

Biotechnological recycling of byproducts in the rice soft beverage industry: a preliminary research

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Abstract. The use of industrial waste as the secondary raw materials is relevant all over the world. The rice sediment is a byproduct of the rice soft beverage industry. The rice mash was obtained by the rice sediment fermentation with α -amylase and ethanol yeast *Saccharomyces cerevisiae*. The rice wort fermentation efficiency was estimated by rice mash ethanol concentration, the visible mass concentration of mash dry substances, mash acidity, total yeast number and yeast budding, yeast cell area. The most intensive fermentation was in the sample with α -amylase. On the 7th day of fermentation, the alcohol concentration in this sample was 5.28volume (%), which is 5 times more than in the sample without α -amylase. Digital morphometric characteristics of yeast correlated with actual fermentation parameters, reflecting yeast adaptive reactions at various ethanol technological stages. The rice mash can be used in the rectification process to obtain new products - ethanol distillate or bioethanol. New methods and expanding technologies for biotechnological rice sediment recycling are required in this field of research.

1 Introduction

Currently, there is a steady increase in the food and processing industry capacity, this results into a waste increase. Waste recycling of food raw materials and food products becomes the actual problem. Food waste is the materials which are intended for human consumption, however, they are subsequently getting discharged, lost, degraded and contaminating the environment. The problem of food waste involves all sectors of waste management, from the collection to its removal; the identifying of sustainable solutions extends to all contributors to the food supply chains, agricultural and industrial sectors, as well as retailers and final consumers [1].

The interest in such a topic is determined by two interrelated aspects: economic and environmental.

The economic aspect is that companies make not only high-quality products from raw materials, but also use byproducts to make new ones. Scientists investigate the possibility of food waste recycling of both plant and animal origin.

Ivanchenko O. et al [2] devoted their research to the problem of improving methods of pectin obtaining from apple pomace by ultrasonic vibrations. The importance of this study was because there is a big waste formation during apple juice production. The apple juice yield is from 50 to 75% during its production, but 25-50% is waste.

Food waste from the salmon industry is the most important part of secondary fish resources. Salmon meat has high nutrition value and includes a lot of polyunsaturated fatty acids (PFA), minerals, vitamins B1, B2, C, E, A. Bazarnova J. et al [3] made the specification of Atlantic salmon byproducts. They researched the possibility of its use in the fish sausage production. Obtained results allowed to optimize functional and technological characteristics of fish sausages "Tender" and "Vienna".

Scientists researched the food industry waste using for the production of heavy metal ion sorbents based on the modified wheat husk [4, 5]. Sorbent kinetic and isotherm characteristics, the influence of pH environment on the efficiency of wastewater treatment from heavy metal ions were studied.

The widespread of corn starch and sugarcane using as glucose sources for ethanol production via fermentation could have a negative influence on farmlands. Nevertheless, alternative sources of fermentable sugars, particularly from lignocellulosic sources, are investigated extensively. Another source of fermentable sugars with substantial potential for ethanol production is the food recycling industry waste: the potato recycling waste, molasses from the beet recycling, whey from cheese production, byproducts of rice and coffee bean processing, and other food processing waste as sugar sources for fermentation to ethanol [6].

Traditionally, wheat and rye are the main raw materials in the production of grain ethanol [7, 8].

The new alternative raw material searching is the main way to increase ethanol production profitability. For example, using rice milling as a byproduct [9]. This work describes the use of rice milling as raw material for bio-ethanol. Starch-rich waste (rice bran, broken, unripe and discolored rice) was individually fermented (20%w/v) through consolidated bio-processing by two industrial engineered yeast secreting fungal amylases. Fermenting efficiency for each byproduct was high (above 88% of the theoretical) and further confirmed on the blend of residues (nearly 52g/l ethanol). These results demonstrated for the first time that the co-conversion of multiple waste streams is a promising option for second generation ethanol production.

Food waste is of particular interest to researchers of renewable energy sources. Many studies indicate various technologies for processing food waste into biofuel. The ethanol production from secondary resources allows solving the problem of expanding the raw material base in the production of ethanol as a biofuel. Various objects could be used as sources - animal waste, agricultural waste, plant shells and stems, substandard wood, as well as food industry waste or, for example, microalgae [10, 11]. Liquid fuel could be as an alternative to gasoline, or as a supplement to traditional autocar fuel. Such a raw material resource, rice production waste is repeatedly mentioned in the literature [12, 13].

Starch is an important constituent of the human diet and is a major storage product of many economically important crops such as wheat, rice, maize, tapioca, and potato. Amylases are one of the main enzymes that have been used in the industry. Such enzymes hydrolyze the starch molecules into polymers composed of glucose units. Amylases have potential application in a wide number of industrial processes such as food, fermentation, and pharmaceutical industries [14].

Alpha-amylase could be obtained from plants, animals, and microorganisms. However, enzymes from fungal and bacterial sources have dominated applications in industrial sectors. The production of α -amylase is essential for the conversion of starches into oligosaccharides. α -amylase is produced from *Bacillus licheniformis*, *Bacillus stearothermophilus*, and *Bacillus amyloliquefaciens* find potential application several industrial processes such as in food, fermentation, textiles, paper, and detergent industries [15]. The properties of each α -amylase such as thermal stability, pH profile, pH stability, and Ca-independency are important in the development of the fermentation process.

Yeast represents valuable sources in food science and microbiology and is a kind of food factories, because of the potentiality of whole cells or for their produced compounds [16]. Yeast performs three main functions. The role of yeast as starter cultures with a special focus on wine [17], ethanol [18, 19]. Yeast is also used as probiotics to regulate the human microbiome [20] in the food production industry and as an enzyme producer [21].

Carbohydrates are about 90% of the total weight of rice grains. Rice is used not only for ethanol production but also in the rice soft beverage industry. Rice milk is one of them. Rice grows in more than 100 countries with 90% of the total global production from Asia. Although there are more than 110,000 cultivated rice varieties differ in quality and nutritional content. After post-harvest processing, rice could be categorized as either white or brown. In addition to calories, rice is a good source of magnesium, phosphorus, manganese, selenium, iron, folic acid, thiamin, and niacin; but it is low in fiber and fat. Although brown rice is promoted as being "healthier" because of bioactive compounds, including minerals and vitamins not present in white rice after polishing, white rice is more widely consumed than brown. This is for several reasons, including cooking ease, palatability, and shelf life. Polished rice has a higher glycemic load and may impact glucose homeostasis but, when combined with other foods, it can be considered part of a "healthy" plate. With the projected increase in the global population, rice will remain a staple [22].

Globally, consumers are concerned about saturated fat levels in foods, lactose intolerance, hormone and antibiotic levels in dairy products, as well as sustainable treatment of the environment and animal food production. New uses of rice in value-added food products are being explored because rice is a lactose-free, cholesterol and gluten-free food [23].

Various methods of rice milk production had been explored, including the method for enzymatic induction by germination of rice grain [24].

Previously, we created a rice soft drink recipe based on boiled rice filtrate, green tea water concentrate, stevia extract as a natural sweetener. The rice suspension obtained was used to prepare a rice soft beverage based on the water green tea concentrate and stevia powder extract. And the rice sediment, remaining after filtration, was used for further biotechnological recycling.

This research aimed to study the possibility of biotechnological recycling the rice sediment – a byproduct of the rice soft beverage industry to obtain a new substance: rice mash that could be used for bio-ethanol production.

2 Materials and methods

Previously, we have developed a rice soft drink recipe based on boiled rice filtrate, green tea water concentrate, stevia extract as a natural sweetener.

Raw materials for the rice suspension, the basis of the rice soft beverage production: white grain rice, milled (commercial product, Russia); leave stevia extract powder (commercial product, Russia); green tea (commercial product, China); green tea leaves water concentrate; drinking water.

The rice was ground in a homogenizer. Water (1500 ml) was added to the rice. The soaked rice was cooked for 20 minutes in a water bath with a constant temperature of + 95°C. The mass was constantly mixed

to avoid uneven heating, starch agglomeration and incomplete gelatinization.

Main technological stages of the rice beverage cooking: cooking rice flour at +180°C for 15 minutes → grinding → heating in a water bath at a constant temperature of +30°C for 2 hours → colloid crushing for 3 minutes → heating at a temperature of +70°C in a water bath for 30 minutes → filtration → rice suspension.

The rice suspension was cooled to room temperature, poured into a blender in order to reduce the particle size and distribute the particles more evenly. Then the rice suspension obtained was used to prepare a rice soft beverage based on the water green tea concentrate and the stevia powder extract. The rice sediment, remaining after filtration, was used for further biotechnological recycling. The rice sediment was diluted with water in the ratio 3:2. For example, 95 g of the rice sediment and 60 ml of H₂O.

Rice sediment polysaccharides were hydrolyzed by bacterial ferment α -amylase BF7658 (*Junsheng Biological Technology Co., Ltd, China*). Ferment dosage was, as recommended by the manufacturer, (100.00±0.005) g/kg of rice sediment. The fermentation took 30 minutes at (65.0±0.5) °C. Then the rice wort was cooled for 30 minutes. Ethanol yeast was added to the cooled rice wort.

Dry ethanol yeast *Saccharomyces cerevisiae*, Angel Yeast (Hubei Anqi Biological Group Co., LTD, China) were used for biotechnological recycling of the rice sediment in these experiments. Dry yeasts were rehydrated with (10.00 ±0.5) ml·g⁻¹ distilled water at (35.0±0.5) °C for 10 minutes. Yeast dosage was, as recommended by the manufacturer, (1.000±0.005) g·dm⁻³ of rice wort.

Yeast cultivation was carried out on two substrate types: diluted rice sediment without preliminary hydrolysis by the α -amylase and diluted rice sediment with preliminary hydrolysis by an α -amylase.

The fermentation took place for 7 days at (30.0±0.5) °C. Data were collected after 3, 5, 7 days of fermentation.

In our preliminary research, the rice wort fermentation efficiency was estimated by rice mash ethanol concentration, visible mass concentration of mash dry substances, mash acidity (pH), total yeast number and yeast budding number, yeast cell area.

The rice mash ethanol concentration was measured by Koloss M, a digital automatic analyzer (*NPP "Biomer", Russia*). This device is used to express-measurement the ethanol concentration, the mass concentration of dry substances in alcohol-containing beverages and solutions. The rice mash filtrate was used to measure physicochemical parameters by the digital analyzer.

Titrimo Plus (Metrohm) titrator was used for pH measurement. The device operates in two modes, potentiometric titration, and pH meter, the later used in the experiment. The time of the assay was no less than 3 min.

During and after the rice sediment fermentation, fixed yeast cell preparations were stained with the

hematoxylin-eosin solution according to a standard protocol. Microscopic analysis of samples was carried out in visible spectral range using *Nikon* microscope equipped with a DS-Fi2 camera providing a total 1000-fold magnification. Images were recorded by a video system and displayed on a computer screen with NIS-Elements Basic Research (*Nikon*) software.

The cell number and yeast digital cytomorphometric characteristics (total cell area, μm^2) were obtained in 30 view fields for each sample by *Nikon* image recognition software "Image J". Data were collected after 0, 3, and 24 hours of fermentation.

All data were reported as the standard error of the mean (SEM). Statistical data processing was made with software GraphPad Prism 6 (GraphPad Software Inc., La Jolla, USA), SPSSH Statistics version 17.0 (IMB, New York, USA).

3 Results

Two samples of the fermented rice wort were the object of the experimental study. The fermentable raw material of the first sample (number 1, control sample) consisted following components: rice sediment + water + yeast. The fermentable raw material of the second sample (number 2, experimental sample) consisted following components: rice sediment + water + α -amylase + yeast. All physical and chemical parameters of the fermented rice wort and rice mash were obtained after 0, 3, 5, and 7 days of fermentation.

The mash ethanol concentration was the main criterion of the yeast efficiency during their cultivation in the rice sediment. Changes in ethanol concentration over time in two types of rice mash during the wort fermentation are presented in *Figure 1*.

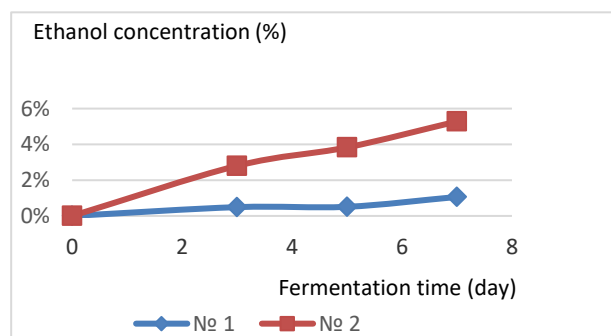


Fig. 1. Rice mash ethanol concentration during the wort fermentation (No 1. Fermented raw materials: rice sediment + water + yeast ; No 2. Fermented raw materials: rice sediment + water + α -amylase + yeast).

Visible mass concentration of mash dry substances and pH value mash are also an important criterion for the yeast fermentation efficiency estimation. Values are presented in the *Table 1* and the *Table 2*.

Table 1. Changes in the visible mass concentration of mash dry substances during the rice sediment fermentation.

No	Fermentation time, days	0	3	5	7
	Rice wort composition	Visible mass concentration of mash dry substances (%)			
1	Rice sediment + water + yeast	3.14±0.01	3.00±0.01	2.9±0.01	2.78±0.01
2	Rice sediment + water + α -amylase + yeast	6.60±0.01	6.00±0.01	5.80±0.01	5.68±0.01

Table 2. Changes in the mash acidity during the rice sediment fermentation.

No	Fermentation time, days	0	3	5	7
	Rice wort composition	pH value			
1	Rice sediment + water + yeast	4.50±0.02	4.44±0.02	3.88±0.02	3.83±0.02
2	Rice sediment + water + α -amylase + yeast	4.20±0.02	3.92±0.02	3.68±0.02	3.66±0.02

Cell budding intensity is one of the main criteria of the yeast functional activity. Photos in *Figure 2* show budding yeast cultivated in two types of rice mash based on: rice sediment without α -amylase (a) and rice sediment with α -amylase adding before fermentation (b).

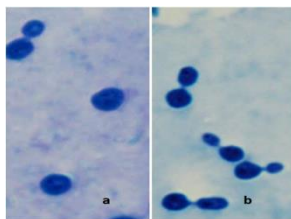


Fig. 2. Yeast cell budding after 24 hours of the rice sediment fermentation (a - mash composition: rice sediment + water + yeast; b - mash composition: rice sediment + water + α -amylase + yeast ; Yeast cell budding has increased (b) when α -amylase was added to the rice wort ; Hematoxylin - eosin staining. Microscope total magnification: x 1000 (a, b)).

The digital microscopic analysis demonstrated the presence of heterogeneous yeast cells during 24 hours of fermentation. Digital cytomorphometric data of yeast cell area changes during the rice sediment fermentation are presented in the *Table 3*.

Table 3. Yeast cell area changes during the rice sediment fermentation.

No	Fermentation time, hours	0	3	24
	Rice wort composition	Yeast cell area (μm^2)		
1	Rice sediment + water + yeast	7.61±1.79	10.47±1.37	9.52±2.81
2	Rice sediment + water + α -amylase + yeast	7.61±1.79	9.59±1.93	7.44±2.02

4 Discussion

Physical and chemical studies of rice mash were carried out. Rice sediment – the byproduct in the rice soft beverage industry – was the substrate for rice mash production. Dry industrial ethanol yeast *Saccharomyces cerevisiae* were used for the diluted rice sediment fermentation.

Bacterial enzyme α -amylase BF7658 was added to the rice substrate before yeast to hydrolyze starch and increase the soluble carbohydrate amount (experimental sample). The rice sediment sample without α -amylase, but with ethanol yeast, was as a control sample. Fermentation was carried out at a temperature of + 30 °C for 7 days.

Starch is the main polysaccharide of rice grains. Yeast does not digest it. It is necessary to carry out starch preliminary hydrolysis in order to obtain carbohydrates available for yeast metabolism. Dextrins, disaccharides and monosaccharides, maltose and glucose are formed during starch hydrolysis. Using of α -amylase BF7658 allowed to increase the dry substance concentration in the fermentation rice wort by 2 times: 3.14 % in the control sample, 6.60 % in the experimental sample. On the 7th fermentation day, compared to the beginning of fermentation, the dry substance concentration in the control sample decreased by 1.2 times, in the experimental sample - also decreased by 1.2 times (Table 1).

Mudrak T. *et al* [25] studied an optimal enzyme complex for hydrolysis of the maize grain components with a starch content of 69.0 %. Fermentation was carried out with 18–30% of dry matters (DM) in the wort, using the osmophilic yeast strain *Saccharomyces cerevisiae* DO-16. The alcohol concentration in fermented mashes was 10.51, 13.35, 15.78% vol., according to the wort DM concentrations 18, 27, 30 %, respectively. In our experiment, the primary dry substance concentration was low because the raw material for fermentation was not rice grain, but the diluted rice sediment - byproduct in the preceding rice beverage production. Thus, the enzyme α -amylase used in our experiment, hydrolyzed components of the rice sediment starch.

It is shown that the most intense fermentation took place in the experimental sample with the addition of the enzyme α -amylase BF7658. On the 7th day of fermentation the ethanol concentration in the experimental sample with α -amylase was 5.28 volume%, which is 5 times more than in the control sample without the enzyme: 1.10 volume% (Fig.1).

During the all fermentation period rice mash pH values slightly decreased both in the control and experimental samples: from 4.50 to 3.83, from 4.20 to 3.66, respectively (Table 2). Obtained data confirms the research Zhangcheng Liang *et al* [26] that during first 5-7 days of rice wine production there was pH decrease of the fermented rice wort against the increase in the alcohol concentration in the rice wort.

The fermentation intensity could be controlled not only by the degree of the carbon dioxide synthesis, ethanol concentration and dry substance reduction in the

mash, but also by the yeast morphology. In yeast suspension, as a rule, all cells are of different ages: budding, young, mature (main), dead, so the suspension is more often characterized as "heterogeneous". Young and old cells have different physiological activity, which is important to take into account during fermentation [27]. Paying attention to the yeast cell morphological features during fermentation, we could assume its age and physiological state.

In our experiments, we noted that the yeast cells are increasing during the first 3 hours, of fermentation in both control and experimental samples (Table 3). Probably, in first three hours yeast cells did not bud, but only adapted, and then grew and increased in size. Moreover, more intensive growth occurred in the control samples without α -amylase. It could be assumed that in the sample with α -amylase yeast cells were more intensively budded. After 24 hours of fermentation we observed a greater number of budding yeast cells (Fig. 2). The formation of daughter cells does not increase the cell size, so the cell area is on average smaller. A larger number of small young cells in the fermenting rice wort with α -amylase, of course, had a positive effect on further fermentation.

The cell number in the control sample without the enzyme is less and they have a thicker cell wall. Most likely, yeast cells in this rice wort had the shortest lag phase. The yeast exponential growth phase also ended earlier. After 24 hours of fermentation, yeast has "entered" the stationary growth phase.

5 Conclusions

1. Rice sediment as the byproduct in the rice soft beverage industry could be used for biotechnological recycling by yeast fermentation.

2. Rice mash with 5.21 volume % ethanol concentration was obtained after rice sediment fermentation by α -amylase and ethanol yeast.

3. Digital yeast cell morphometric characteristics correlate with fermentation parameters, correctly representing yeast adaptive reactions at various ethanol production stages.

4. The rice mash could be used for further rectification to obtain new products: ethanol distillate or bioethanol.

5. Further research is required in the study of new methods and conditions for biotechnological rice sediment recycling, ethanol yeast sedimentary processing.

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