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OBTAINING OF RICE MALT WITH THE USE OF PLASMA-CHEMICALLY ACTIVATED AQUEOUS SOLUTIONS

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Abstract. Innovative technological solutions in the processing of rice and production of rice malt cause an increased interest of the specialists of brewery industry. Study of the intensifying effect of plasma-chemically activated aqueous solutions on the rice malting process is an object of attention of scientists and brewers. The paper shows the peculiar features of the production of rice malt, namely, the process of its humidification, with the use of plasma-chemically activated aqueous solutions with different concentrations of hydrogen peroxides. Studies related to the duration and temperature of soaking with solutions activated under action of non-equilibrium contact plasma are presented. Processing as above results in formation of hydrogen peroxide microparticles which, when in contact with the grain raw materials, are capable of producing active oxygen, a stimulating agent accelerating the transport of water into the middle of the rice grain and a number of biochemical transformations. These aspects allow activating the germination process significantly. Optimal hydrogen peroxide concentration in the solutions and temperature of rice soaking were determined (650 mg/l, at 25°C) and further used to obtain high-quality rice malt. It is proved that rice malt obtained with the use of plasma-chemically activated aqueous solutions features higher quality indicators compared to the control. Owing to the use of plasma-chemical solutions of the specified concentration, extractability of rice malt increased and reached 85.6%. Duration of rice malt saccharification decreased to 15 minutes, that is, more than three times. Acidity of wort produced from rice malt decreased to pH 5.2. The content of amino nitrogen was 48.8 mg/100 ml. This can be explained by the fact that activation of solutions promotes the acceleration of biochemical transformations and, as a result, accumulation of enzymatic systems. Deeper hydrolysis of starch and nitrogen-containing compounds occurs. It is proved that antiseptic properties of the activated aqueous solutions allow additional disinfection of the grain raw materials, and pathogenic contamination of malt is reduced. We carried out two-way analysis of variance without repetition for the process parameters obtained during our studies. The paper shows the malting process parameters, which can be used in the industrial processing of rice into malt, and gives technological recommendations for the use of plasma-chemically activated aqueous solutions in the process of rice malt production.

Keywords: rice, malt, germination, plasma-chemically activated aqueous solutions, enzymatic activity, two-way analysis of variance without repetition.

Introduction. Formulation of the problem

Production of malt from various grain crops, apart from classical barley, attracts a lot of attention from the experts in the fermentation industry all over the world. The reason is the need to find high-quality alternative to the imported barley for countries where growing of this crop is not possible or makes no economic sense.

Rice with high content of starch is widely used in the world today. It is a promising raw material for the fermentation industry of many countries, where no barley is grown. For example, rice and products of its processing have already become important components in the brewery industry. In the Asian countries, barley is grown in small quantities or not grown at all, while rice is the main crop. Thus, substitution for the imported and expensive raw material (barley malt) is an important issue to be solved.

Malt (germinated grain) is the main raw material used in the production of beer, kvass, alcohol, mono- and polymalt extracts. It is used to produce a wide range of foodstuffs. This can be explained by the fact that the germinated grain possesses valuable nutritional properties and has a positive effect on the human body. Malt grain contains microelements such as phosphorus, magnesium, selenium, calcium, manganese, B-group vitamins and vitamin E [1-5]. Various grain crops, which can effectively expand the range of brewing malts and, accordingly, diversify the beer choice cause an increased interest of malt producers and brewers [6]. Therefore, rice malt production is a promising technological direction, as it allows expanding the range of products and replacing barley malt in the countries where there is a shortage of it.

There is a classical brewing technology using barley malt, however, the use of other cereals is a promising and interesting research trend for scientists in the brewery industry. Priority here is given to the obtaining of high-quality and safe product (i.e. beer). Rice malt beer is a gluten-free drink of light color with a vague organoleptic profile. Therefore, the possibilities of production of special malts, namely caramelized and dark malts, from rice are being additionally explored [7]. Beer made from rice malt is tasty, and resembles barley lager from the organoleptic point of view [8]. Gluten-free beer can be produced based of rice, although in the modern brewing the latter is used mainly as an additive to barley malt. Nevertheless, one cannot overlook the potential of use of rice malt for the brewing of rice malt beer in different conditions of the germinated grain production [9]. Furthermore, the scientists are studying the prospects of wide use of rice malt to obtain the various functional products [10].

Thus, there is an urgent issue of further implementation of innovative technologies for the obtaining of high-quality rice malt in the processes of the brewery industry for operators involved in the production of various beer brands.

Analysis of recent research and publications

Rice having a huge socio-economic impact on the human civilization is a representative model of cereal food crops [11], and its wider use in the processing and production of high-quality and ecological food is advisable.

Production of rice malt of different varieties of rice, which could be used as an ingredient in gluten-free food products, was studied, and this is of particular importance for the brewing process. The results show that high-quality rice malt can be obtained from rice, even if it has the lower enzymatic activity compared to barley [12].

For example, rice malt as a brewing raw material will increase the availability of grain materials for use in the production of gluten-free beer, potentially making this drink more sustainable, cheaper and widely available one [13].

Beer made entirely from rice will be especially appealing to people with celiac disease because rice does not contain gluten proteins. In addition, rice malt can also contribute to the creation of new beer brands and flavors [14].

Many rice varieties are suitable for processing into malt, but it is necessary to find an individual technological approach to processing, taking into account the varietal characteristics of the raw materials. Currently Thai rice is actively studied with regard to its sweetening for use in brewing. For example, the content of amylolytic enzymes in rice malt was evaluated, and the results showed that the use of rice grain for the production of malt under certain process conditions (duration of soaking and temperature) allowed obtaining a product with high enzymatic activity [15].

Germinated brown rice is considered healthier than white rice because it is richer in essential nutrients (vitamins, minerals, dietary fiber and essential amino acids) and contains more bioactive components such as ferulic acid, γ-oryzanol and gamma-aminobutyric acid [16].

The scientists studied the production of prebiotic oligosaccharides during rice germination. The germinated rice was used to produce rice malt syrup by mashing. After saccharification, rice malt syrup contained different concentrations of sugars and oligosaccharides, in particular, isomaltose, panose, and isomaltotriose possessing prebiotic properties [17]. It demonstrates that rice malt could have a wide application in the functional nutrition. Thus, rice-malt extracts are used quite widely in the industry, which allows considering rice malt as a versatile raw material [18].

Malted rice was studied with a view to obtaining rice malt with high diastatic power for the production of sugar syrup from cassava flour. Malting of rice for 10 days and preparation of malt from germinated rice seeds with feather and roots can give rice malt with high diastatic power for hydrolysis of starch in sugar syrup production [19].

Since rice is rather specific raw material for the malt production, scientists are working on the creation of the innovative technologies to obtain a product with specified properties by using various types of effects on the grain material germinated on an industrial scale (chemical, physical, biological, biochemical, and complex effects). Besides, an important aspect is the intensification of the rice germination process. The latest technological developments in this regard are considered below.

Rice malt has never been used commercially in brewing because of low free α-amino nitrogen (FAN) content. Studies were conducted to optimize the substitution of barley malt for rice malt in the wort production and to improve FAN by adding α-amylase and protease. Addition of protease increased the amount of FAN in the wort owing to improved release of amino nitrogen. Moreover, rice germination time had the greatest impact on FAN of the wort. The process of optimization of the wort production with the addition of enzymes and rice malt was successful. The ratio of rice malt was increased to 90% using five-day germinated rice malt, while α-amylases and proteases were added in the amount of 0.40 g/100 g of malt [20].

Chinese rice wine is a traditional fermented alcoholic drink of China with the history of more than 5000 years. The rice soaking process is the most important step during the CRW brewing process, as the quality of soaking directly determines the quality of the CRW. However, water for soaking of rice would result in significant water consumption. The longer the rice soaking time, the higher the content of biogenic amine; it has a huge impact on the human health. To eliminate the long rice soaking process, an innovative brewing technology was used. This problem was solved by addition of Lactobacillus [21]. Therefore, reduction of the soaking process and saving of water resources is an important technological problem, requiring the additional process solutions.

To improve the rice grain malt quality, rice pade (Jasmine 85) is purposely held in the air for 2-8 hours during soaking and malted for 12 days at 28oC. Rice grains left in the air absorb 0.5-5% more moisture compared to untreated ones. Grains kept in the air for six hours after 48 hours of soaking absorbed the highest moisture content – 36.6. The highest germination energy (93%) and diastatic activity were recorded in 6-hour grains held in the air. Furthermore, the malt resulting from this treatment showed its optimal enzymatic activity on the 8th day after germination, which was observed earlier than the control, reaching its peak on the 10th day. Rice treatment in the air during malting improves its hydrolytic potential [22].

The process of anaerobic germination of rice was studied, and while much remains to be learned about biochemical and molecular fundamentals of the anaerobic rice germination, ability of rice to support an active enzymatic metabolism (providing the glycolytic pathway with readily fermentable carbohydrates) is crucial [23].

The effect of magnetic treatment on rice malting was studied in the laboratory conditions. The seeds were exposed to magnetic fields of 150 and 250 mT. Unexposed seeds were used as a control. The chronic exposure to magnetic field of 150 mT increased ($p < 0.05$) both the rate and percentage of germination compared to unexposed seeds. In addition, the seeds were subject to moistening with water treated magnetically by static and dynamic methods. Dynamic and static water treatment improved the seed germination relative to the control, but significant differences ($p < 0.05$) were obtained for the dynamic method only (16% in 48 hours) [24].

The attempts have been made to promote the germination of old rice grains by treating them with lanthanum nitrate. The use of this component allowed accelerating the rate of respiration and the activity of enzymes, and reduced the permeability of the plasma membrane. This suggests that lanthanum can be used to pre-treat seeds before germination [25].

Gibberellic acid and surfactants (sodium dodecyl sulfate (SDS) (1.0 g/l) and Triton-X−100 (1.0 ml/l)) were also used in the rice grain germination technology. For example, positive biochemical changes during germination of rice grains (Oryza sativa L. subsp. indica var. Mottaikaruppan) and improvement of the germination rate were recorded. During germination, an increase in the amylolytic and proteolytic activity was observed. The effect of different concentrations of gibberellic acid on the germination of rice grains was evaluated, and 0.1 g/l was found to promote germination. When the effect of gibberellic acid (0.1 g/l) and surfactants was assessed jointly and individually, the control experiment (grain germinated in distilled water) showed the higher germination rate, while gibberellic acid and surfactants decreased the germination rate [26]. Hormonal signals, in particular, signals of abscisic acid (ABA) and gibberellin (GA) play a dominant role in the process of rice germination regulation [27].

Solutions with nanoparticles have an effect on the intensification of the rice germination process as well. The potential effect of SiO2 (10-20 nm) and Mo (<100 nm) nanoparticles on the rice seed germination was studied. A positive dynamics of germination is recorded [28]. Zn nanoparticles are also used for the physiological correction of rice germination processes, namely, to improve germination [29].

A new method of rice processing is the treatment of grains with large germs by soaking and gas treatment. After soaking for 3 hours the grain is treated with gas during 21 hours at 35°C, the content of γ-aminobutyric acid (GABA) was higher compared to the conventional soaking method (10.1 mg/100 g). While the number of microorganisms on the rice surface increased during soaking, 20-minute steaming and treatment with ethanol for 3 minutes sterilized the rice completely and did not reduce the GABA amount [30].

The purpose of all studies of the process of rice germination is to determine the optimal malting conditions for the production of rice beer. Therefore, all research efforts are confined to the selection of certain conditions for obtaining rice malt from the available rice raw materials [31].

Improvement of the malting qualities of rice malt will increase its potential and sphere of use in the brewery industry. Modern discoveries show that soaking of rice grains for 48 hours promotes the absorption of water, which is important for the maximum production of diastase, necessary for the higher conversion of starch into simple sugars. Therefore, brewing and other related industries can use rice malt in the development of their products [32].

Presence of water significantly affects the mechanism of transformation of grain into malt. High moisture content promotes the enzyme production and emergence of seedlings. In the study of this effect on rice malt, rice grains were soaked for different periods in order to establish the optimum having a significant effect on the final malt quality. Moisture content beyond soaking, losses during soaking, germination energy, shoot length and diastatic activity of malt were evaluated. Statistical analysis showed that the soaking period correlated positively with the rate of water absorption and losses during soaking. The energy of germination was dependent on the soaking period with 48-hour and 72-hour treatment, with the highest energy recorded at 91 and 96%, respectively. 48-hour grains gave the highest average shoot length and high diastatic activity. Soaking of rice grains for 48 hours during malting significantly improves its overall hydrolytic capacity to ensure better conversion of starch to fermentable sugar [32,33].

Soaking of grain raw material in water is the main method of influencing its structure; however, this process can be limited to the breakdown of water-soluble proteins only. Therefore, to completely break the bonds of starch with other components of raw materials, chemical solutions of type depending on the protein content in the grain are used. It results in forced denaturation of proteins and changing of the structure of protein substances, which contributes to the release of starch grains. Besides, the effect of chemical preparations makes cellular structures permeable for the diffusional transport of soluble substances into a liquid environment, that is, into water [6,33].

Activation of water and aqueous solutions by plasma-chemical treatment is the first step towards use of the properties of water without its forced chemization by foreign chemicals [34]. All processes that occur during activation are processes taking place directly in water without addition of external chemical components. Reactogenic properties of the activated water are of great interest to scientists, since the properties of water arising after activation may have prospects in various directions of the modern nanotechnologies. Water activated under action of non-equilibrium contact plasma possesses antiseptic and antibacterial properties [34]. However, it should be noted that this water being a cluster structure after plasma treatment may exhibit completely new properties previously little studied but interesting from a practical standpoint [6]. The resulting activated water has a specific composition, and the reaction products determining the water reactivity are the most easily detectable ones. It applies mainly to hydrogen peroxide and superoxide compounds, excited particles and radicals, which play an important role in redox processes [35,36]. It should also be noted that this water after plasma treatment may exhibit new properties, previously little studied, for example, it may accelerate moisture transport into grain material and correct biochemical transformations in the malt grain [36]. A special role in this case is given to the studies of the effect of activated water on the parameters of some processes in the food, biochemical and biotechnological industries.

Final components in water and aqueous solutions after their treatment with non-equilibrium contact plasma represent an important aspect of the course of technological processes. They can be imagined as a mixture of hydrogen peroxide, superoxide components, active radicals and particles. However, their quantitative characteristics (mkg and parts per million) are at the level that cannot harm the human health in any way. Peculiar action of these components lies in several directions. In the first place, when tracing the process of grain raw material treatment, one can find very useful processes aimed at intensification of moistening of rice grains by changing the very structure of water (water clusters are actively crushed under action of the contact plasma, with the effect of more active penetration of such clusters through membranes and shells of the malt grain in the process of its soaking and subsequent malting) [33]. Such clusters contain microparticles of hydrogen peroxide, which, when in contact with raw materials, are capable of forming active oxygen and water, according to the appropriate reaction, and oxygen is positively consumed as a stimulating agent of biochemical transformations to activate the germination process [6,33,37].

All this happens without addition of any complex chemical compounds, as a rule, of inorganic or organic origin and any additional energy demands. This aqueous environment (plasma-chemically activated solutions) inspires the researchers to create environmentally friendly "green" technologies for the production of rice malt. There is an antibacterial effect of water treated with non-equilibrium contact plasma against mold fungi under conditions of high humidity of the germinated grain material. This is only a small part of positive effects observed in the malt technology [33,38,39].

Purpose of this study consists in obtaining of rice malt with the use of plasma-chemically activated aqueous solutions as an intensifier of the germination process.

Objectives of the study:

1. Studying of the chemical composition of rice grain, namely, the content of starch, proteins, fat, fiber, mineral substances and moisture.
2. Investigation of the effect of plasma-chemically activated aqueous solutions on the process of rice malting, analysis of the duration of soaking and changes in the moisture content of the material.
3. Determination of the energy of rice grains and their ability to germinate with the use of plasma-chemically activated aqueous solutions.
4. Investigation of the dynamics of changes in the amylolytic and proteolytic activity of malt with the use of plasma-chemically activated aqueous solutions.

5. Research of rice malt parameters: extractivity, saccharification time, pH, amino nitrogen content.
6. Analysis of the disinfecting effect of plasma-chemically activated aqueous solutions on the pathogenic complex of rice malt.

Research materials and methods

We used long-grain Vietnamese rice as an object of research.

Activation of water for moistening of rice grains was carried out using the laboratory plasma-chemical unit [34].

Tap water was activated in plasma discharges of reduced pressure with the voltage of 1000–1200 V, current of 30.0–200.0 mA, with the subsequent transition (as the electrical conductivity increased) to the mode of non-equilibrium contact plasma with the following parameters: voltage of 400 to 600 V and current of up to 150 mA.

The content (concentration) of hydrogen peroxide in the activated water was determined by iodometry method [40].

To analyze the rice grain, the methods below were used. The starch content was determined by the polarimetry method regulated by DSTU ISO 6493:2008 in Ukraine; protein in rice was determined by the Kjeldahl method which is an arbitration method with several main stages (sample preparation, mineralization, distillation and titration) [40]; fat content was determined by the extraction method, an arbitration method for the food products [41]; total crude fiber content was found by the extraction method [41]; the content of mineral substances was found by determining the ash content by the weight method; moisture content of rice grain was determined according to DSTU 7734:2015 (Metrology. Hot-air plants for measurement of the moisture content of grain and grain processing products) [40,41].

Growing of rice malt was carried out in the laboratory box malting system. Grain moisture during the period of moistening and growing was monitored with the use of electronic digital meter of grain and seed moisture ВСП-100. In addition, standard indicators such as rice grain energy and ability to germinate were determined [40].

Thermometry of rice malt growing conditions was performed as well. Malting was carried out in four temperature regimes – 15, 20, 25, 30°C.

Amylolytic activity of rice malt was determined by the Windisch-Kolbach method [41], while activity of proteolytic enzymes of rice malt was determined by the modification method [40].

Green rice malt was dried in the drying cabinet for 24 hours to the final moisture content of 4-6%. The drying temperature ranged from 35°C at the beginning of drying to 85°C at the end, with the uniform temperature increase in the malt layer. The wort was obtained from the finished malt.

Dry malt extractivity was determined using the standard (congress) method [40]; the duration of malt saccharification was determined by a visual method using the iodine solution. pH was measured by the potentiometric method [40]. These parameters were recorded during the entire time of germination, namely from the 2nd to 5th day of the process.

The content of amino nitrogen in rice malt was determined by the copper method. This method is based on the ability of amino acids to form soluble compounds with copper, the amount of which is found by iodometric titration [40].

Microbiological indicators of rice malt, namely, the presence of pathogens (*Aspergillus, Alternaria, Penicillium, Fusarium, Mucor*) were determined using generally accepted microbiological methods [42,43].

For mathematical processing of experimental materials and substantiation of conclusions of the study, we used the analysis of variance without repetition, which establishes the effect of two factors on the effective feature [44-46]. It is assumed that the considered factors have, respectively, M and N gradations, that is, sample observations are denoted as X_{mn}, m=1..M, n=1..N. The general mean over the sample is defined as $\overline{X}$. Let $\overline{X_m}$ is the average value for the gradation of the m factor that correspond to the data rows, $\overline{X_n}$ – average value for the gradation of the n factor, that correspond to the data columns.

Variance of the effective feature by the factor corresponding to the rows of the data table is calculated as

$$MS_{rows} = N \cdot \Sigma_{m=1..M} (\overline{X_m} - \overline{X})^2 / (M-1), \quad (1)$$

the variance of the effective feature by the factor compared to the columns of the data table is found by the formula

$$MS_{columns} = M \cdot \Sigma_{n=1..N} (\overline{X_n} - \overline{X})^2 / (N-1), \quad (2)$$

the variance of errors not explained by the considered factors is equal to

$$MS_{error} = \Sigma_{m=1..M} \Sigma_{n=1..N} (X_{mn} - \overline{X_m} - \overline{X_n} + \overline{X})^2 / ((M-1)(N-1)).$$

Conducting of the analysis of variance in comparing the calculated Fisher's criterion

$$F_{rows} = MS_{rows} / MS_{error} \text{ або } F_{columns} = MS_{columns} / MS_{error}$$

with the critical value of F_{crit} with the significance level α. If

$$F_{rows}>F_{crit} \text{ або } F_{columns}>F_{crit}, \quad (3)$$

then the effective feature significantly depends on the dynamics of the corresponding factor. If the inequality

$$F_{rows} \le F_{crit} \text{ або } F_{columns} \le F_{crit},$$

is true, the corresponding factor does not have a significant effect on the effective feature.

All calculations of two-way analysis of variance without repetition were carried out using spreadsheet tools at the significance level α=0,05.

Results of the research and their discussion

At the first stage of research, we analyzed the rice grains determining their chemical characteristics. For example, the indicators of moisture content, content of starch, proteins, fats, fiber, mineral substances were recorded (Fig.1).

As shown by comparative studies of the selected long-grain rice and malting barley, rice contains more starch (64.8%) compared to barley (59.1%) and does not significantly differ in the content of protein substances. That is, we can make a reasonable conclusion that rice grain can be used to obtain high-quality rice malt with the excellent extractive yield.

Characteristic of plasma-chemically activated water used for soaking of rice grain is given in Table 1.

The first stage of the work was to determine the optimal concentration of hydrogen peroxides by using solutions of different concentrations (from 300 to 700 mg) during soaking of rice grains. The results of study are shown in Table 2.

Table 1 – Characteristics of water activated by non-equilibrium contact plasma

Experiment	Water	Activation time, minutes	Concentration of hydrogen peroxide, mg/l
1 (control)	Tap water	-	-
2	Activated water	10	300
3	Activated water	20	400
4	Activated water	30	600
5	Activated water	40	650
6	Activated water	60	700

When we analyze the research results shown in Table 3, it should be noted that germination processes are activated when the moisture content of grain material reaches 42–48% [1]. Therefore, the minimum required value of moisture is 42%. After consideration of the obtained data, we can conclude that the optimal temperature for soaking of rice grains is 25°C. Concentration of peroxides in the solutions, which contributed to the fastest penetration of moisture into the grain, was 650 mg/l, since with the use of plasma-chemically activated solutions with this concentration it took only 20 hours for moistening. Such minimization of the time of rice soaking rice to the optimal moisture level will allow to significantly shorten the process of growing of rice malt.

To identify the dependence of moisture content in rice grains under the impact of temperature, hydrogen peroxide concentration and duration of soaking, two-way analysis of variance without repetition was applied to the data in Table 2. Three series of calculations were performed.

First, the temperature was recorded and dynamics of a pair of other factors was monitored. The main calculation results are shown in Table 3. Based on the ratios $F>F_{crit}$ (3) it is found that both the concentration of hydrogen peroxide (from 0 to 700 mg/l) and duration of soaking (from 10 to 80 hours) cause the appearance of significantly different indicators of moisture content in rice grains. However, at all temperature levels (from 15 to 30ºC) variances MS (1) and (2) for the duration of soaking prevailed over the dynamics of hydrogen peroxide concentration, that is, moisture content in rice grains changed more as a result of soaking. The largest and smallest fluctuations in moisture content at different hydrogen peroxide concentrations were recorded at T=20ºC and T=30ºC. However, as evidenced by MS, the largest and smallest fluctuations in moisture content under the effect of soaking duration were found at T=15ºC and T=25ºC.

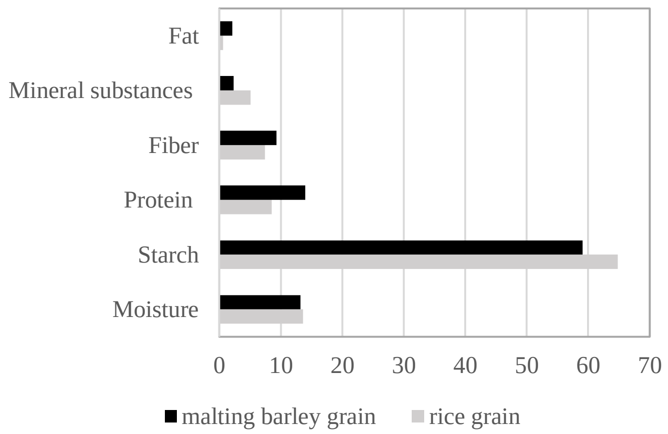

Fig. 1 – Chemical composition of rice grain and malting barley grain

Table 2 – Moisture content of rice grains during soaking in plasma-chemically activated aqueous solutions, % (п=3)

T, °C	Concentration of hydrogen peroxide, mg/l	Duration of soaking, hours								
		0	10	20	30	40	50	60	70	80
1	2	3	4	5	6	7	8	9	10	11
15	-	14 1	17	20	24	27	30	33	35	36
	300		19	23	30	33	35	37	38	40
	400		20	25	31	34	37	40	**42**	**44**
	600		21	26	33	36	39	**42**	**45**	**47**
	650		22	28	35	38	42	44	46	48
	700		21	27	34	37	40	**42**	**44**	**45**
20	-	14	19	21	25	28	32	32	36	37
	300		22	26	35	38	40	**42**	**44**	**46**
	400		25	31	37	40	**42**	**44**	**46**	**48**
	600		29	35	39	**42**	**43**	**45**	**47**	**48**
	650		30	38	**42**	**43**	**44**	**45**	**47**	**49**
	700		28	36	41	**42**	**43**	**44**	**46**	**48**
25	-	14	21	23	27	30	34	37	40	**44**
	300		23	31	36	39	**42**	**43**	**44**	**46**
	400		26	33	40	**42**	**43**	**44**	**46**	**47**
	600		32	36	**42**	**44**	**45**	**46**	**47**	**48**
	650		37	**42**	**44**	**45**	**46**	**48**	**49**	**50**
	700		36	41	**42**	**44**	**45**	**46**	**48**	**49**
30	-	14	21	24	28	31	35	39	**43**	**46**
	300		22	30	37	40	**42**	**44**	**45**	**46**
	400		25	31	39	**42**	**43**	**45**	**46**	**47**
	600		32	34	**42**	**43**	**44**	**45**	**47**	**48**
	650		34	41	**42**	**44**	**45**	**46**	**48**	**49**
	700		33	39	40	**42**	**43**	**45**	**46**	**48**

Table 3 – Results of analysis of variance of moisture content in rice grains depending on hydrogen peroxide concentration and duration of soaking

T,°C	Rows – Concentration of hydrogen peroxide			Columns – Duration of soaking		
	MS	F	F_{crit}	MS	F	F_{crit}
15	109.1	95.9	2.5	408.1	358.6	2.3
20	199.5	92.1	2.5	285.4	131.7	2.3
25	184.2	40.2	2.5	222.6	48.6	2.3
30	107.9	19.3	2.5	262.6	47.0	2.3

Secondly, concentration of hydrogen peroxide was recorded, and dynamics of a pair of other factors was considered. The main calculation results are presented in Table 4. Based on the ratios $F>F_{crit}$ (3) it is found that both the temperature (from 15 to 30 ºC) and duration of soaking (from 10 to 80 hours) result in significantly different indicators of moisture content in rice. However, at all considered concentrations of hydrogen peroxide (from 300 to 700 mg/l) variances MS (1) and (2) for the duration of soaking prevailed over the temperature dynamics, i.e. the moisture content of rice grains changed more due to the duration of soaking. The largest and smallest fluctuations in moisture content due to temperature dynamics were found when the concentration of hydrogen peroxide was equal to 700 and 400 mg/l. As indicated by MS, the largest and smallest fluctuations in moisture content under effect of soaking duration occurred at the concentration of hydrogen peroxide at the level of 300 and 700 mg/l. Variance MS as a measure of fluctuations in the moisture content of rice grains depending on the dynamics of the soaking duration decreased consistently as the concentration of hydrogen peroxide increased from 300 to 700 mg/l.

Table 4 – Results of analysis of variance of moisture content in rice grains depending on temperature and duration of soaking

Concentration of hydrogen peroxide, mg/l	Rows – Temperature			Columns – Duration of soaking		
	MS	F	F_{crit}	MS	F	F_{crit}
300	70.2	87.4	3.1	256.5	319.2	2.5
400	62.8	60.2	3.1	241.5	231.5	2.5
600	67.3	20.7	3.1	180.8	55.6	2.5
650	78.1	14.2	3.1	142.2	25.9	2.5
700	84.4	18.7	3.1	139.1	30.9	2.5

Thirdly, we recorded the duration of soaking and studied the dynamics of a pair of other factors. The main calculation results are accumulated in Table 5.

Based on the ratios $F>F_{crit}$ (3) it is found that both the concentration of hydrogen peroxide (from 0 to 700 mg/l) and the temperature (from 15 to 30ºC) cause significantly different indicators of moisture content in rice grains. Only during soaking for 10 hours, the temperature dynamics led to greater changes in the moisture content parameter than the hydrogen peroxide concentration dynamics. However, the concentration of hydrogen peroxide became the more influential factor in the magnitude of variance MS (1) and (2) for all subsequent soaking periods from 20 to 80 hours. The largest and smallest fluctuations in moisture content conditioned by the dynamics of temperature and concentration of hydrogen peroxide were observed simultaneously at the time of 20 and 80 hours of soaking of rice grains.

Table 5 – Results of analysis of variance of moisture content in rice grains depending on hydrogen peroxide concentration and temperature

Duration of soaking, hours	Rows – Concentration of hydrogen peroxide			Columns – Temperature		
	MS	F	F_{crit}	MS	F	F_{crit}
10	85.4	15.2	2.9	98.2	17.5	3.3
20	127.1	29.6	2.9	107.6	25.0	3.3
30	115.9	72.1	2.9	67.7	42.1	3.3
40	98.7	99.8	2.9	53.9	54.5	3.3
50	68.3	99.1	2.9	34.7	50.4	3.3
60	57.4	32.5	2.9	25.5	14.4	3.3
70	43.6	19.8	2.9	22.3	10.1	3.3
80	35.5	9.1	2.9	21.3	5.5	3.3

How can we explain accelerated transport of moisture or diffusion of the activated aqueous solutions into grain raw materials? The intensity of water absorption in the first periods of soaking affects the further process of germination. Semi-permeable seed coat allows water only to diffuse into the grain. Ions penetrate through the cracks of the seed coat into the middle of the grain and have the ability to affect the germ. The chaotic movement of ions in the activated water allows accelerating the diffusion of water into the middle of the grain due to more active inflow of charged particles to the grain surface. This aspect confirms that when we use the activated water as a moistening agent, due to its specific composition, more active transport of moisture into the middle of the grain takes place. That is, activated water quickly diffuses into the grain (is absorbed) [6]. In addition, one should not forget about migration of charged particles in the middle of the grain. Water absorption takes place mainly through the vessels that exit at the basal end of the grain. After penetration of water into the middle of the grain, transfer of water from the endosperm to the germ begins. Migration of charged particles in the grain leads to the inflow of negatively charged particles into the germs and outflow of positively charged particles. These processes increase the permeability of grain structures for water and nutrients. Capillary condensation occurs quickly; activated water is absorbed with the formation of condensate in the grain capillaries [36].

One of the important indicators of the quality of rice and other grain crops used in the malt production is the vital activity of grains. Domestic and foreign researchers note the dependence of the quality of finished malt on the energy and ability of the grain to germinate [47-48]. At the moment of germination the germ is activated and a complex of enzymes is formed, comprising mainly amylolytic ones, providing hydrolysis of polysaccharides, starch, and low-molecular compounds (mono- and disaccharides), fermented by yeast with the formation of ethyl alcohol and carbon dioxide [6,48]. Therefore, in order to evaluate the intensifying ability of plasma-chemical aqueous solutions, basic indicators such as the rice grain energy and ability to germinate were evaluated. The results are given in Table 6.

As shown in Table 6, the use of activated solutions allows increasing the energy of germination from 85 to 95%, and the ability to germinate from 96 to 100%. This effect allows us to talk about the prospects of intensification of the rice malting process by using plasma-chemically activated aqueous solutions. The optimal concentration for intensification of rice grain germination is 650 mg/l, since this concentration gives the maximum effect of increasing the indicators under study, namely, energy and ability to germinate. The activation of the germination process can be explained as follows. Plasma-chemically activated water accelerates the flow of moisture and, as a result, nutrients from the endosperm to the germ, and stimulates its awakening to active vital activity, which can accelerate the process of accumulation of a complex of cytolytic, proteolytic and amylolytic enzymes. Therefore, the next stage of research was the determination of enzymatic activity.

In the process of germination of rice malt, we recorded the change in the amylolytic activity of the grain material. The results are shown in Table 7.

Table 6 – Rice grain energy and ability to germinate

Experiment	Water	Activation time, minutes	Concentration of hydrogen peroxide, mg/l	Indicators, %	
				Energy of germination	Ability to germinate
1 (control)	Tap water	-	-	85	96
2	Activated water	10	300	89	98
3	Activated water	20	400	92	99
4	Activated water	30	600	93	100
5	Activated water	**40**	**650**	**95**	**100**
6	Activated water	60	700	93	98

Table 7 – Amylolytic activity of grain material, unit/g

T, °C	Experimental samples	Time, day				
		1	2	3	4	5
1	2	3	4	5	6	7
15	Control	35	58	155	194	210
	Experiment	**89**	**157**	**206**	**248**	**287**
20	Control	39	71	176	210	221
	Experiment	**99**	**172**	**268**	**295**	**320**
25	Control	48	99	197	242	248
	Experiment	**120**	**229**	**270**	**341**	**357**
30	Control	47	100	187	191	193
	Experiment	**109**	**150**	**161**	**193**	**242**

Enzymatic activity was monitored for samples treated with plasma-chemically activated aqueous solutions with the concentration of 650 mg/l.

The results show (Table 7) increased activity of amylolytic enzymes with the use of plasma-chemically activated aqueous solutions during soaking of rice grains. We observe stable increase in enzymatic activity throughout the malt growing period. Samples of grain material treated with plasma-chemically activated solutions feature higher activity, both compared to the control and results obtained in similar papers [6,49-55]. Increase in amylolytic activity compared to the control was 2–2.5 times, which is a positive technological effect. Such a significant increase in the indicator contributes to intensification of the saccharification process, that is, we have better quality indicators in the finished malt [49].

For the mathematical substantiation of dependence of the amylolytic activity of grain material under the effect of temperature and germination time, we applied two-way analysis of variance without repetition to the data in Table 7. The main calculation results are given in Table 8. Based on the ratios $F>F_{crit}$ (3), it can be concluded that the temperature (from 15 to 30ºC) and growing time (from 1 to 5 days) cause the different course of amylolytic activity of the grain material of both experimental samples compared. However, owing to the experimental measures, the amylolytic activity in the experiment became significantly more sensitive to the increase in temperature, which is indicated by the larger value of the variance MS.

Table 9 shows the data on changes in proteolytic activity. The process of breakdown of malt grain proteins depends on the germination conditions of the grain material [56]. The degree of cleavage of protein substances may vary depending on the activity of proteolytic enzymes.

Dynamics of increase in the activity of proteolytic enzymes was also established with the use of plasma-chemically activated aqueous solutions. On the 4-5th day of growing, an increase in the accumulation of proteolytic enzymes in samples treated with activated water is clearly visible, which indicates activation of the process of dissolution of protein substances. Therefore, extractivity of the wort obtained from this malt will have the high percentage, which is an important aspect in the production of beer.

For the mathematical justification of dependence of proteolytic activity of grain material under the effect of the temperature and growing time, we applied two-way analysis of variance without repetition to the data in Table 9.

The main calculation results are given in Table 10.

Table 8 – Results of analysis of variance of amylolytic activity of grain material depending on temperature and growing time

Experimental samples	Rows – Temperature			Columns – Growing time		
	MS	F	F_{crit}	MS	F	F_{crit}
Control	1147.5	6.3	3.5	25117.2	137.3	3.3
Experiment	8060.5	12.4	3.5	24259.3	37.4	3.3

Table 9 – Proteolytic activity of grain material, mg/100 g

T, °C	Experimental samples	Time, day				
		1	2	3	4	5
1	2	3	4	5	6	7
15	Control	8	19	79	128	139
	Experiment	**26**	**46**	**84**	**111**	**100**
20	Control	10	24	80	130	141
	Experiment	**28**	**59**	**121**	**216**	**154**
25	Control	18	35	78	144	128
	Experiment	**30**	**74**	**176**	**263**	**245**
30	Control	19	38	90	118	120
	Experiment	**29**	**68**	**131**	**137**	**121**

Table 10 – Results of analysis of variance of proteolytic activity of grain material depending on temperature and growing time

Experimental samples	Rows – Temperature			Columns – Growing time		
	MS	F	F_{crit}	MS	F	F_{crit}
Control	30.6	0.4	3.5	12160.7	150.5	3.3
Experiment	6328.2	5.9	3.5	16503.7	15.4	3.3

Based on the ratios $F<F_{crit}$ (3) it is found that the temperature factor did not significantly change the proteolytic activity of the grain material of the control sample. In other cases, the proteolytic activity changed significantly changes, and the growing time was more influential, as indicated by the values of variance MS (1) and (2). However, owing to the experimental measures, the proteolytic activity of the grain material in the experiment became significantly more sensitive to the increase in temperature, which was confirmed by higher values of the variance MS.

The final stage of the research was the determination of technological parameters of the wort obtained from rice malt. The results are shown in Table 11. Rice malt dried to the moisture content of 5% was used to obtain wort.

Table 11 – Composition of rice malt wort

No.	Indicator	Rice malt	
		Control	**Experiment**
1	Weight fraction of extract (extractivity), %	77.5	85.6
2	Saccharification time, minutes	40	15
3	Acidity (pH)	5.7	5.2
4	Amino nitrogen content, mg/100 g	24.7	48.8

Analyzing the data in Table 11, we may can conclude that as a result of use of plasma-chemical solutions of the stated concentration, extractability of rice malt increased and reached 85.6%. The duration of saccharification of rice malt was reduced to 15 minutes, i.e. more than three times. The acidity of rice malt wort decreased to pH 5.2. The content of amino nitrogen was 48.8 mg/100 ml. It can be explained by the fact that the use of activated solutions promotes acceleration of biochemical transformations and, as a result, accumulation of enzymatic systems. Deeper hydrolysis of starch and nitrogen-containing compounds occurs. All this contributes to the production of high-quality rice malt from a technological point of view.

Furthermore, it should be noted that plasma-chemically activated aqueous solutions have antiseptic properties, which additionally reduces the microbial contamination of rice malt and has a positive effect on the microbio logical indicators of the finished product. For example, the number of infected malt grains is significantly reduced, when using the activated aqueous solutions (Table 12).

With the use of plasma-chemically activated aqueous solutions, the pathogenic contamination of malted rice grain decreased (Table 12), and the possibility of absolute destruction of mold microflora at high concentration of peroxides in the solutions is noted.

To visualize the reduction of microbial contamination of rice malt we used analogs of Lorentz curves [57] for each of 5 pathogens under the effect of hydrogen peroxide with the concentration from 0 mg/l (or Y_1=0%) to 500 mg/l (or Y_6=100%). For the construction of Lorentz curves we compare the percentage X_{ij} of malt where the i-th pathogen has been destroyed, i=1..5, with the concentration of hydrogen peroxide Y_j, j=1..6. The relevant calculation data and graphic illustrations are presented in Table 13. It is found that all pathogens die faster than the concentration of hydrogen peroxide increases. In order to compare their resistance, an analogue of the Hoover index was calculated, according to the formula

$$H_i = \max_{j=1..6}(X_{ij} - Y_j),\ i=1..5.$$

The higher the Hoover index is, the less resistant the pathogen is to hydrogen peroxide [57]. Therefore, according to the results of Table 13, the list of pathogens under study in order of increasing resistance (by decreasing Hoover index) looks like: *Fusarium, Penicillium, Alternaria, Aspergillus, Mucor*.

There are no peroxides in rice malt after disinfection, which confirms the chemical purity and safety of the disinfectant [58], as well as the possibility to obtain competitive raw materials meeting the European standards and requirements for the food products.

Table 12 – Effect of plasma-chemically activated aqueous solutions on the pathogenic complex of malt, % of infected grains

Pathogens	Control	Concentration of peroxides in plasma-chemically activated aqueous solutions, mg/l						
		100	**200**	**300**	**400**	**500**	**600**	**700**
Aspergillus	92	65	34	15	0	0	0	0
Alternaria	35	21	15	5	0	0	0	0
Penicillium	23	11	7	3	0	0	0	0
Fusarium	11	5	3	1	0	0	0	0
Mucor	37	27	15	11	7	0	0	0

Table 13 – Dynamics of destruction of malt pathogens, % of infected grains

Pathogens	Concentration of hydrogen peroxide, mg/l						Hoover index, percentage points
	0	100	200	300	400	500	
Aspergillus	0	29	63	84	100	100	24
Alternaria	0	40	57	86	100	100	26
Penicillium	0	52	70	87	100	100	32
Fusarium	0	55	73	91	100	100	35
Mucor	0	27	59	70	81	100	19

Approbation of results of the study. Plasma-chemically activated aqueous solutions were prepared on the basis of the specialized laboratory for plasma treatment of technological solutions of food processors. All studies were performed in the Research and Production Laboratory of the Department of Technology of Storage and Processing of Agricultural Products of the Dnipro State Agrarian and Economic University and in the conditions of the private meat processing enterprise.

Conclusion

The paper proves the feasibility of use of plasma-chemically activated aqueous solutions in the production of high-quality rice malt:

1. Rice grain chemical composition has been studied. Rice contains more starch (64.8%) compared to barley (59.1%) and does not significantly differ in the content of protein substances. It allows using rice grain to obtain high-quality rice malt with the excellent extractive yield.
2. We investigated the effect of plasma-chemically activated aqueous solutions on the process of rice malting. The optimal temperature for soaking of rice grain is 25°C, while the concentration of peroxides in the solutions, which contributes to the fastest penetration of moisture into the grain, was 650 mg/l; duration of soaking decreased to 20 hours.
3. Rice grain energy and ability to germinate with the use of plasma-chemically activated aqueous solutions was determined. It allows increasing the energy of germination from 85 to 95%, and the ability to germinate from 96 to 100%. The optimal peroxide concentration for intensification of rice grain germination is 650 mg/l.
4. The dynamics of changes in the amylolytic and proteolytic activity of malt with the use of plasma-chemically activated aqueous solutions was studied. Increase in amylolytic and proteolytic activity was 2-2.5 times, which is a positive technological effect.
5. Parameters of rice malt were studied: extractability of rice malt increased and reached 85.6%, duration of saccharification of rice malt decreased to 15 minutes, acidity of rice malt wort decreased to pH 5.2, the content of amino nitrogen was 48.8 mg/100 ml.
6. We analyzed the effect of plasma-chemically activated aqueous solutions on the pathogenic complex of rice malt during its processing. With the use of plasma-chemically activated aqueous solutions, the pathogenic contamination of malted rice grain decreased. It is necessary to note the possibility of absolute destruction of mold microflora at high concentration of peroxides in the solutions (400 mg/l and more).

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ОТРИМАННЯ РИСОВОГО СОЛОДУ З ВИКОРИСТАННЯМ ПЛАЗМОХІМІЧНО АКТИВОВАНИХ ВОДНИХ РОЗЧИНІВ

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Анотація. Інноваційні технологічні рішення при переробці рису і виробництві рисового солоду викликають підвищений інтерес фахівців галузі пивоваріння. Дослідження інтенсифікуючого впливу плазмохімічно активованих водних розчинів на процес солодорощення рису стало об’єктом підвищеної уваги вчених та пивоварів. У роботі наведено особливості виробництва рисового солоду, а саме процесу його зволоження, при використанні плазмохімічно активованих водних розчинів з різною концентрацією пероксидів водню. Наведено дослідження щодо тривалості та температури зволоження зерна рису розчинами активованими під дією контактної нерівноважної плазми. У процесі такої обробки утворюються мікрочастки пероксиду водню, які при контакті з зерновою сировиною здатні утворити активний кисень, який є стимулюючим агентом, що пришвидшує транспорт вологи в середину рисового зерна і прискорює ряд біохімічних перетворень. Ці всі аспекти дозволяють значно активізувати процес пророщування. Визначено оптимальну концентрацію пероксиду водню в розчинах і температурі замочування рису (650 мг/л, при 25°С), які в подальшому використовувались для отримання рисового солоду високої якості. Доведено, що рисовий солод отриманий з використанням плазмохімічно активованих водних розчинів має більш високі якісні показники в порівнянні з контролем. Так в результаті використання плазмохімічних розчинів заявленої концентрації підвищилась екстрактивність рисового солоду і досягла 85,6%. Тривалість оцукрювання рисового солоду скоротилася до 15 хв, тобто більше ніж в три рази. Кислотність сусла з рисового солоду знизилась до pH 5,2. Вміст амінного азоту склав 48,8 мг/100 мл. Це все можна пояснити тим, що активація розчинів сприяє прискоренню біохімічних перетворень, і як результат, пришвидшення накопичення ферментативних систем. Відбувається більш глибокий гідроліз крохмалю і азотовмісних сполук. Доведено, що антисептичні властивості активованих водних розчинів дозволяють додатково дезінфікувати зернову сировину, знижується патогенна зараженість солоду. Проведено двох-факторний дисперсійний аналіз без повторів для технологічних показників, отриманих при дослідженнях. У роботі висвітлено технологічні параметри процесу солодорощення, які можуть бути використані при промисловій переробці рису на солод. Наведено технологічні рекомендації щодо використання плазмохімічно активованих водних розчинів в процесі виробництва рисового солоду.

Ключові слова: рис, солод, пророщування, плазмохімічно активовані водні розчини, ферментативна активність, двох-факторний дисперсійний аналіз без повторів.