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IMPACT OF BURNET (*Sanguisorba officinalis* EXTRACTS FOR a *Lactobacillus* GROWTH

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Abstract. One of the approaches for the ensuring of an effective quantitative and qualitative composition with biochemical activity of normal microflora, i t is recommended to use for food fortification by rod-shaped bacillus milk – *Lactobacillus.* Nevertheless, probiotic bacteria are sensitive for oxygen that in fermented milk products has an inhibitory effect. This problem is often solved by using the antioxidants. Biologically active substances of medicinal plants have a positive effect on the growth parameters of *Lactobacillus*. The unique property of burnet (*Sanguisorba officinalis*) is the ability to accumulate phenolic compounds and other biologically active substances that mainly available in rhizomes and roots. Application of microwave radiation for the

burnet (*Sanguisorba officinalis*) extraction allows for the obtaining probiotic foods with natural antioxidants. The microwave extraction at 90W was more effective than 180W and boiling in terms of extractives and melanin. The ratio - raw material per extractant particularly 2:10 has a total antioxidant activity for 15 % higher that compared to the extract obtained in the similar conditions with a ratio 0.8:10. Adding of burnet extract at a concentration of 10^{-3} g/cm^3 and 10^{-5} g/cm^3 actually stimulates *Lactobacillus* growth. The following growth rates μ were calculated: burnet extract 10^{-5} g/cm^3 = 0.60 h^{-1}, burnet extract 10^{-3} g/cm^3=0.63 h^{-1}, and control = 0.41 h^{-1} that confirms a stimulating effect of burnet extract for *L.acidophilus*. In this regard burnet extract at concentrations of 10^{-3} g/cm^3 and 10^{-5} g/cm^3 maybe recommended for the development of a functional fermented milk products.

Key-words: antioxidants, biologically active substances, burnet, extract, Lactobacillus, probiotic

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ҚАНДЫШӨП СЫҒЫНДЫЛАРЫНЫҢ (*Sanguisorba officinalis*) *Lactobacillus* ӨСУІНЕ ӘСЕРІ

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Аннотация. Қалыпты микрофлораның биохимиялық белсенділігінде тиімді сандық және сапалық құрамды қамтамасыз ету тәсілдерінің бірі ретінде тағамды сүт таяқша тәрізді бациллалармен – *Lactobacillus*-пен байыту ұсынылады. Дегенмен, пробиотикалық бактериялар оттегіге сезімтал, ол ашытылған сүт өнімдерінде ингибиторлық әсерге ие. Бұл мәселе көбінесе антиоксиданттармен шешіледі. Дәрілік өсімдіктердің биологиялық белсенді заттары *Lactobacillus* өсу қарқынына оң әсер етеді. Дәрілік қандышөптің (*Sanguisorba officinalis*) ерекше қасиеті-фенолдық қосылыстар мен басқа биологиялық белсенді заттарды жинақтау қабілеті, олар негізінен тамырлар мен түйнектерде болады. Қандышөп сығындысын алу үшін микротолқынды сәулелендіруді қолдану (*Sanguisorba officinalis*) табиғи антиоксиданттармен пробиотикалық өнімдер алуға мүмкіндік береді. Экстракция мен меланин үшін 90 Вт-та микротолқынды экстракция 180 Вт және қайнатуға қарағанда тиімдірек болды. Шикізат / экстрагент қатынасында атап айтқанда 2:10 жалпы антиоксиданттық белсенділік 0,8:10 қатынасында ұқсас жағдайларда алынған сығындыға қарағанда 15 % жоғары. Қандышөп сығындысын 10^{-3} г/см^3 және 10^{-5} г/см^3 концентрациясына енгізу іс жүзінде Lactobacillus өсуін ынталандырады. Келесі өсу қарқыны μ есептелді: қандышөп сығындысы 10^{-5} г/см^3 = 0,60 сағ$^{-1}$, қандышөп сығындысы 10^{-3} г / см^3 = 0,63 сағ$^{-1}$ және бақылау = 0,41 сағ$^{-1}$, бұл *L.acidophilus* үшін қандышөп сығындысының ынталандырушы әсерін растайды. Осыған байланысты функционалды ашытылған сүт өнімдерін жасау үшін 10^{-3} г/см^3 және 10^{-5} г/см^3 концентрациясындағы қандышөп сығындысы ұсынылуы мүмкін.

Түйін сөздер: антиоксиданттар, биоактивті заттар, қандышөп, сығынды, лактобактериялар, пробиотик

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ВЛИЯНИЕ ЭКСТРАКТОВ КРОВОХЛЕБКИ (*Sanguisorba officinalis*) НА РОСТ *Lactobacillus*

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Аннотация. В качестве одного из подходов для обеспечения эффективного количественного и качественного состава при биохимической активности нормальной микрофлоры рекомендуется использовать для обогащения продуктов питания палочковидными бациллами молока – *Lactobacillus*. Тем не менее, пробиотические бактерии чувствительны к кислороду, который в кисломолочных продуктах оказывает ингибирующее действие. Эту проблему часто решают с помощью антиоксидантов. Биологически активные вещества лекарственных растений положительно влияют на показатели роста *Lactobacillus*. Уникальным свойством кровохлебки лекарственной (*Sanguisorba officinalis*) является способность накапливать фенольные соединения и другие биологически активные вещества, которые в основном имеются в корневищах и корнях. Применение микроволнового излучения для экстракции кровохлебки (*Sanguisorba officinalis*) позволяет получать пробиотические продукты с природными антиоксидантами. Микроволновая экстракция при 90 Вт была более эффективной, чем 180 Вт и кипячение в отношении экстрактивных веществ и меланина. При соотношении сырье/экстрагент в частности 2:10 общая антиоксидантная активность на 15 % выше, чем у экстракта, полученного в аналогичных условиях при соотношении 0,8:10. Внесение экстракта кровохлебки в концентрации 10^{-3} г/см^3 и 10^{-5} г/см^3 фактически стимулирует рост *Lactobacillus*. Были рассчитаны следующие скорости роста μ: экстракт кровохлебки 10^{-5} г/см^3 = 0,60 ч$^{-1}$, экстракт кровохлебки 10^{-3} г/см^3 = 0,63 ч$^{-1}$ и контроль = 0,41 ч$^{-1}$, что подтверждает стимулирующее действие экстракта кровохлебки для *L.acidophilus*. В связи с этим экстракт кровохлебки в концентрациях 10^{-3} г/см^3 и 10^{-5} г/см^3 может быть рекомендован для разработки функциональных кисломолочных продуктов.

Ключевые слова: антиоксиданты, биологически активные вещества, кровохлебки, экстракт, лактобактерии, пробиотик

Introduction

Currently, probiotics are included in many biologically active substances (BAS) and are applied in the obtaining functional food products (Granato et al., 2010), prescriptions for improving the quality and maintaining human health. For example, in Europe, Japan, USA as such products breakfast cereals, muesli, ice cream, cheeses, various drinks and meat products are available. Japan is the only country that has legal indications and regulated requirements for foods that include probiotics under the «Foods for Specified Health Use» (FOSHU). According to the FOSHU system, scientific v erification is

required, waiting for the development of functional health products (Awaisheh, 2012). Such control of the functional characteristics of food products makes it possible to use their quality depending on specific conditions or human diseases that are associated with violations of the microbiocenosis of the large intestine (Utebaeva et al., 2018).

Lactobacillus is among the beneficial microbiota of the human body. They produce organic acids, mainly lactic acid, peroxide, antibiotic substances, bacteriocins — lactocins B, F, J, M, lactobrevin, plantaricin, etc., showing their antibacterial effect against representatives of pathogenic and opportunistic flora. *Lactobacillus* has high proteolytic, β-galactosidase, glycolase and lactate dehydrogenase activities and phenomena in the processes of digestion (Dobson et al., 2007).

The genus *Lactobacillus*, belonging to the family *Lactobacillaceae*, includes 56 species of microaerophilic, gram-positive rod-shaped cultures, facultative anaerobes. Typical species of *Lactobacillus* are *L.fermentum*, *L.plantarum*, *L.acidophilus*, *L.caseu*, *L.reuteri*, *L.ramnosum*, which are often included in probiotics, dietary supplements, and food products (Burmasova et al., 2017).

Bacteria *L. acidophilus* better perceive a decrease in the content of phosphorus, content, content in the human body, and have an increased attention to the production of vitamins Utebaeva et al., 2016 b). They have high anti-inflammatory and antitumor activity. It has been identified that *L.acidophilus* has an immunomodulatory property and can to modulate the expression of TLR2 and TLR4 in intestinal HT29 epithelial cells caused by *Salmonella enterica serovar Enteritidis* (SesE) (Moshiri et al., 2017).

It has been established that *L.acidophilus* breaks down cholesterol in serum lipids. They also fix cholesterolemia by blocking the enzyme hydroxymethylglutarate-CoA reductase, limiting the rate of cholesterol synthesis (Novokshonov and Sokolova, 2012).

L.acidophilus provides the accumulation of amino acids such as Valine, Glycine, Histidine that stimulate the development of the human intestinal microflora. The antagonistic properties of *L.acidophilus* bacillus are due to both the acid produced by the action and antibiotics — *L.acidophilus* and *L.lactocidi*n (Utebaeva et al., 2018).

Normal intestinal microflora forms a relatively stable ecological system of the human body. However, with age or appearance, the balance of this system may be disturbed. One of the approaches for the regularity an effective quantitative and qualitative composition with biochemical activity of normal microflora is recommended to use for food enriched by rod-shaped bacillus milk — *Lactobacillus* (Burmasova et al., 2017).

The purpose of the presented work is to study the effect of dried burnet roots extract from on the growth of *Lactobacillus* in order to develop functional food products based on them.

Plant extracts have a therapeutic effect that is due to the complex of biologically active substances contained in them. Substances contained in extracts can stimulate a growth of lactic acid microorganisms, being growth factors (Utebaeva et al., 2016 a).

Probiotic bacteria are sensitive for oxygen that in fermented milk products has an inhibitory effect on probiotic bacteria. This problem is often solved by using the antioxidants (Alibekov et al., 2018).

In biotechnology and the food industry, at least two dozen different compounds

related to the antioxidants are used, such as ionol, α-tocopherol, ascorbic acid, phenols or polyphenolic compounds (Alibekov et al., 2019; Shingisov et al., 2022). Flavonoids, such as quercetin, resveratrol, catechins, carotenoids, tannins, anthocyanins, coumarins, hydroxycinnamic acid derivatives, and others, are promising antioxidants for use (Shahidi et al., 2019).

Mostly, the action of antioxidants is indicated in ultra-low concentrations, 10^{-12}–10^{-13} M and below. This effect was established for antioxidants of various origins and different chemical nature: for synthetic and natural antioxidants, for flavonoids, phenols, alcohols, and oxypyridines. A number of authors believe that when a biologically active substance is introduced into the body in doses of 10^{-12} – 10^{-13} M, the cell will contain at least 1–10 molecules of this substance and ensure their action (Venturella et al., 2021).

It is known that biologically active substances of medicinal plants can have a positive effect on the growth parameters of *Lactobacillus* that is due to the multicomponent nature of food sources (Kokina et al., 2018).

The unique ability of burnet (*Sanguisorba officinalis*) is the ability to accumulate phenolic compounds in large quantities, which accumulate mainly in rhizomes and roots. The polyphenolic complex of burnet rhizomes and roots includes mainly hydrolysable tannins of the pyrogallic group — up to 23 %, ellagic and gallic acids, pyrogallol, catechin and gallocatechin. The rhizomes and roots of the burnet (*Sanguisorba officinalis*) is contain starch (up to 30 %), essential oil (1.8 %), saponins: sanguisorbin and pyoterin — up to 4 %, dyes, calcium oxalate salts, flavonoids derivatives of quercetin and kaempferol, hyperin, 3, 7-diramnoside kaempferol, 3-galactosido-7-glucoside catechins, chromones. Roots and rhizomes also contain steroids: β-sitosterol, β-D-glucoside of β-sitosterol, stigmasterol; carotenoids, vitamin C; macroelements (mg/g): potassium - 5.8, calcium - 23.1, magnesium - 2.9, iron - 0.4; trace elements (μg/g): manganese - 0.47, copper - 0.59, zinc - 1.02, cobalt - 0.04, chromium - 0.03, aluminum - 0.31, barium - 5.71, vanadium - 0.12, selenium - 1.39, nickel - 1.15, strontium - 6.14, lead - 0.06, iodine - 0.1, boron - 2.0. Roots and rhizomes are capable of concentrating zinc, nickel, selenium, and especially barium and strontium (Janovska et al., 2003).

It has been experimentally proven that the extract from the rhizomes and roots of the plant, when applied topically, has anti-inflammatory and vasoconstrictive properties. The tannins that make up the plant assist to improve digestion and normalize the work of the entire gastrointestinal tract, since they have anti-inflammatory, antimicrobial and astringent effects (Utebaeva et al. 2016b).

Extracts from the rhizomes and roots of burnet (*Sanguisorba officinalis*) have a pronounced antimicrobial activity, in particular against the bacteria Escherichia coli, Pseudomonas aeruginosa and Staphylococcus aureus. The pronounced bactericidal effect was noted in relation to various microbes of the dysentery and paratyphoid groups, a detrimental effect on Trichomonas, fungi of the genus Candida and Giardia. It has been established that the infusion of rhizomes and roots of burnet exhibits antiprotozoal activity, and the alcoholic extract exhibits antibacterial activity. The antiviral activity of burnet officinal is against the hepatitis B virus has also been reported (Zhang et al., 2012).

Materials and methods

For the obtaining a dry extract of burnet, raw burnet root was used (Producer "Zerde-Fito" LLP, Shymkent).

Lyophilized culture of microorganisms *Lactobacillus acidophilus* n.v. Ep 317/402, not less than $1x10^9$ CFU/g. TR RA16076867.186-2003.

Dry extract of burnet (*Sanguisorba officinalis*), obtained from burnet roots by microwave extraction according to the method (Sysoeva, 2011), was used in the study at concentrations of 10^{-10}, 10^{-5} and 10^{-3} g/cm^3.

The cultivation of *Lactobacillus* was carried out for 72 hours in a thermostat at the temperature of 37±1°C on a standard MRS medium (Man Rogosa Sharpe). The quantitative accounting of the grown microorganisms was carried out using the dilution method (GOST 10444.11).

The content of free phenols was determined on the "UNICO HV/VIS 2800" spectrophotometer at λ=500 nm (Poliudek-Fabini and Beirih, 1981).

Calibration graph was drawn by using pyrocatechol solutions (from 1 to 10 μg/ml). The total antioxidant activity was determined by the phosphomolybdenum method by using an INFINITE M200PRO plate reader ("TECAN", Austria). The calibration graph was built within the concentration range of 0-0.1 mg/ml of ascorbic acid (Tusevski et al., 2014).

Statistical data processing was carried out using the "Statistica 6.0" software package. Data are presented as means and standard error of the mean value.

Results and discussion

Preparations of burnet officinal are effectively used in the treatment of diarrhea and eliminate its characteristic symptoms, as they normalize the motor function of the intestine, inhibit its peristalsis; normalize the work of the central nervous system; reduce inflammatory processes. It should be noted that burnet is used not only as a medicine, but also used in cooking. Mainly, in the Caucasus, burnet leaves are used, which have a pleasant cucumber smell and are part of some salads. The dried leaves are used as a condiment to make a delicious tea. At the end of the 19th century, in Siberia, the Yakuts added dried and crushed roots of the burnet officinal to flour, and such flour was used to prepare a dish called "Butugas" (Sibbesson, 2022).

During the extraction of plant raw materials, a mass of substances passes from one medium to another, that is, the transition of a substance from raw materials to an extractant. The process of transition of biologically active substances into the extractant is complicated by many factors, such as the high molecular weight of the substance or the charge of colloidal particles. The mass transfer of substances into the extractant will be affected by the size of the crushed raw material, the filling density of the raw material in the extractor, the nature of the extractant, the temperature and/or the duration of the extraction process (Ivashchuk et al., 2021).

Taking into account the above mentioned factors, two extraction methods were used for burnet extraction: boiling raw materials (control method) and microwave extraction.

The use of microwave extraction proved to be more effective than the traditional method, boiling within 60 min (Table 1). Microwave radiation made it possible to

intensify the extraction process and to obtain a higher yield of the product. Under the influence of microwave, a high speed and uniformity of heating of raw materials with an extractant and saving thermal energy are achieved. Microwave processing of the product makes it possible to increase the rate and degree of extraction and improve the quality of the extract, since many biologically active substances in the extracts are not destroyed and retain their properties (Guseinova et al., 2011).

For the process intensify, the finished extracts were infused using microwave treatment in the 180W mode in order to increase the diffusion of substances in solution. However, this factor did not greatly affect the increase in extractive substances, their yield increased only by 8 % (Table 1).

The degree and nature of grinding has an important influence on the extraction process. The smaller the particle sizes of the material, the larger the contact surface with the extractant, the faster the extraction. However, for each raw material, depending on the anatomical structure, composition and localization of active substances, the degree and nature is selected individually. In this case, grinding to d ≤ 2 mm did not give the proper result, and the yield of extractives from raw materials with finer particle sizes, less than 2 min, remained at the level of their yield from raw materials with particles of 2–7 mm during boiling and slightly changed during extraction by using microwave (Table 1).

As shown in Table 1, microwave extraction at 90W was more effective than 180W and boiling in terms of extractives and melanin.

Table 1.

The content of extractive substances in depends of various extraction methods

Sample	Extraction conditions		Grinding, mm	Dry residue, g/ml	Ash content, g/ml
	Microwave mode, °C	Boiling, min			
1	-	60	≤2	0,067±0,004	0,0018±0,001
			2-7	0,068±0,003	0,0019±0,002
2	180W, 2 min	-	≤2	0,059±0,001	0,0016±0,002
			2-7	0,063±0,001	0,0018±0,002
3	90W, 9 min	-	≤2	0,075±0,001	0,0020±0,001
			2-7	0,080±0,001	0,0021±0,001

The difference in the concentrations of substances in the raw material and the extractant is the driving force of the extraction process. During extraction, it is necessary to strive for the maximum difference in concentrations that was achieved by changing the ratio - raw materials: extractant. At the selecting this extraction parameter, the ratio of 2:10 turned out to be the most effective. The yield of extractive substances in this case increased by 2 times compared with the use of the ratio - raw materials: extractant 0.8:10 and almost three times compared with the control.

Table 2.

Dependence of extractive substances on the extract concentration

Sample	Concentration, g/ml	AOA, mg.eq. ascorbic acid/ml	Dry extract, g
1	8g/100	1,76±0,13	0,0735±0,003
2	20g/100	2,04±0,29	0,192±0,004
3	25g/100	-	0,135±0,001

Burnet extract with a high content of extractives obtained by using microwave extraction in the 90W mode and the ratio — raw materials: extractant 2:10 has AOA (antioxidant activity) 15 % higher compared to the extract obtained under similar conditions with a ratio - raw materials: extractant 0.8:10.

Requirements for the quality indicators for burnet dry extract have been developed for the purpose of its introduction into production, for the production of dietary supplements and functional foods. On the basis of the studies of the physicochemical properties of the burnet extract, the fundamental indicators that characterize it, in terms of suitability for use in fermented milk products technologies and determine its high quality, are selected: the mass fraction of moisture, the amount of free phenolic substances, and the total antioxidant activity. The requirements for quality indicators of burnet extract are shown in Table 3.

Table 3.

Quality requirements for burnet dry extract

Object	Definition	Indicator name	Unit. measurements	Value
Dried burnet extract	Extract from burnet roots	Mass fraction of moisture, not more than	%	8,00
		The amount of free phenolic substances, not less than	mcg/ml	18,00
		Total antioxidant activity, not less than	mg equivalent of ascorbic acid/g of extract	0,2

It was found that the introduction of burnet (*Sanguisorba officinalis*) extract at a concentration of 10^{-3} g/cm^3 and 10^{-5} g/cm^3 activates the growth of *L.acidophilus bacillus* compared with the control (Fig.1).

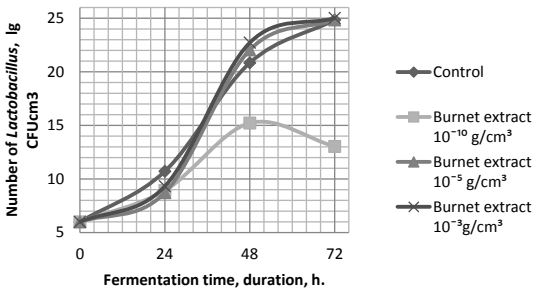

Fig. 1. Growth rates of *L.acidophilus* with burnet extract concentration

An analysis of the growth curves of *Lactobacillus* shown in Figure 1 showed that the introduction of burnet extract at a concentration of 10^{-3} g/cm^3 and 10^{-5} g/cm^3 actually stimulates their growth. The growth rates μ of these objects of study were calculated: burnet extract 10^{-5} g/cm^3 = 0.60 h^{-1}, burnet extract 10^{-3} g/cm^3=0.63 h^{-1}, and control = 0.41 h^{-1}. This confirms the stimulating effect of burnet extract at concentrations of 10^{-3} g/cm^3 and 10^{-5} g/cm^3. Thus, during the research, a positive effect of burnet extract on *L.acidophilus* was established.

Conclusion

Application of microwave radiation for the burnet (*Sanguisorba officinalis*) extraction allows for the obtaining probiotic foods with natural antioxidants. The microwave extraction at 90W was more effective than 180W and boiling in terms of extractives and melanin. The ratio - raw material per extractant particularly 2:10 has a total antioxidant activity for 15 % higher that compared to the extract obtained in the similar conditions with a ratio 0.8:10. Adding of burnet (*Sanguisorba officinalis*) extract at a concentration of 10^{-3} g/cm^3 and 10^{-5} g/cm^3 actually stimulates *Lactobacillus* growth. The following growth rates μ were calculated: burnet extract 10^{-5} g/cm^3 = 0.60 h^{-1}, burnet extract 10^{-3} g/cm^3=0.63 h^{-1}, and control = 0.41 h^{-1} that confirms a stimulating effect of burnet extract for *L.acidophilus*. In this regard burnet extract at concentrations of 10^{-3} g/cm^3 and 10^{-5} g/cm^3 maybe recommended for the development of a functional fermented milk products.

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