a decrease in the intensity of the increase in acidity of beverages after 7 days of storage. During the 10 days of study, no whey separation was observed in the sample with the addition of psyllium, while partial syneresis was observed in the control sample.

A microbiological evaluation of the optimal sample of fermented beverage was also conducted. The results of the studies are presented in Table 4. Neither Escherichia coli nor yeast were detected in the studied samples.

*Table 4 – Microbiological studies [20]*

| Indicator name | Requirements | Actual content |
|---|---|---|
| Bacteria of the Coliform Group in 0.1 cm $^3$ | not allowed | not detected |
| Yeast, CFU in 1 cm $^3$ | no more than 50 | not detected |

The energy value of the optimal sample with 60% walnut milk whey and 1.5% psyllium was calculated based on the chemical composition of the raw material components (Figure 1) and their content in the developed product (Table 5).

*Table 5 – Chemical composition of raw materials [21]*

| Raw | Content of components in 100 g of product | | |
|---|---|---|---|
| | Proteins, g | Fats, g | Carbohydrates, g |
| Walnut milk | 0,420 | 3,511 | 0,615 |
| Cow 's milk | 1,130 | 0,870 | 1,690 |
| Psyllium | 0,023 | 0,009 | 1,275 |
| Together | 1,573 | 4,390 | 3,580 |

The energy value of the developed product was calculated according to formula (1), based on the energy value of proteins, fats and carbohydrates, respectively:

$$EV=(P\times4)+(F\times9)+(C\times3{,}8) \quad (1)$$

where EV is the energy value;

B – protein content in 100 grams of product;

F – fat content in 100 grams of product;

C – carbohydrate content in 100 grams of product.

Thus, the resulting fermented drink based on a mixture of nut and cow's milk with the addition of 1.5% psyllium is tasty, of high-quality and safe for the consumer. Its calculated energy value is 60 kcal (251 kJ), which correlates with the nutritional value of the base product - traditional yogurt on cow's milk, the energy value of which is 51-66 kcal [22].

The drink is enriched with beneficial walnut components and dietary fiber from the husk of plantain seeds, which additionally give the drink prebiotic properties, bind water, and have an enveloping and anti-inflammatory effect on the intestinal mucosa.

**Conclusions.** The method of obtaining a fermented drink containing 60% nut milk by double grinding of walnuts, using dry bacterial starter "Yogurt Vivo" and adding psyllium 30 minutes after the start of fermentation has been theoretically substantiated. The most appropriate concentration of the psyllium additive has been determined, which is 1.5%, which gives the finished product high organoleptic indicators and nutritional and biological value. The physicochemical and microbiological indicators of the drinks have been analyzed, the resulting products comply with regulatory documentation and are safe for the consumer, the energy value of the drink has been calculated - 60 kcal.

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# ФІЗИКО-ХІМІЧНІ ТА МІКРОБІОЛОГІЧНІ ПОКАЗНИКИ ЯКОСТІ ФЕРМЕНТОВАНОГО НАПОЮ НА ОСНОВІ ГОРІХОВОГО МОЛОКА

*Необхідність розробки нових видів напоїв на основі рослинного молока обумовлена обмеженістю молочних ресурсів в Україні та збільшенням кількості людей, хворих на гіполактазію (непереносимість лактози). У роботі досліджуються суміші коров'ячого молока з горіховим, що дає змогу отримати низьколактозну продукцію.*

*Метою статті є розробка ферментованого напою на основі горіхового молока, збагаченого лузгою насіння подорожника та оцінка його якості за фізико-хімічними, органолептичними та мікробіологічними показниками.*

*Проведене дослідження включало:*

*1. Виготовлення та аналіз горіхового молока і ряду сумішей його з коров'ячим.*

*2. Ферментація одержаних сумішей за різного дозування псиліуму.*

*Горіхове молоко виготовлялось шляхом двократного подрібнення горіхового ядра у водному середовищі. Ферментацію проводили за використання сухої бактеріальної закваски «Йогурт Vivo» та внесення псиліуму як стабілізатора консистенції та джерела корисних харчових волокон через 30 хв після початку сквашування. Дослідження показників якості ферментованих напоїв з комбінованим складом проводили для сумішей 50:50 та 60:40 об'ємних відсотків горіхового і коров'ячого молока. Контролем виступав напій на коров'ячому молоці. Вміст лушпиння насіння подорожника становив 1; 1,5 та 2 %. Аналіз отриманих даних показав, що додавання псиліуму сприяє збільшенню густини продукту на 17-67 кг/м$^3$ залежно від його концентрації для сумішей 50:50 та на 10-50 кг/м$^3$ –для сумішей 60:40. Найвище значення титрованої кислотності було у контрольного зразка, а зі збільшенням вмісту псиліуму кислотність напою знижується на 30 %.*

*Визначено найбільш доцільну концентрацію добавки-псиліуму, яка становить 1,5 %, що надає готовому продукту високі органолептичні показники та харчову й біологічну цінність. Отриманий продукт відповідає нормативній документації та є безпечними для споживача, розрахована енергетична цінність напою- 60 кал /100 г продукту.*

***Ключові слова:*** *горіхове молоко; ферментований напій; псиліум; рослинна сировина; фізико-хімічні показники; харчова цінність.*

*Табл.: 5. Рис.: 3. Бібл.: 22.*

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