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# DEVELOPMENT OF A PROBİOTİC GLUTEN-FREE KVASS USİNG BUCKWHEAT MALT EXTRACT

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**Abstract.**. Non-alcoholic beverages constitute one of the key groups of functional food products**.** When developing a technology, the scientific justification and selection of primary raw materials and functional ingredients are of paramount importance. Buckwheat is a valuable cereal crop that occupies an important place among agricultural crops. Buckwheat grain is cahracterised by a unique chemical composition and is considered as essential for a nutritionally balanced diet. Buckwheat proteins have high biological and nutritional value, containing albumin, globulin, and balanced and complete amino acids. Buckwheat has a high level of amino acids due to the presence of essential amino acids and the high biological value of its proteins, including tryptophan (2.16) and the limiting one, threonine (0.80). It is close to unity in other amino acids, surpassing barley, rice, corn and wheat in isoleucine, lysine, methionine, cystine. At the same time, the presence of allergens in buckwheat should be taken into account, and sprouts should be kept under control when growing its seeds. The amylolytic activity of freshly germinated buckwheat malt is 1.7 times lower than that of barley and amounts to 264 (w–k) units. The proteolytic activity was slightly higher compared to freshly germinated barley malt. The proteolytic activity of barley and buckwheat malt enzymes was reached its maximum on the 6$^{th}$ day of germination and amounted to 28 units/g in both. The amylolytic activity also reached its maximum on the sixth day of germination, amounting to 440 W–K units in barley and 270 W–K units in buckwheat. A gluten-free probiotic kvass formulations have been developed on the basis of powdered buckwheat malt extract. The formulation incorporated lemon balm (Melissa officinalis) extract, bifidobacteria, and other components. The newly developed kvass is intended for consumers with gluten intolerance.

**Keywords:** barley, buckwheat, antioxidant, gluten-free, kvass

## Introduction. Formulation of the problem

Modern medicine pays great attention to the interaction between human health and its nutritional properties. Recently, food has been viewed not only as a source of energy and nutrition, but also as a means of determining the normal functioning of all body systems and the prevention of diseases. In this regard, expanding the range of special food products and creating a new generation of food products with preventive properties is an urgent task. Despite all this, in recent years, the health of the population has been characterized by negative trends: mortality from cardiovascular and oncological diseases is increasing, the problem of vitamin and micronutrient deficiency (iodine, iron, fluorine, selenium) and the increase in non-communicable diseases associated with this is acute.

Among the various food products currently consumed by the population, non-alcoholic beverages are of great interest from the point of view of creating a product with high nutritional value. These beverages can be considered as an optimal form of food products. Since they are used to enrich the diet of any person with essential nutrients, as well as biologically active substances, they have a beneficial effect on the functional state, metabolism and immune stability of the body. One of the technological ingredients for intensive technologies of drinks with approved functional properties are biologically active additives of plant origin. Extracts that can be used in the diet, depending on the balance of their functional components, including powdered malt and polymalt extracts obtained from various grain raw materials, are considered.

Soft drinks play the most technological basis for obtaining new functional types of products. Adding new functional ingredients to the drink is not a big problem, and thermal processing of the drink allows you to preserve the beneficial substances and vitamins in it. Medicinal plants with pronounced biologically active properties are used to develop the recipe for functional drinks. For example, molasses herb can be used as a sedative with antidepressant, spasmolytic,

immunomodulatory, antioxidant, antiviral, antiallergic, antimicrobial properties.

Lemon balm (Melissa officinalis), a perennial herb belonging to the Lamiaceae family, has been known for its health properties and healing functions for a century. It is part of the Mediterranean and some parts of Asia, and it has gone through cultivation and subsequent uses in traditional formulations by the indigenous communities for hundreds of years [1]. M. officinalis is a major medicinal herb, usually used as a potent anxiolytic, anticonvulsant, and sedative agent [2]. M. officinalis contains diverse bioactive compounds, such as phenolic acids, flavonoids, rosmarinic acid (RA), and essential oils (EO) [3], which are associated with antioxidant, antimicrobial, anti-inflammatory, neuroprotective, and antiviral properties [4,5].

Buckwheat is a valuable grain crop, occupying a significant place among agricultural crops. Buckwheat grain is unique due to its chemical composition and is considered a vital element of a complete diet [6]. Buckwheat has antioxidant, cardioprotective, anticancer, hepatoprotective, antihypertensive, antitumor, anti-inflammatory, antidiabetic, neuroprotective, and cholesterol-lowering properties [7,8,9].

The bioactive compounds in buckwheat are flavonoids (i.e., rutin, quercetin, orientin, isoorientin, vitexin, and isovitexin), fatty acids, polysaccharides, proteins, and amino acids, iminosugars, dietary fiber, fagopyrins, resistant starch, vitamins, and minerals. Buckwheat possesses high nutritional value due to these bioactive compounds [10,11]. Phytosterols, especially rutin, were detected in buckwheat. These compound exhibit antioxidant activity, while fagopyrins, microelements, and both soluble and insoluble dietary fibers are biologically active [12,13].

Buckwheat is considered a promising raw material in the production of beverages intended for dietary nutrition, especially for individuals whose diets exclude gluten from barley, wheat and rye [14].

## Analysis of recent research and publications

In order to increase the range of functional non-alcoholic beverages, it is of great interest to use polyunsaturated and non-traditional grain and bean sprouts, to pulverize them and prepare beverages based on them. There is considerable interest in expanding the range of functional non-alcoholic beverages through the use of polymalt and non-traditional cereal and legume sprouts, their processing into powdered forms, and the subsequent production of beverages on the basis of these materials.

Cereals and legumes are often used in beverage technologies [15]. It is important to balance the above-mentioned components, including wheat, barley, rye, corn and alternative raw materials such as buckwheat and peas [16, 17]. Buckwheat is a pseudocereal crop plant with high nutritional value that can be included as an alternate food in our diet [18]. Buckwheat is used as a gluten-free raw material [19, 20]. The article highlights the potential of buckwheat for the development of gluten-free products for people with celiac disease (1.4% of the world's population) and other conditions [21, 22].

To enable the use of the mentioned food crops in beverage technology, their malts are produced [23].

Phytosterols, especially rutin, were detected in buckwheat. These compound exhibit antioxidant activity, while fagopyrins, microelements, and both soluble and insoluble dietary fibers are biologically active [24,25].

**Purpose and objectives of the study**

The purpose of this study is to develop a recipe for functional kvass using buckwheat. To achieve this goal, the following objectives were set:

- to study the dependence of the amylolytic and proteolytic activity of barley and buckwheat malt on the malt maturation period;
- to study changes in the antioxidant content and moisture content of malt during soaking;
- to develop an optimal kvass recipe for functional purposes;

## Research materials and methods

The study employed barley, buckwheat, malts obtained there from, powdered buckwheat malt extract, medicinal lemon balm (Melissa officinalis) extract, dry purified bread culture, the lactic acid bacterium strain Lactobacillus plantarum EP-A3, and a pure technical dry bread culture of Bifidobacterium bifidum.

Comparative organoleptic and physicochemical parameters of barley and buckwheat were studied. Their characterization was carried out according to the following indicators: the amount of impurities in the composition, the natural form of the grain , the absolute mass of the grain, the mass fraction of moisture, starch content, germination capacity, extractability, acidity and hull content (Table 1).

**Table 1– Organoleptic and physicochemical parameters of grains from which malt will be grown**

| Parameters | Parameter values | |
|---|---|---|
| | Barley | Buckwheat |
| Colour | Light yellow | Greyish brown |
| Odour | Free from foreign odours | Free from foreign odours |
| Moisture, % | 6.8 | 8.0 |
| Impurities, % | 1.0 | 1.0 |
| In its natural form (as is), g/dm³ | 651.3 | 504.8 |
| Hull content, % | 1.0 | 24.1 |
| 1000 grain weight, g (dry matter) | 42.6 | 23.4 |
| Germination capacity, % | 98.7 | 93.0 |
| Starch content, % | 57.0 | 52.6 |
| Extract content, % (in dry matter) | 78.3 | 67.9 |
| Acidity degree | 2.5 | 2.5 |
| Protein content, % | 10.7 | 12.2 |

## Results of the research and their discussion

When optimal parameters (τ = 6 days, w = 44%, t = 15.60 C) were expected for buckwheat germination, the enzymatic activity of the amylase complex provided the technological parameters required for extract preparation.

Figure 1 and Figure 2 show the dependence of the amylolytic (AA) and proteolytic activities of barley and buckwheat malts on the malting period in the optimal regime. The amylolytic activity of freshly germinated buckwheat malt was 264 (w-k) units (1.7 times less than barley). The proteolytic activity was slightly higher compared with freshly germinated barley malt, which is related to the properties of the enzymatic complex and the physicochemical composition of the cereals.

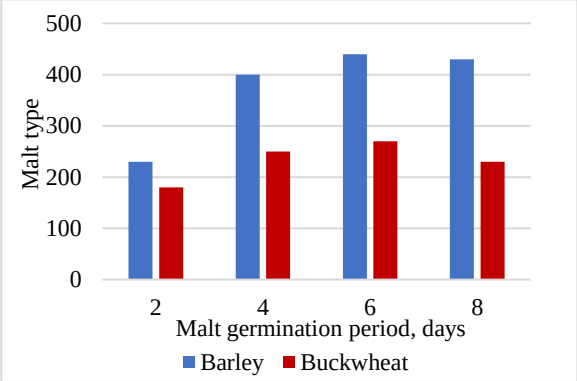


**Fig. 1. Changes in the amylolytic enzyme activity (WK units) of freshly germinated malt**

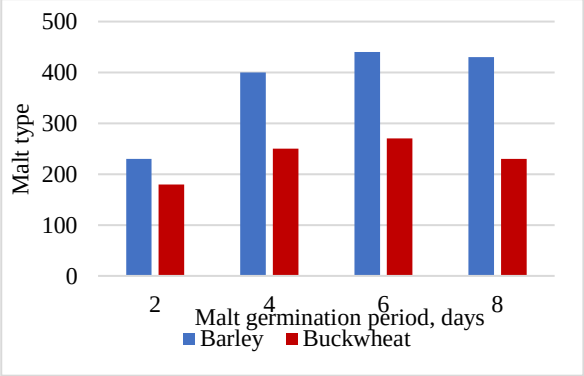


**Fig. 2. Changes in proteolytic enzyme activity (units/g) of freshly germinated malt**

The amylolytic and proteolytic activity of enzymes of freshly germinated malts increase depending on the germination period, with the proteolytic activity is reaching its maximum on the 6$^{th}$ day of germination, amounting to 28 units/g in both barley and buckwheat. Amylolytic activity reached its maximum on the 6$^{th}$ day of germination, amounting to 440 W-K units in barley and 270 W-K units in buckwheat.

Flavonoids have antioxidant protective properties and high biological activity. High antioxidant activity is considered one of the important factors in the preparation of functional products [11]. The changes in the amount of antioxidants during the cultivation process of barley and buckwheat malts were studied by using liquid chromatography (Table 2).

**Table 2 – Changes in antioxidant content and moisture content of malt during soaking**

| Malt type | Malt germination period, days | | | |
|---|---|---|---|---|
| | | | | |
| | 2 | 4 | 6 | 8 |
| Barley | 8.5/9 | 8.8/13 | 9.5/29 | 9.5/29 |
| Buckwheat | 9.0/20 | 5.7/27 | 10/30 | 10/30 |
| *NOTE: Antioxidant content (mg/100 g) are presented above the slash (/) and moisture content (%) are under the slash.* | | | | |

The amount of antioxidants in buckwheat is 2 times higher than in barley. The antioxidant content of the studied grains decreases sharply during soaking as a result of the intensification of oxidation processes and the influence of physical and biochemical factors for the nutrition of the embryo. The minimum amount of antioxidants correlates with the maximum soaking (42, 45%) for both grains. The amount of natural antioxidants in the grain is also reduced due to oxidation reactions with atmospheric oxygen. A continuous increase in antioxidants is observed starting from the second day of germination of the malt. On the sixth day of germination, the parameters had increased compared with the initial measuremnets by 46% for barley, 31% for buckwheat. This is related to the biochemical synthesis process of natural antioxidants in germinated grains.

The main quality criterion for malt in the preparation of powdered malt and polymalt is the presence of active enzymes, mainly amylase, in freshly germinated malt. This is proven by the amount of amylolytic activity. Malts made from different grains do not have the same amylolytic activity, which should be taken into account when choosing a rational malting regime (Table 3).

Beverages with functional properties, depending on their purpose, should contain oligosaccharides, mono- and disaccharides, amino acids, peptides, organic acids (lactic acid, acetic acid), minerals (microelements - zinc, iron, calcium, sodium, etc.), antioxidant enzymes, bioflavonoids, B-group vitamins (thiamine $B_1$, riboflavin $B_2$, choline $B_4$), vitamins C and E, and as well as probiotic bacteria. For this reason, the amount of components in powdered buckwheat malt extract was determined (Table 4).

The content of amino acids, vitamins, and macro- and microelements in powdered buckwheat malt extract was determined (Fig. 3, 4 and Table 5).

**Table 3 – The main physicochemical parameters of freshly germinated malts**

| Parameters | Barley malt | Buckwheat malt |
|---|---|---|
| Mass fraction of extract (calculated on dry matter of malt) | 80.1 | 67.5 |
| Mass fraction of protein (calculated on dry matter of malt) | 11.0 | 11.5 |
| Laboratory-prepared juice: colour, colour unit; acidity , acid degree; clarity (visual) | 0.18<br>0.68<br>Slightly cloudy | 0.33<br>0.41<br>Slightly cloudy |
| Saccharification time, min | 14.7 | Not saccharified |
| Amylolytic activity (WK) units | 442 | 264 |

A sample of kvass was prepared on the basis of the buckwheat malt extract. Experimentally prepared kvass samples complied with current standard requirements.

**Table 4 – Amount of components in powdered buckwheat malt extracts**

| Amount of nutrients in product of 100 g, 1/100g | Powdered buckwheat malt extract |
|---|---|
| Proteins | 11.73 |
| Carbohydrates | 79.1 |
| Tryptophan | 0.11 |
| Lysine | 0.38 |
| Methionine | 0.27 |
| Calcium | 0.900 |
| Magnesium | 0.270 |
| Phosphorus | 0.250 |

The kvass sample had a harmonious sweet-sour taste, a characteristic aroma of a fermented beverage, and light fruity notes. The physicochemical parameters of kvass samples are given in Table 6.

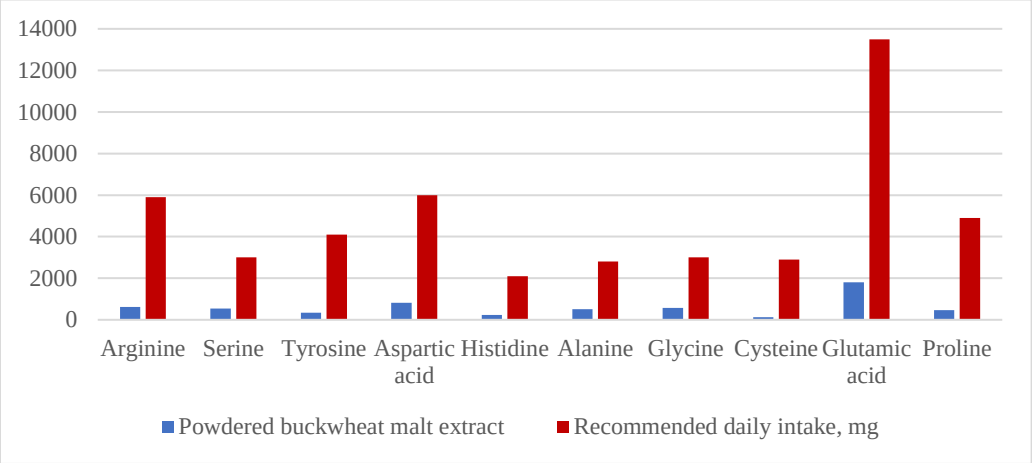


**Fig. 3. The amount of amino acids in powdered buckwheat malt extract and recommended daily intake, mg**

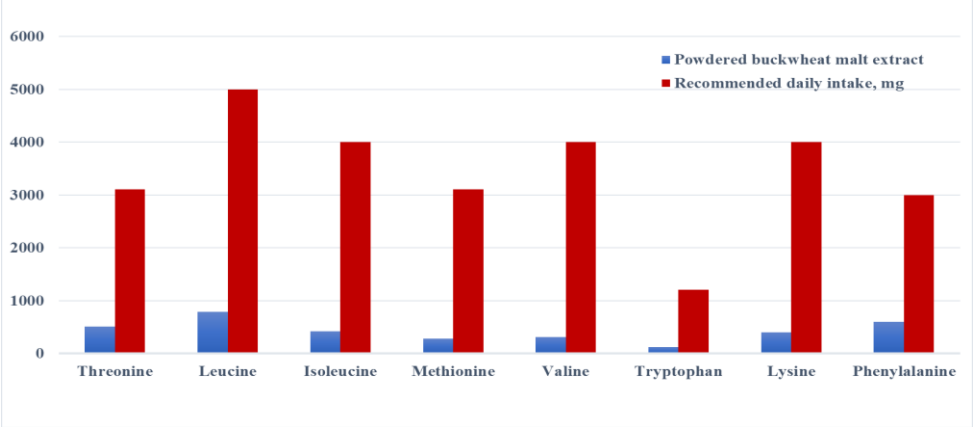


**Fig. 4. The amount of essential amino acids in powdered buckwheat malt extract and recommended daily intake, mg**

**Table 5 – The amount of functional components in powdered buckwheat malt extract and weighting factors for solving the design problem**

| Beneficial components (mg/100 g) | Amount of matters in the extracts, mg% | |
|---|---|---|
| | Powdered buckwheat malt extract | Recommended daily intake, mg |
| $B_1$(thiamine) | 0,92 | 1,5-2,0 |
| $B_2$(riboflavin) | 0,77 | 2,5-3,5 |
| $B_4$(choline) | 9,7 | 500-1000 |
| Amount of macroelements | | |
| Calcium | 905 | 800-1000 |
| Phosphorus | 255 | 800-1500 |
| Sodium | 244 | 4000-6000 |
| Potassium | 350 | 2000-4000 |
| Magnesium | 271 | 300-400 |
| Amount of Microelements | | |
| Zinc | 0,039 | 5-20 |
| Copper | 0,004 | 1-3 |
| Iron | 0,030 | 15 |

**Table 6 – Physicochemical parameters of kvass samples**

| Parameter | Parameter values | |
|---|---|---|
| | 100% kvass juice concentration (control) | 100% powdered buckwheat malt extract (experiment) |
| Mass fraction of dry matter, % | 6.03 | 6.03 |
| Acidity, c. unit | 2.68 | 2.98 |
| Volume fraction of alcohol, % | 0.51 | 0.51 |

A gluten-free kvass formulation has been developed (on the basis of powdered buckwheat malt extract). This is intended for a special group of consumers - consumers with gluten intolerance. Medicinal plants with pronounced biologically active properties are used to develop formulation for functional beverages. Melissa extract was used in the new kvass formulation. Melissa officinalis can be used as a sedative due to its antidepressant, antispasmodic, immunomodulatory, antioxidant, antiviral, antiallergic, and antimicrobial properties (Table 7).

**Table 7 – Raw material consumption for gluten-free kvass production (per 100 dal of kvass)**

| Raw material | Mass fraction of dry matters in raw material, % | Raw material consumption (as is), kg |
|---|---|---|
| Sugar | 99.91 | 49.89 |
| Powdered buckwheat malt extract | 96.83 | 20.87 |
| Medicinal lemon balm extract (Melissa officinalis), dm³ | - | 0.105 |
| Dry purified bread culture | 90.15 | 0.004 |
| Lyophilised biomass of lactic acid bacteria strain Lactobacillus plantarum EP-A3 | 90.07 | 0.003 |

It is also possible to use bifidobacteria in a gluten-free, probiotic kvass formulation (Table 8).

It should be noted that the inclusion of the therapeutic ingredient melissa (the extract has a calming effect) in the formulation has a biological effect. The presence of lactic acid bacteria and bifidobacteria determines the immunobiological properties of the beverage.

**Table 8 –Raw material consumption for gluten-free probiotic kvass production (per 100 dal of kvass)**

| Raw material | Mass fraction of dry matter in raw material, % | Raw material consumption (as is), kg |
|---|---|---|
| Sugar | 99.90 | 50.7 |
| Powdered buckwheat malt extract | 97.13 | 20.79 |
| Medicinal lemon balm extract (Melissa officinalis), dm³ | - | 0.105 |
| Pure technical dry bread culture Bifidobacterium bifidum, dm³ | - | 40.08 |

## Conclusion

The amylolytic activity of freshly germinated buckwheat malt was 1.7 times lower (264 (w-k) units) than that of barley malt. Its proteolytic activity was slightly higher than that of freshly germinated barley malt, which is related to the properties of the enzymatic complex and the physicochemical composition of the cereal.

The amount of antioxidants in buckwheat is 2 times higher than in barley. The minimum amount of antioxidants correlates with the maximum soaking (42, 45%) for both grains.

Beginning from the second day of malt germination, a continuous increase in antioxidants is observed. Considering that germination was at its beginning on the sixth day, the indicators increased as follows: 46% for barley, 31% for buckwheat. This is related to the biochemical synthesis process of natural antioxidants in germinated grains.

Powdered buckwheat malt can be considered as an element of gluten-free product formulation.

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