Effect of electrochemically activated aqueous solution on the yield of reducing agents during enzymatic hydrolysis of green buckwheat starch

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Abstract. The present study was conducted to investigate the effect of complex amylolytic preparations on green buckwheat starch in the environment of oxidized and reduced fractions of electrochemically activated solutions. The novelty of the work lies in the study of the effect of catholyte and anolyte obtained from tap water on the accumulation of reducing substances in the process of enzymatic hydrolysis of green buckwheat starch with the use of preparations AmyloLux ATS and Maltogenase 2X L. The practical significance of the work is related to the possibility of improving the technology of grain drinks or preparation of buckwheat malt in the production of gluten-free beer. It was found that enzymatic hydrolysis of green buckwheat starch with the use of complex amylolytic preparations proceeded most intensively when using the oxidized anodic fraction of electrochemically activated solutions. The level of reducing substances in the anolyte environment exceeded the analogous index in the samples based on water and catholyte by 1.2-1.3 times, already after 30 minutes and before the end of the process.

1 Introduction

Buckwheat grain is an important raw material source that increases the nutritional and biological value of food. "Green" buckwheat is obtained by sequential peeling of cereals, without exposing to heat and steam (hydrothermal treatment). Green buckwheat flour is produced from purified green buckwheat groats [1]. Heat treatment is required to increase the storage time of buckwheat [1], however, this process is accompanied by losses of vitamins and biologically active substances [2]. In general, the biochemical composition of green buckwheat is superior to that of heat-treated buckwheat.

The chemical composition of buckwheat is characterized by a high protein content, including all essential amino acids. Green buckwheat is rich in B vitamins, vitamins E and P [2]. A wide range of phenolic compounds, flavonoids (rutin, quercetin, orientin, vitexin,

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isovitexin, isoorientin) gives buckwheat antioxidant properties. Green buckwheat ranks third among many cereals in terms of the content of lignans, phytochemical phenolic compounds acting as phytohormones and phytoestrogens [3-5]. Sodium, magnesium, phosphorus, potassium, calcium, manganese, iron, copper, zinc and molybdenum are contained in quantities that make up significant proportions of the daily human need for these macro- and microelements [5-6].

The uniqueness of the carbohydrate composition of buckwheat, especially the embryo, is due to the content of hexatomic alcohol D-chiro-inositol and its derivatives, which have a proven ability to reduce blood glucose levels and activate insulin [3-4, 7-8]. The most representative mono- and disaccharides of buckwheat are glucose, fructose, arabinose and xylose, as well as sucrose and maltose [5]. Green buckwheat grain contains 5-11% soluble and insoluble dietary fibers, including cellulose, non-starch polysaccharides formed by glucuronic acid, mannose, arabinose, galactose, glucose [3-4, 8-9]. Approximately 33-33.5% of starch is in the form of resistant starch, which recommends buckwheat as a potential ingredient for the production of products with a low glycemic index [8-9]. The content of resistant starch is influenced by the production stages of autoclaving, cooking or boiling [8].

Buckwheat contains more starch than other pseudo-cereals, and the caloric content (343 cal/100 g) of buckwheat is similar to cereals and legumes [8]. The endosperm of buckwheat grain contains from 60 to 70% starch, the amount of which is directly proportional to the weight of the grains. In commercially available buckwheat seeds, the weight of a thousand grains varies from 17.6 to 25.9 g [8, 10]. Buckwheat starch granules are small in size, according to various data 2-6 microns or 3-10 microns, similar to tuberous and grain starch. The surface of the granules is smooth, the shape is polygonal or irregular in a compact package [8, 9]. The granules contain 24-25% amylose and 75-76% amylopectin, corresponding to the usual ratio of grain starch fractions [8, 10].

Due to the presence of hydrophilic high-molecular compounds (proteins, starch, dietary fibers) in the composition, green buckwheat has a high water-binding ability, the ability to swell, which makes it possible to regulate the structural, mechanical and rheological properties of the food systems being developed and the consumer properties of finished products [3].

Buckwheat grain of various degrees of processing serves as an independent food product, as well as raw materials for the production of protein isolates, resistant starch, bioflavonoid rutin [1]. Buckwheat is used in baking [2, 11], for malting in brewing [10], for the preparation of grain-based beverages [1, 12], a variety of functional foods, pharmaceuticals [1, 10, 13]. Buckwheat is an important component of gluten−free diets for people with gluten intolerance [10, 14]. In order to develop the technology of gluten-free thermophilic fermented starter culture for the further production of gluten-free bakery products, the saccharification of green buckwheat flour was carried out at a dosage of enzyme preparations: 0.18% glucavamarin and 0.36% alfalift by weight of the brewed flour [11]. Buckwheat is used to produce gluten-free malt and beer with high antioxidant activity. To ensure the high quality of beer made entirely from buckwheat malt, the mandatory addition of enzymes is required [10]. Non-steamed buckwheat is a promising ingredient for the production of grain-based herbal beverages (vegetable "alternative milk") with increased nutritional value and bioavailability of nutrients. The advantage over rice or corn grain used for this purpose is due to its rich chemical composition, including a high protein content with a balanced composition of essential amino acids [1]. The disadvantage of vegetable drinks based on grain is a bland specific taste and starch astringent taste, therefore, to modify the carbohydrate composition and improve the organoleptic properties of the drink, it is advisable to conduct directed biocatalysis of starch and other biopolymers using complex enzyme preparations [12]. Electrochemical reactions at the cathode and

anode transfer the corresponding fractions of the electrochemically activated solutions (ECAS) - catholyte and anolyte, to a metastable state with excess potential energy. The activated state of the aquatic environment is characterized by a change in its reactivity, the rate of chemical and biochemical processes with its participation. ECAS can affect the activity of enzymes by changing the ionization of the active center, the degree of stability of the tertiary structure of the protein, leading to a decrease in the activation energy and acceleration of the enzymatic reaction [15].

It is known that ECAS fractions are capable of intensifying biocatalytic processes in food technologies [15]. ECAS intensify the germination of seeds and grains of plants, including during hydroponic cultivation, due to the activation of redox, hydrolytic and other classes of enzymes involved in this process [16-18]. The treatment of green buckwheat grains during germination with slightly acidic electrolysis water (pH 5.83, ACC 20.3 mg / l) contributed to a more intensive accumulation of gamma-aminobutyric acid (GABA) and rutin in the seedlings.It is believed that the accumulation of GABA and rutin in sprouted buckwheat treated with ECAS is due to an increase in the activity of glutamic acid decarboxylase and phenylalanine monialiase, that is, the effect on the secondary metabolic pathway of phenylpropanoids [16].

It seems advisable to use ECAS as an aqueous environment for enzymatic hydrolysis of starch. ECAS can affect the activity of enzymes by changing the ionization of the active center, the degree of stability of the tertiary structure of the protein, leading to a decrease in the activation energy and acceleration of the enzymatic reaction. The mechanism of enzymatic hydrolysis of starch is associated with the direct participation of water molecules as a reagent [15]. Previously, it was experimentally confirmed that ECAS fractions themselves have an effect on starch hydrolysis. With a significant number of works devoted to the influence of ECAS on the course of biochemical processes in food technologies, the hydrolysis of green buckwheat starch in the ECAS environment is practically not represented. The present study was conducted in order to study the effect of complex amylolytic drugs on green buckwheat starch in an environment of oxidized and reduced fractions of electrochemically activated solutions. The novelty of the work is to study the effect of catholyte and anolyte obtained from tap water on the accumulation of reducing substances in the process of enzymatic hydrolysis of green buckwheat starch using Amylolux ATS and Maltogenase 2X L. The practical significance of the work is related to the possibility of improving the technology of grain drinks or the preparation of buckwheat malt in the production of gluten-free beer.

2 Objects and methods of research

2.1 Electrochemically activated solutions

ECAS was obtained from drinking tap water (pH 8.8±0.1, redox potential (RP) 44±0.5 mV) in an electroactivator "SUPER PLUS" (Russia) for 30 minutes. Untreated drinking tap water served as a control. Anolyte is a fraction of water electrochemically treated in the anode chamber of an electroactivator for 30 minutes: pH 4.0±0.1, RP 530±0.5 mV. Catholyte is a fraction of water electrochemically treated in the cathode chamber of an electroactivator for 30 minutes: pH 10.7±0.1, RP -203±0.5 mV. Captions should be typed in 9-point times. They should be centred above the tables and flush left beneath the figures.

2.2 Substrate

Buckwheat kernels (unpeeled) "Green" of the highest grade with a starch content of 61.8% (Mistal Trading LLC, Russia).

2.3 Enzyme preparations

Amylolux ATS (alpha-amylase thermostable) (Sibbiopharm Software LLC, Russia): amylolytic activity at 30 °C − 3000±200 units / ml, at 90 °C − 30,000±2000 units / ml. Optimal conditions of action: pH 4.0-7.0, temperature 80-90 °C. Operating conditions: 30-97 °C. The main enzyme α-amylase catalyzes the hydrolysis of α-1,4-glycoside bonds of starch, providing preparation of the substrate for the action of glucoamylase. The end products of the action of α-amylase on starch are low molecular weight soluble dextrins with a low content of mono- and disaccharides (glucose and maltose).

Maltogenase 2X L (maltogenic amylase) (Novozymes, Denmark). Maltogenase® 2X L is an exoactive α-amylase, the main product of the reaction is α-maltose. Maltogenase® 2X L also hydrolyzes the trisaccharide maltotriose to maltose and glucose. When used individually, it can produce up to 70% maltose. It is used to increase the dextrose equivalent in glucose production, create improved taste characteristics and increase the natural sweetness of the drink. The optimal reaction temperature is 70-80 °C.

2.4 Method of determination of reducing substances

The accumulation of reducing substances was controlled by the modified Bertrand-Schoorl method. The method is based on the ability of substances having a free−OH group to reduce copper ions to copper oxide in an alkaline environment during boiling and in the presence of ferrotic salt, which is then determined by iodometric titration. A sample with a volume of 0.5; 1.0 or 2.0 cm^3 (depending on the expected RS content) is quantitatively transferred to a conical flask per 100 cm^3, 9.5; 9.0 or 8.0 cm^3 of distilled water (up to a total volume of 10 cm^3) is added, respectively, and then 10 cm^3 of Fehling I solution (69.27 g sulfuric acid 5-aqueous copper $CuSO_4 \cdot 5H_2O$ in 1 dm3 of distilled water) and 10 cm^3 of Fehling II solution (346 g of ferrotic salt (potassium-sodium tartaric acid) and 100 g of caustic soda NaOH to 1 dm3 distilled water). The flask is boiled for 2 minutes, counting from the beginning of boiling, after which it is quickly cooled in a bowl with cold water. 15 cm^3 of 20% sulfuric acid H_2SO_4 is added to the cooled flask, then 0.5 g of potassium iodide KI and immediately after that the released iodine is titrated with 0.1 n. solution of 5-aqueous sodium thiosulfate $Na_2S_2O_3 \cdot 5H_20$. When the solution changes its mustard color to pale milky, 3 drops of starch solution are added to it and titrated until the blue staining disappears (milk coloring). In parallel, a control experiment is conducted in which 10 cm^3 of distilled water is taken instead of the test sample and then the same operations are performed as with the test sample (starting with the addition of Fehling solutions).

To calculate the number of RS, the value X is calculated:

$$X = A - B \tag{1}$$

A - the volume of 0.1 n. sodium thiosulfate solution used for titration of the control sample, cm^3; B - the volume of 0.1 n. sodium thiosulfate solution used for titration of the experimental sample, cm^3.

The amount of RS in % (Q) is calculated by the formula:

$$Q = X - K \tag{2}$$

K - the correction to the titer of thiosulfate.

The formula is given for a sample volume of 1 cm^3; for a sample volume of 2 cm^3, the result is divided by two, and for a sample volume of 0.5 cm^3, multiplied by two.

3 Results and Ddiscussion

Pre-collapsed grains of non-steamed buckwheat were ground to flour in a non-serial laboratory planetary mill PL-10 (Russia). The size of starch granules ranged from 6 to 90 microns. In order to preserve useful substances, the grain was not pretreated.

To assess the effect of ECAS fractions on the degree of starch hydrolysis, the enzyme preparations Amylolux ATS, Maltogenase 2X L were sequentially introduced into a suspension containing 15% crushed green buckwheat in control water, anolyte or catholyte and kept at a temperature of 60-65 °C for 2.5 hours. Then the suspension was filtered, the total content of reducing substances (RS) was determined in the filtrate the modified Bertrand-Shoorl method. Data on the accumulation of the RS are shown in Figure 1.

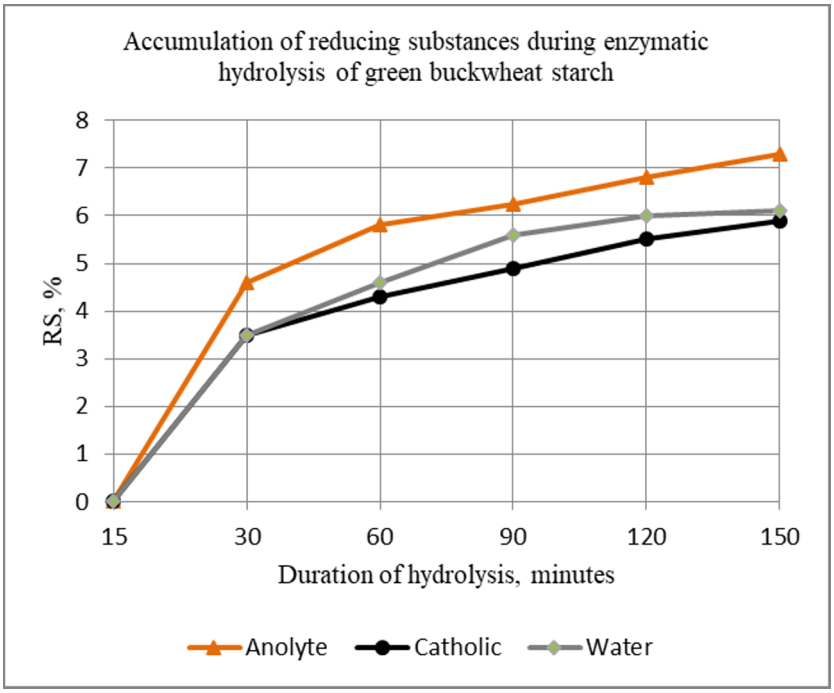

Fig. 1. The effect of ECAS on the accumulation of reducing substances during enzymatic hydrolysis of green buckwheat starch.

It can be seen from the figure that with similar kinetics and the nature of the curves, the intensity of RS accumulation is higher in the anolyte-based sample. After 30 minutes, the amount of RS was detected in the anolyte 1.3 times more than in water-based and catholyte-based samples (4.6% vs 3.6% and 3.6%, respectively). The gap persisted until the end of the process. It can be assumed that the main stimulating effect of the anolyte was manifested at the initial stage of hydrolysis. By the end of hydrolysis, the RS content in the anolyte (7.2%) was 1.2 times higher than the RS content in water-based suspensions (6.0%) and catholyte (6.0%).

4 Conclusion

Thus, under experimental conditions, the enzymatic hydrolysis of green buckwheat starch revealed the activating effect of the anolytic fraction of the ECAS. Earlier, a study [15] showed a threefold acceleration of the hydrolysis reaction of corn and rye starch in a mildly acidic anolyte obtained by RS of chloride or calcium acetate using the enzyme preparations Amylosubtilin and Glucalux. The authors explain the established effect by electron and proton transfer at the site of interaction of the active centers of the amylase enzyme with oxygen and carbon atoms at the C1 position involved in the formation of a chemical bond between glucose monomers. Presumably, the high concentration of active electrons in the anolyte contributes to a decrease in the energy of electronically excited states of the enzyme-substrate complex [15].

It was found that the enzymatic hydrolysis of green buckwheat starch using complex amylolytic prerarates Amylolux ATS and Maltogenase 2X L proceeded most intensively when using an oxidized anode fraction of ECAS. The level of reducing substances in the anolyte environment exceeded the same indicator in water- and catholyte-based samples by 1.2-1.3 times after 30 minutes and before the end of the process.

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References

1. G.M. Kerimova, I.A. Fomenko, A.R. Pivchenko, Development of technology for a grain drink based on non-steamed buckwheat. New technologies, NT, **19** (**2**), 57-71, (2023) https://doi.org/10.47370/2072-0920-2023-19-2-57-71
2. S. Gur'ev, V. Ivanova, V. Korovyansky, A. Novichenko, I. Kostin, Rheological properties of wheat dough with the addition of green buckwheat flour, E3S Web of Conferences, **420**, 01010 (2023) https://doi.org/10.1051/e3sconf/202342001010
3. L.E. Glagoleva, I.V. Korotkov, Investigation of the influence of process conditions and environment properties on the kinetics of the swelling process of green buckwheat flakes, Bulletin of VGUIT, **1**, 134-137 (2015)
4. L.E. Glagoleva, A.S. Gubin, A.V. Alexandrova, I.V. Korotkov, Determination of functional groups of vegetable complexes of green buckwheat by IR spectroscopy, Bulletin of the RAS, **4**, 57-60 (2017)
5. M. Nesovic, U. Gasic, T. Tosti, N. Horvacki, N. Nedic, M. Sredojevic, Z. Tesic Distribution of polyphenolic and sugar compounds in different buckwheat plant parts, RSC Advances, **11**(**42**), 25816–25829 (2021) doi:10.1039/d1ra04250e
6. M. Neacsu, S. De Lima Sampaio, H.E. Hayes, G.J. Duncan, N.J. Vaughan, W.R. Russell, V. Raikos, Nutritional Content, Phytochemical Profiling, and Physical Properties of Buckwheat (Fagopyrum esculentum) Seeds for Promotion of Dietary and FIBC **2**, 287–305 (2022) https://doi.org/10.3390/crops2030021
7. A.V. Mityaev, A.S. Fedyunina, E.E. Kurdyukov, M.I. Ulanova, Some representatives of the genus Fagopyrum: chemical composition, pharmacological effects, prospects of use, COPRM, **1**, 31-56 (2024) doi:10.14258/jcprm.20240112933
8. S.A. Sofi, N. Ahmed, A. Farooq, S. Rafiq, S.M. Zargar, F. Kamran, T. A. Dar, S.A. Mir, B.N. Dar, A. Mousavi Khaneghah, Nutritional and bioactive characteristics of

buckwheat, and its potential for developing gluten-free products: An updated overview, FSN, **11**, 2256–2276 (2023) https://doi.org/10.1002/fsn3.3166

9. J. Yang, Z. Gu, L. Zhu, L. Cheng, Z. Li, C. Li, Y. Hong, Buckwheat digestibility is affected by the chemical and structural features of its main components, FH, **96**, 596–603 (2019)
10. A. Dabija, M.E. Ciocan, A. Chetrariu, G.G. Codină, Buckwheat and Amaranth as RMfBR. Plants, **11**, 756 (2022) https://doi.org/10.3390/plants11060756
11. O.I. Parakhina, L.I. Kuznetsova, O.A. Savkina, T.A. Gavrilova, M.N. Makarchuk, M.A. Anuchina, Development of gluten-free thermophilic fermented starter culture technology. BoR, **2**, 27-31 (2022) doi: 10.37443/2073-3569-2022-1-2-27-32
12. I.Yu. Sergeeva, L.V. Permyakova, V.S. Rainik, O.A. Rybintseva, Investigation of the kinetics of enzymatic hydrolysis of plant polymers to increase the bioavailability of beverages from cereals, ACoR, **29**, **2**, 247-253 (2022) doi: 10.55934/2587-8824-2022-29-2-247-253
13. Z. Luthar, A. Golob, M. Germ, Tartary Buckwheat in Human Nutrition. Plants, **10**, **4**, 700 (2021)
14. M.A.N. Khairuddin, O. Lasekan, Gluten-Free Cereal Products and Beverages: A Review of Their Health Benefits in the Last Five Years. Foods, **10**, **11**, 2523 (2021)
15. V.V. Aksenov, A systematic approach to the intensification of the processes of bioconversion of native starches and starch-containing raw materials, Message II. Conducting bioconversion of native starches in electroactivated solutions, Bulletin of KrasGAU, **6**, 176-181 (2008)
16. J. Hao, T. Wu, H. Li, W. Wang, H. Liu, Dual effects of slightly acidic electrolyzed water (SAEW) treatment on the accumulation of γ-aminobutyric acid (GABA) and rutin in germinated buckwheat, FC, **201**, 87-93 (2016) doi: 10.1016/j.foodchem.2016.01.037
17. H.X. Wang, Y.Q. Zhang, H.T. Jiang, J.K. Cao, W.B. Jiang, A comprehensive review of effects of electrolyzed water and plasma-activated water on growth, chemical compositions, microbiological safety and postharvest quality of sprouts, TiFST, **129**, 449-462 (2022) doi:10.1016/j.tifs.2022.10.017
18. Y. Li, S. Liu, J. Hao, H. Rao, D. Zhao, X. Liu, Antioxidant Benefits and Potential Mechanisms of Slightly Acidic Electrolyzed Water Germination in Sesame, Foods, **12**, **22**, 4104 (2023) https://doi.org/10.3390/foods12224104