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Vegan alternative for fermented milk products: preparation and some properties

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Abstract. Rice is relatively easy to digest by humans, and the presence of starch provides the organism with slow energy. In addition, the absence of lactose makes it possible to provide a complete diet for people suffering from allergies or individual intolerance to cow's milk components. Therefore, the creation of analogues to traditional dairy and fermented milk products is a timely and in-demand task. The use of biotechnology methods allows not only to obtain a product with original organoleptic characteristics, but also to enrich it with metabolites of lactic acid bacteria, to give pre- and probiotic properties. The issues of obtaining fermented rice base (FRB) as a basis for alternative vegan products have been studied. It is shown that with the ratio of solid and liquid phases in the rice base – 1:2, respectively, favourable conditions were created for the development of lactic acid bacteria. It was found that additional treatment (partial enzymatic hydrolysis at a temperature of 40 ± 2 ° C for 60 minutes) mixtures before fermentation improves the adaptation of the microbiota to this substrate. In addition, the taste of the rice base improves: a pronounced sweetness appears, the flour flavour disappears, the consistency becomes more viscous. The process of fermentation (for 12 hours at a temperature of 38-40 ℃) of rice base was studied, the dynamics of acid accumulation depending on the introduced cultures of lactic acid bacteria was studied. The expediency of applying pectin (1% and 2% by weight) is shown to regulate the consistency of the FRB. The FRB samples received a high sensory evaluation.

1 Introduction.

Foods based on cereals and grain components open up opportunities for the inclusion of probiotics, prebiotics and fibre in the human diet. An increasing number of consumers are choosing plant-based milk substitutes for medical reasons or moral and ethical reasons. Medical reasons include lactose intolerance with a worldwide prevalence of 75% and allergy to cow's milk. Also in countries where mammalian milk is scarce and expensive, vegetable milk substitutes serve as a more affordable option [1-3].

Consumer interest and research in plant-based dairy analogues have been growing in recent years due to the increasingly negative effects of animal products on human health, animal welfare and the environment. However, analogues of plant-based dairy products have

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a number of disadvantages: unsatisfactory sensory properties, undesirable taste, poor texture [4-9].

Considering the above, it is relevant and timely to search and evaluate new opportunities for the use of plant raw materials in food production technology.

The correct choice of raw materials can be based on the following criteria:

- accessibility - does not depend on external circumstances;
- "traditional" - to have a good receptivity among the population, rooted in the consciousness due to the historical and cultural peculiarities of the development of the people;
- manufacturability - the ability to be processed by various methods of modern industry, including biotechnological.

Rice was chosen as the raw material for obtaining analogues of fermented products. Rice is one of the most common and affordable cultivated crops worldwide. Rice is a source of important amino acids, vitamins and trace elements. It has less saturated fat, compared to other products, it is easily digested. In addition, the presence of complex carbohydrates in rice contributes to the formation of a long-term feeling of satiety. And the absence of gluten and lactose allows people with intolerance to these components to eat rice and products based on it.

In this article, fermentation is considered as a viable way to develop pure analogues of plant-based dairy products with satisfactory consumer properties.

Thus, the purpose of this study was to study the suitability of rice milk for the preparation of fermented rice base (FRB) for vegan fermented dairy alternatives products.

Based on the aim, the following tasks were set:

1) obtaining rice base (RB) for subsequent fermentation;
2) pretreatment (hydrolysis) and fermentation of RB;
3) Obtaining the FRB and its analysis.

2 Materials and Methods.

Description of samples. Rice base (RB) is a mixture consisting of crushed rice and water in a certain ratio. Fermented rice base (FRB) is hydrolyzed RB (with a hydromodule of 1:2), then treated with LAB.

"Control" is a FRB obtained by treatment with LAB that does not contain pectin.

"Experience-1", "Experience-2" – FRB obtained by treatment with LAB and containing 1% or 2% pectin, respectively.

Obtaining RB. Round-grain rice was used as a raw material for the preparation of RB. Rice was pre-soaked and ground in a high–speed laboratory grinder (JustBuy, China), at a rotation speed of 36,000 rpm for 3 min (approximate particle size – from 50 to 300 Mesh (0.3-0.05 mm)). Then RB was prepared with hydromodules 1:2, 1:3 and 1:4.

Pretreatment of RB. RB (hydromodule 1:2) were treated with the preparation "Alfalad BN" (low-temperature α–amylase, activity 2000 units / cm3, operating range: pH 5.0 – 9.0; temperature 30-80 ° C, Russia) for partial starch degradation and an increase in the level of free simple sugars. After inactivation of the enzyme (by heating the mixture to 95 ° C with an exposure of 5 min), RB was cooled and treated with lactic acid bacteria.

As starter cultures (dosage of 5% by weight of the mixture) were used:

- bacterial starter culture of *Streptococcus thermophilus* ("Lactosynthesis", Russia);
- bacterial starter culture of *Lactobacillus delbrueckii subsp. bulgaricus* ("Lactosynthesis", Russia);
- bacterial starter culture of *Lactobacillus acidophilus* ("Lactosynthesis", Russia);

- complex bacterial starter culture "Kefir": *kefir fungi, Lactococcus lactis, Bifidobacterium lactis, Leuconostoc mesenteroides, Streptococcus thermophilus, Lactobacillus acidophilus, Lactobacillus delbrueckii ssp. bulgaricus*, ("VIVO", Russia);
- complex bacterial starter culture "Symbilact": *Streptococcus thermophilus, Lactobacillus delbrueckii ssp. Bulgaricus, Lactobacillus acidophilus, Bifidobacterium lactis, Lactococcus lactis ssp. cremoris, Lactococcus lactis ssp. lactis, Lactococcus lactis ssp. lactis var. diacetylactis* ("VIVO", Russia).

Commercial apple pectin ("Pektowin", Poland) was used as a thickener.

Determination of the properties and parameters of the FRB. Titrated acidity was determined by the potentiometric method. The active acidity was determined using a pH meter HI 2211-02 (HANNA Instruments, Germany). The analysis of the content of protein, fat, solids and total sugars in the samples was carried out on an infrared analyzer "InfraLUM FT-12" (Russia) with the appropriate software and calibration data.

All studies were conducted in at least three repetitions, followed by statistical data processing.

The organoleptic evaluation of the samples was carried out by an untrained non-smoking group of 20 subjects (10 men and 10 women aged 19 to 65 years). The subjects were asked to evaluate the samples by appearance, colour, taste, smell, consistency and general acceptability.

3 Results and Discussion.

1) *Obtaining rice base (RB) for subsequent fermentation*. Rice and products based on it have long been used in the diet in the world. However, its non-standard use as a raw material required additional research.

At the initial stage, studies were conducted to select the ratio of rice and water, the so-called "hydromodule", suitable for basic technological operations.

The selection of the hydraulic module was reduced to solving several tasks:

a) ensuring a sufficient amount of dry substances in the Republic of Belarus, necessary for the vital activity of starter microorganisms;

b) formation of satisfactory organoleptic and physico-chemical indicators of RB;

c) obtaining RB with satisfactory structural and mechanical properties.

To solve the tasks set, RB samples were prepared with a ratio of 1:2, 1:3 and 1;4 solid and liquid phases, respectively. The resulting RB was fermented with cultures of Bulgarian bacillus, as one of the most used in the technology of classical fermented milk products. Considering the use of a specific substrate for lactobacilli, it was of interest to study their behavior under these conditions. Fermentation was carried out at a temperature of 40 ° C with temporary control of titrated acidity and pH until the expected plateau biomass growth was achieved.

Before fermentation, the physico-chemical properties were studied (Table.1) indicators of all samples of RB.

Table 1. Physico-chemical indicators of RB.

Hydro-module	Fat, %	Protein, %	Dry matter, %	Density, kg/m3	Titratable acidity, °T	Active acidity (pH)
1:2	1,47±0,10	4,62±0,24	10,52±0,53	1040,3±52,0	3,0±0,15	7,19±0,37
1:3	1,35±0,10	3,23±0,18	8,74±0,44	1030,7±51,5	3,3±0,17	7,21±0,37
1:4	0,78±0,04	2,01±0,10	6,39±0,32	1020,0±51,0	3,1±0,16	7,11±0,36

As a result of the physico-chemical analysis, it was found that all samples had a neutral pH medium and, accordingly, a low titratable acidity. RB with hydromodule 1:2 was characterized by a relatively high protein content and density, which is natural, and is associated with a high content of dry substances.

The results of the organoleptic evaluation showed that RB with a hydromodule of 1:4 had a weak taste and smell, the colour was white. The consistency of this sample was liquid, watery. This sample received the lowest rating.

RB with hydromodule 1:3 had a neutral taste and smell, the color was milky white. However, the consistency was also liquid, slightly tightened.

RB with a 1:2 hydromodule received the highest rating among the other samples. The taste and smell were neutral, the colour was white, milky. But the consistency was more pronounced – tear-like, viscous.

However, all samples had an insufficiently sweet taste.

2) *pretreatment (hydrolysis) and fermentation of RB*. Considering that RB is an uncharacteristic environment for the development of lactic acid microorganisms, in particular due to the low sugar content, it seemed appropriate to subject the test sample to enzymatic hydrolysis in order to increase the amount of free sugars.

Enzymatic hydrolysis was carried out using the drug "Alfalad BN" at a temperature of 40 ± 2 ° C for 60 minutes. The enzyme was introduced after heating the mixture to 20 ° C, which was the beginning of the hydrolysis time.

During the processing of RB, the temperature of the process was controlled and changes in the content of soluble solids and total sugar were monitored by the express method on the BIC analyzer.

In this case, the qualitative composition of sugars was not important, because the analysis of the literature data suggests that in the process of starch hydrolysis, mainly reducing sugars are formed. The results are shown in Table 2.

Table 2. Hydrolysis of RB.

Time, min	Temperature, °C	Active acidity (pH)	Titratable acidity, °T	Total sugar, %
0	20±2	6,69±0,37	4,0±1,5	1,1±0,1
10	40±2	6,56±0,35	4,1±1,6	3,8±0,7
20	40±2	6,71±0,34	4,1±1,6	4,3±1,2
30	40±2	6,73±0,34	4,4±1,6	4,7±1,3
40	40±2	6,48±0,32	5,1±1,7	4,8±1,3
50	40±2	6,54±0,33	5,2±1,6	5,3±1,4
60	95±2	6,55±0,34	5,9±1,6	8,6±1,4

The hydrolysis process proceeded stepwise, with the formation of intermediate dextrins. In this case, the purpose of hydrolysis was not the complete decomposition of starch to the simplest sugars (maltose and glucose). The task was to correct the taste and facilitate the adaptability of the LAB to the substrate at the initial stage of fermentation. To inactivate the enzyme, the mixture was heated to 95 ° C and kept for about 5 minutes. The amount of sugars did not change in the future. As a result of hydrolysis, a pronounced sweet taste appeared in the sample.

At this preliminary stage, one of the classic LAB cultures, *Lactobacillus delbrueckii subsp. bulgaricus*, was used to study the fermentation process of RB. As a result of fermentation, the titrated acidity increased to 25 degrees. (pH – 4.90). The consistency of the product has changed. It has become more viscous, close to the consistency of traditional low-fat fermented milk products.

Thus, as a result of hydrolysis, the organoleptic properties of RB were corrected, the possibility of the development of LAB on a rice substrate was shown.

3) *Obtaining the FRB and its analysis*. After cooling the mixture to the fermentation temperature (38 ± 2 ° C), starter cultures were introduced in an amount of 5% by weight of the mixture. They were kept for fermentation for 12 hours at a temperature of 38-40 °C, after

which the samples were thermostated at a temperature of 4-6 ° C for 24 hours to form a clot. Preliminary studies have shown that, although the consistency has become more viscous, we consider it insufficient for the formation of a stable structure. To stabilize the FRB and correct the texture, apple pectin was used as a thickener, as one of the common stabilizers of food products [9-12]. It was added after fermentation (in the amount of 1% and 2% by weight of the mixture). After the introduction and distribution of pectin in volume, exposure and stabilization of the FRB, an analysis was carried out according to physico-chemical, organoleptic and rheological parameters.

The results of the physico-chemical analysis of FRB samples (Table. 3) showed that the introduction of pectin contributed to a decrease in the pH of the medium. Before the introduction of pectin, the pH of the mixture was in the range from 7.33 to 6.69 units. After applying pectin – from 4.40 to 4.00 units. The dry matter content also increased as expected.

Table 3. Physico-chemical indicators of FRB.

Sample	Dry matter, %	Titratable acidity, °T	Active acidity (pH)
Streptococcus thermophilus starter culture			
Control	20,87±0,59	22±1	5,16±0,23
Experience-1	21,27±0,66	43±1	4,40±0,24
Experience-2	21,83±0,61	63±1	4,06±0,21
Lactobacillus delbrueckii subsp. bulgaricus starter culture			
Control	20,69±0,51	23±1	5,31±0,24
Experience-1	21,12±0,57	31±1	4,40±0,23
Experience-2	21,79±0,60	50±1	4,02±0,23
Lactobacillus acidophilus starter culture			
Control	19,83±0,76	29±1	4,70±0,24
Experience-1	20,48±0,88	41±1	4,31±0,22
Experience-2	21,11±0,89	58±1	4,00±0,21
Complex bacterial starter culture "Kefir"			
Control	20,86±0,59	20±1	4,94±0,23
Experience-1	21,46±0,67	31±1	4,41±0,23
Experience-2	22,00±0,62	45±1	4,01±0,23
Complex bacterial starter culture " Symbilact "			
Control	20,70±0,76	23±1	5,12±0,27
Experience-1	21,34±0,82	37±1	4,41±0,22
Experience-2	21,79±0,80	55±1	4,08±0,27

It should be noted that the highest values of titrated acidity were recorded when using *Lactobacillus acidophilus* (one of the strongest acid-forming agents), as well as the complex starter culture "Symbilact" (probably due to the large species diversity of the LAB in the composition) in the samples of Experiment-2.

Sensory analysis (Table.4) showed a colour change in all samples. This is due to the addition of pectin, which has a pronounced cream tint. It should also be noted the great influence of the starter culture on the consistency of the samples. So, the samples on the sourdough "Kefir" had the most liquid consistency characteristic of kefir. The samples on the "Symbilact" starter culture had a more homogeneous and viscous consistency, which is due to the presence of *ssp. cremoris in Lactococcus lactis*. These samples received the highest organoleptic rating.

Table 4. Sensory indicatorsof FOB samples.

Sample	Consistency	Colour	Taste	Smell
Streptococcus thermophilus starter culture				
Control	Homogeneous, liquid	Milky white	Sweet, with an acidic aftertaste	Fermented milk
Experience-1	Homogeneous viscous without delamination	Light cream	Pronounced with sourness	Fermented milk
Experience-2	Homogeneous, thick with a velvety consistency	Light cream	Harmonious, with a pronounced sourness	Fermented milk, expressed
Lactobacillus delbrueckii subsp. bulgaricus starter culture				
Control	Homogeneous, weakly expressed	Milky white	Neutral, with a slight sourness	Fermented milk, weak
Experience-1	Homogeneous viscous, without delamination	Light cream	Sweet and sour, weak	Fermented milk, weak
Experience-2	Homogeneous viscous, without delamination	Light cream	Sweet and sour, harmonious	Fermented milk, weak
Lactobacillus acidophilus starter culture				
Control	Homogeneous, liquid	Milky white	Sweet and sour	Fermented milk
Experience-1	Homogeneous, thick with a velvety consistency	Light cream	Harmonious, with a pronounced sourness	Fermented milk
Experience-2	Homogeneous, thick with a velvety consistency	Light cream	Harmonious, with a pronounced sourness	Fermented milk, expressed
Complex bacterial starter culture "Kefir"				
Control	Homogeneous, liquid	Milky white	Characteristic of kefir – spicy, sweet	Kefir, pronounced
Experience-1	Homogeneous, watery	Light cream	Characteristic of kefir – spicy, sweet	Kefir, pronounced
Experience-2	Homogeneous, slightly viscous	Light cream	Characteristic of kefir – spicy, sweet	Kefir, pronounced
Complex bacterial starter culture " Symbilact "				
Control	Homogeneous, slightly viscous	Milky white	Sweet, with a slight sourness	Fermented milk, weak
Experience-1	Homogeneous, viscous	Light cream	Pronounced with sourness	Fermented milk
Experience-2	Viscous, enveloping, thick	Light cream	Harmonious, with a pronounced sourness	Fermented milk, expressed

4 Conclusion.

In the work, some aspects of obtaining FRB from vegetable raw materials – rice were studied. The ratio of the solid and liquid phases of RB is theoretically justified, providing the necessary number of components for subsequent operations.

The necessity of hydrolysis of RB and its positive effect not only on the further development of lactic acid bacteria, but also on structural-viscous, physico-chemical and organoleptic parameters are shown. Thus, the acidity increases significantly, the sugar content increases, the viscosity of "rice milk" improves, as well as its taste. Thus, not only technological properties are formed, but also satisfactory consumer characteristics of the FRB.

The introduction of apple pectin in an amount from 1% to 2% significantly affects the properties of the FRB, primarily on the consistency and acidity and pH. Lowering the pH (4.4 – 4.0 units) is a positive moment for the development of lactic acid bacteria during the adaptation period. The results of the sensory evaluation also showed that the FRO samples with the addition of pectin had a better consistency, a more harmonious, rounded taste.

Thus, the example of the FRB shows the high potential and the prospect of using rice as a basis for new fermented milk products. These can be alternative drinking and thick yogurt-like products, dressing-type sauces, etc.

Further research will be continued and aimed at studying the mechanism of formation of the matrix of a "vegetable" clot during fermentation and maturation. It is necessary to study the rheological properties of the clot, as well as its durability during use, cooking and storage.

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