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INVESTIGATION OF PREBIOTIC PROPERTIES OF AQUEOUS-ALCOHOLIC EXTRACTS OF ROOTS OF CRAMBE KOTCHIANA AND BUNIAS ORIENTALIS

Abstract: *Nutrition is one of the fundamental factors of human health. Today the use of biologically active food additives, enrichment of products with nutraceuticals, consumption of target foods is justified due to the increasing effect of stress along with unbalanced diet and lifestyle. The main resource of nutrients is plant foods and substances derived from them. The use of additional plant components in the manufacture of food products contributes to increasing its biological and nutritional value. Non-traditional plant species have a high potential along with traditional crops. A lot of research is aimed at identifying the positive effects of bioactive substances of plants on the body and the possibility of using them to expand the range of useful food products. We investigated the prebiotic properties of the underground part of plants from the Brassicaceae*

family. In the course of the study we applied traditional microbiological methods of research. As the results of the experiments showed, the plant extracts promoted the growth of lactobacilli, and the prebiotic activity was not inferior to the common preparations - lactulose. The obtained data will be used for further research for the development of technology for obtaining products with a positive effect on the normoflora of the intestine and implying the inclusion of prebiotic substances in the formulation.
Key words: *fermented milk bacteria, plant extracts, prebiotic properties.*

Introduction

The realities of modern life, associated with widespread deterioration of environmental conditions, changes in eating habits, hypodynamia, lifestyle, constant stress are one of the causes of a wide range of different pathological conditions. Nutrition is one of the factors predetermining the quality of life, intellectual and physical working capacity of a person [1]. Prolonged violation of the balance of nutrients in the body leads to metabolic disorders and as a result affects its functional state. Therefore, nutritional correction is particularly important in the treatment and prevention of certain disorders and pathologies [2]. Enrichment of foods with vitamins, minerals, bioactive compounds is a widespread approach to improve their quality, nutritional and biological value. The inclusion of physiologically active substances in the composition of finished products or raw materials can change the characteristics of the product. There are many different components that are introduced in the production of flour and bakery products, confectionery products, sour dairy products, canned foods, sausages and beverages [3-4].

The inclusion of prebiotics in the formulation allows the product to be enriched with components that contribute to the normalisation of gut microflora, excretion of toxins, thereby manifesting an immunostimulating and detoxifying effect [5]. Prebiotics can include substances of different nature, which are actively digested by microorganisms inhabiting the human intestine. One of the sources of prebiotic substances is different parts of plants rich in soluble and insoluble dietary fibres and complex saccharides. The issue of research into the prebiotic properties of different species of traditional and non-traditional food plants does not lose its relevance [6]. According to our previous studies, plants from the Brassicaceae family have a significant potential as food raw materials and contain a sufficiently high level of soluble and insoluble dietary fibres along with the main nutrients [7-8], which makes them promising for development as a prebiotic additive for the production of milk and bakery products.

The aim of our research was to evaluate the prebiotic potential of roots of plants belonging to the Brassicaceae family – Crambe cotchiana and Bunias orientalis

Materials and methods

To evaluate the effect of plant raw materials on the growth of probiotic microorganisms, water-alcoholic extracts of roots of the plants under study (Crambe cotchiana and Bunias orientalis) were used. Commonly known prebiotic substance disaccharide – lactulose (Sigma production) was used as control groups.

Plant raw materials were collected in ecologically clean areas of East Kazakhstan, Arkharly tract. Roots were dried, dry raw material was stored in a cool dry place. To obtain the extract, dry roots were ground on a laboratory mill, dry raw material 1 g was poured into 50% aqueous ethanol solution, left in a dark place at room temperature for 20 hours for extraction of substances. After extraction, the liquid extract was filtered and dried in a rotary evaporator to dry residue. The dry extract was stored in a dark container. It was diluted with 50% aqueous ethanol solution before experimentation. The following concentrations 10mg/ml (1% solution), 15 mg/ml (1,5% solution), 20 mg/ml (2,0% solution), 2,5 mg/ml (2,5% solution) were used for the studies. Control preparations were dissolved in water and used in similar concentrations

Lactic acid bacteria culture test: *Lactococcus* lactis PP*, Limosilactobacillus fermentum* A15, *Lactobacillus acidophilus* IMV were used as objects to evaluate the effect of plant extracts on probiotic microorganisms. Lactic acid bacteria were cultured on MRS medium.

The activity of the extract was determined by the method of diffusion into agar from wells. For this purpose, $1x10^5$ CFU/ml of test microorganism suspension was added to the melted and cooled to 40°C nutrient medium for every 100 ml of medium. It was mixed thoroughly and poured into Petri dishes in the amount of 25 ml. After solidification, 10 mm diameter wells were prepared with a special block cutter.

Water-alcoholic extracts of roots were added to the wells in an amount of 0.3 ml (up to the edge of the well) in three repetitions. The dishes were cultured at 37°C for 48 hours. The diameter of the zones of growth suppression or stimulation of test cultures was measured.

To determine the optimal prebiotic concentration, solid media with the addition of different concentrations of lactulose and plant extracts as a carbon source were used. The cultivation time was 48 hours at 37.0±0.5°C.

The concentration of test bacteria after cultivation was determined by spectrophotometric method. Microorganisms were washed off the surface of nutrient agar with physiological sodium chloride solution. The concentration of microbes in the resulting suspension was determined by measuring the optical density of microbial suspensions at a wavelength of transmitted light of 620 nm [9-10].

Statistical processing of data**.** The obtained results were statistically processed using Microsoft Excel programme, calculating the arithmetic mean of the parameter and the mean square deviation. Taking into account the Fisher-Student criterion, the registered changes in the parameters were considered reliable at $p \leq 0.05$.

Results and discussion

The results of the study are presented in Table 1. As can be seen from the table, plants from the Brassicaceae family have an inhibitory effect on the growth of lactococci, while the introduction of plant extracts into the culture of lactic acid bacilli showed growth activation. It should be noted that the extract of Bunias orientalis had a slightly greater effect than that of Crambe cotchiana.

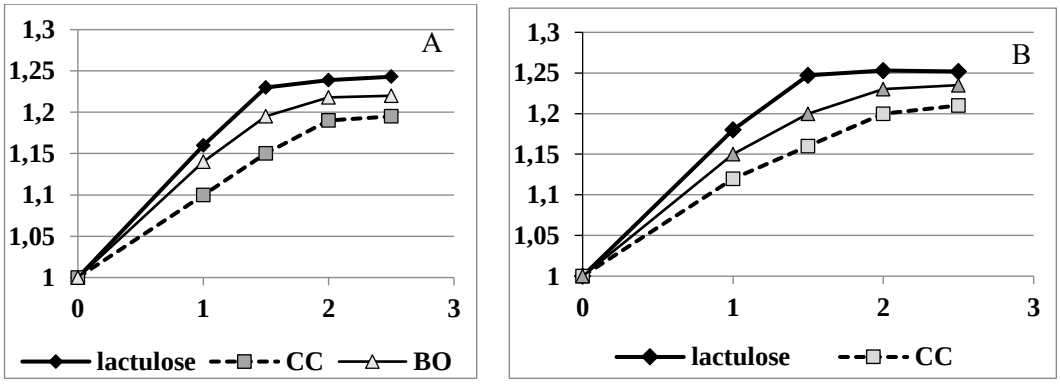
Table 1 – Effect of plant extracts on the growth of lactic acid bacteria

№	Variant / Test	Crambe cotchiana		Bunias orientalis	
		Suppression zone	Stimulation zone	Suppression zone	Stimulation zone
1	*Lactococcus lactis* PP	16±1	-	12±0,5	-
2	*Limosilactobacillus fermentum* A15	-	16±0,5		20±1
3	*Lactobacillus acidophilus* IMV	-	20±0,2		23±1

To determine the optimal concentration of the extract for the stimulating effect, the effect of their increasing concentrations on the growth of *Limosilactobacillus fermentum* and *Lactobacillus acidophilus* cultures was investigated. Lactulose solutions were used for comparison.

Lactic acid bacilli digest most carbohydrates: lactose, sucrose, glucose, salicin, galactose, maltose, dextrin, raffinose. Lactulose is a structural isomer of lactose, so it is absorbed by microorganisms along with other disaccharides as a source of energy and carbon when added to the medium [11-12]

The study on the prebiotic properties of Crambe cotchiana and Bunias orientalis root extract is presented in Figure 1.



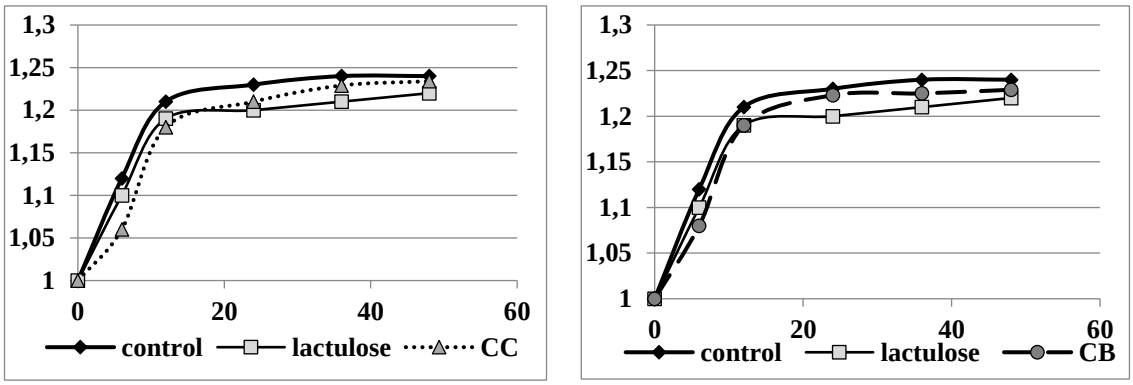
Abscissa axis: optical density of the suspension, ordinate axis: concentration of extract and disaccharide, %

Figure 1 – Study of optimal extract concentration for culture of Limosilactobacillus fermentum A15 (A), Lactobacillus acidophilus IMV (B)

As can be seen from the figure, when lactulose and extracts were introduced into the nutrient medium for cultivation of test cultures of fermented milk bacteria in increasing concentrations, significant growth of bacterial cultures was observed at the concentration of lactulose equal to 1,5%, whereas the optimal concentration for plant extracts was 2%. Increasing the concentration of the above mentioned ones did not lead to intensification of bacterial growth. In comparison, it can be said that the stimulatory activity of the extracts of Bunias orientalis and Crambe cotchiana was much inferior to that of lactulose. However, when comparing the extracts, it was found that the extract of Bunias orientalis roots had a higher stimulating effect than that another one extract.

As shown in the study, 2% concentrations of the extracts of Crambe cotchiana and Bunias orientalis were optimal for growth stimulation for both test cultures, whereas for lactulose this figure was much lower at 1,5%

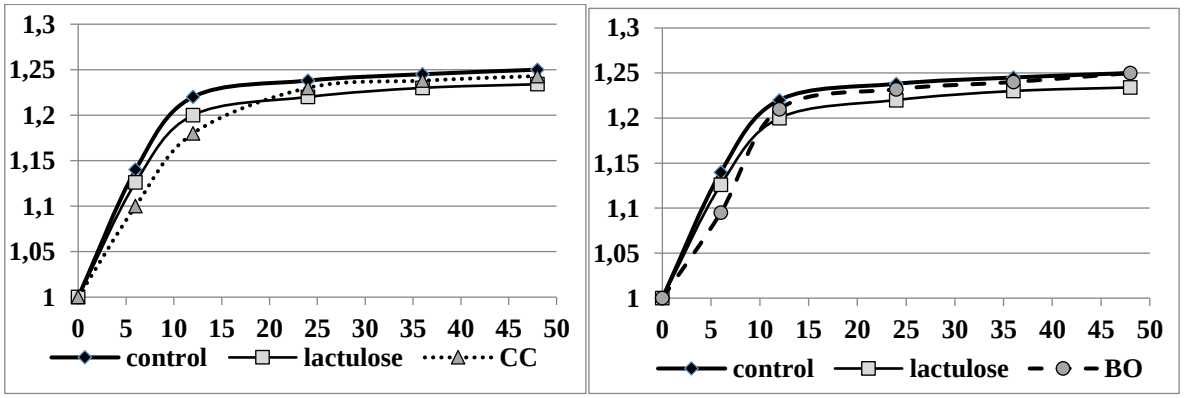
For further studies of lactic acid bacteria growth dynamics, the above-mentioned concentrations of the tested substances were used. The intensity of biomass accumulation was measured at time intervals of 6, 12, 24, 36 and 48 hours. The results of the study are presented in Figures 2-3. Standard MRS medium was used as a control.



Abscissa axis: optical density of suspension, ordinate axis: cultivation time, hours
(CC – Crambe cotchiana, BO – Bunias orientalis)

Figure 2 – Study of growth dynamics of *Limosilactobacillus fermentum A15* culture

As can be seen from Figure 3, in all cultures L.fermentum reached stationary phase after 12 hours of cultivation, but the biomass accumulation was slightly different. The comparison revealed that the test cultures assimilated lactulose less intensively compared to the control. Analysis of growth dynamics on media with plant extracts showed that after culturing for 6 hours, the growth rate of the culture was lower almost 2-fold when Crambe cotchiana extract was added to the medium, and 1.5-fold when the extract was added to the medium. After 12 hours of cultivation, biomass accumulation increased and became almost at the same level with samples cultivated on media with added lactulose. After 24 hours, the level of microbial biomass accumulation was almost identical to the control.



Abscissa axis: optical density of suspension, ordinate axis: cultivation time, hours
(CC – Crambe cotchiana, BO – Bunias orientalis)

Figure 3 – Study of growth dynamics of *Lactobacillus acidophilus IMV* culture

Practically similar tendency is observed in the test culture of L. acidophilus which can be seen from Figure 4. When the Crambe cotchiana extract was introduced, the log phase was molted for 24 hours. For both tested extracts in the initial phase the biomass growth was lower by 1,5 times than in the control and in the media with lactulose.

In the media with the inclusion of Bunias orientalis extract after 12 hours of cultivation, the biomass index for Crambe cotchiana extracts after 24 hours of cultivation was almost similar to the control values. Most likely, the low growth at the initial stages is due to the fact that the extracts contain a mixture of saccharides, of which the readily available forms are consumed first, with adaptation to other types of carbohydrates. We attribute the rather high rates of biomass accumulation to the complex composition of the extracts, which, in addition to sugars, includes other bioactive substances, dietary fibers and oligosaccharides, which favourably affect the growth intensity of lactobacilli.

Conclusion

Plants are a source of various nutrients and bioactive substances with a high potential impact on the human body. The use of bioactive components of plants in the development of functional products is a modern trend in the field of healthy nutrition and dietary enrichment [13]. There are about 14,000 plant species in Kazakhstan, 111 species of which are potentially suitable as a food resource [14]. At the moment, the issue of careful development of local resources of food raw materials is quite acute. Crambe cotchiana and Bunias orientalis plants are poorly studied and have great potential for further research and practical applications. Their biological and nutritional value has been shown [7-8], our studies were aimed at evaluating the prebiotic properties of extracts of the named species. Thus, as our studies have shown, root extracts of the studied plants had an inhibitory effect on lactococci cultures and are able to stimulate the growth of lactic acid bacilli. The study of the optimal concentration for cultivation and growth dynamics when introduced into culture will be a scientific basis for further development of technology of application of the investigated species of non-traditional raw materials in order to obtain new products of target purpose.

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КОЧИ КАТРАНЫ ЖӘНЕ ШЫҒЫС МАЙРАКЕБІСІНЕН АЛЫНҒАН СУЛЫ-СПИРТТІ СЫҒЫНДЫЛАРЫНЫҢ ПРЕБИОТИКАЛЫҚ ҚАСИЕТТЕРІН ЗЕРТТЕУ

Тамақтану – адам денсаулығын құраушы негізгі факторларының бірі. Бүгінгі таңда биологиялық белсенді қоспаларды тағамға қосымша қолдану, тағамдарды нутрицевтиктермен байыту, функционалды тағамдарды тұтыну қажеттілігі теңгерімсіз тамақтану және заманауи өмір салтымен қатар стресстің жоғарылауына байланысты туындап отыр. Қоректік заттардың негізгі ресурсы- өсімдіктекті тағамдар және олардан алынған қосылыстар болып келеді. Азық-түлік өндірісінде қосымша өсімдік компоненттерін қолдану оның биологиялық және тағамдық құндылығын арттыруға көмектеседі. Дәстүрлі емес өсімдік түрлері дәстүрлі дақылдармен қатар жоғары әлеуетке ие. Көптеген зерттеулер өсімдіктердің биоактивті заттарының ағзаға оң әсерін және оларды пайдалы тағамдардың ассортиментін кеңейту үшін пайдалану мүмкіндігін анықтауға бағытталған. Атаулы мақалада қырыққабат тұқымдасына жататын өсімдіктердің жер асты бөлігінің пребиотикалық қасиеттерін зерттеу жұмыстарының нәтижесі келтірілген. Тәжірибе барысында дәстүрлі микробиологиялық зерттеу әдістері қолданылды. Жүргізілген эксперименттердің нәтижелері көрсеткендей, өсімдік сығындылары лактобактериялардың өсуіне оңынан ықпал етті, ал пребиотикалық белсенділігі бойынша олар жалпыға бірдей танымал препарат – лактулозадан кем түспеді. Алынған нәтижелер ішек нормофлорасына оң әсер ететін және рецептураға пребиотикалық заттарды қосуды көздейтін өнімдерді алу технологиясын әзірлеу үшін ғылыми негіз ретінде пайдаланылады.

Түйін сөздер: *сүтқышқылды бактериялар, өсімдік сығындылары, пребиотикалық қасиеттер.*

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ИССЛЕДОВАНИЕ ПРЕБИОТИЧЕСКИХ СВОЙСТВ ВОДНО-СПИРТОВЫХ ЭКСТРАКТОВ КОРНЕЙ КАТРАНА КОЧИ И СВЕРБИГИ ВОСТОЧНОЙ

Питание – один из основополагающих факторов здоровья человека. Сегодня применение биологически активных добавок к пище, обогащение продуктов нутрицевтиками, потребление

целевых продуктов питания оправдано в связи с возрастающим действием стресса наряду с несбалансированным питанием и образом жизни. Основным ресурсом питательных веществ является растительная пища и вещества полученные из них. Применение дополнительных растительных компонентов при изготовлении пищевых продуктов способствует повышению его биологической и пищевой ценности. Нетрадиционные виды растений обладают высоким потенциалом наряду с традиционными культурами. Множество исследований направлено на выявление положительных эффектов биоактивных веществ растений на организм и возможности их использования для расширения ассортимента полезных пищевых продуктов. Нами исследовано, пребиотические свойства подземной части растений из семейства Капустные. В ходе исследования применяли традиционные микробиологические методы исследования. Как показали результаты проведенных экспериментов, экстракты растений способствовали росту лактобацилл, и по пребиотической активности не уступали общеизветсному препарату – лактулозе. Полученные данные будут использованы для дальнейших исследований для разработки технологии получения продуктов обладающих положительным эффектом на нормофлору кишечника и подразумевающих включение в рецептуру пребиотических веществ.

Ключевые слова: *кисломолочные бактерии, экстракты растений, пребиотические свойства.*

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