doi.org/10.29327/217514.6.12-8

ORGANOLEPTIC AND PROTEIN ANALYSIS OF RICE BRAN FERMENTATION WITH *Saccharomyces cerevisiae* YEAST

ANÁLISES ORGANOLÉPTICAS E PROTEICA DA FERMENTAÇÃO DO FARELO DE ARROZ COM A LEVEDURA *Saccharomyces cerevisiae*

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RESUMO: O arroz é o segundo cereal mais consumido do mundo e o Brasil é um dos maiores produtores. O farelo de arroz é o resíduo do arroz (a camada externa do grão) produzido durante o processo de polimento do arroz. Esse material orgânico apresenta quantidades significativas de carboidratos, lipídios, proteínas, vitaminas e sais minerais. Porém, o farelo de arroz ainda possui seu valor comercial baixo devido ao odor e sabor de ranço durante armazenagem. Além disso, o farelo contém fator antinutricional fitato que interferem na digestibilidade, absorção ou nutrientes que podem limitar sua utilização na ração animal. Para aumentar o valor comercial do farelo, a tecnologia da fermentação por meio da aplicação de levedura pode resultar no enriquecimento de proteína, eliminação de fitato, além da eliminação da enzima que causa odor e sabor de ranço. Este trabalho tem o objetivo de analisar as transformações físico-químicas da fermentação do farelo de arroz com *Saccharomyces cerevisiae*. Trataremos o farelo de arroz com diferentes concentrações de *S. cerevisiae* de 2%, 4% e 6% w/w durante 72 horas. O farelo não fermentado foi utilizado como o controle. Observaremos mudanças de pH e suas mudanças de propriedades sensoriais tais como textura, cor, sabor e aroma. O conteúdo de proteína de farelo fermentada foi analisado pelo método Kjedahl. A fermentação aumentou o conteúdo de proteína de 13% (controle) a 15.5% (2% da levedura), 19% (4% da levedura) e 21% (6% da levedura).

Palavras- chave: Farelo de arroz, Fermentação, *Saccharomyces cerevisiae.*

ABSTRACT: Rice is the second most consumed cereal in the world and Brazil is one of the largest producers. Rice bran is the waste of the rice (the outer layer of the grain) produced during rice polishing process. This organic material has significant amounts of carbohydrates, lipids, proteins, vitamins, and minerals. However, rice bran still has a low commercial value due to rancid smell and taste during the storage. In addition, the bran contains anti-nutritional factor phytate that interferes digestibility, absorption or nutrients that may limit its use for animal

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feed. To increase the commercial value of the bran, fermentation technology through the application of yeast can result in protein enrichment, elimination of phytate, also the elimination of the enzyme that causes rancid odor and taste. This work aims to analyze the physical-chemical transformations of the fermentation of rice bran with *Saccharomyces cerevisiae*. Rice bran was fermented with different concentrations of *S. cerevisiae* 2%, 4% and 6% w/w for 72 hours. Non fermented bran was use as control. The changes of pH and its sensory properties such as texture, color, flavor, and aroma. The protein content of bran fermentation was analyzed using Kjedahl method and compared to the control. The fermentation increased the protein content from 13% (non fermented) to 15.5% (2% of yeast), 19% (4% of yeast) and 21% (6% of yeast).

Keyword: Rice bran, Fermentation, *Saccharomyces cerevisiae*

INTRODUCTION

Rice is among the most consumed cereals in the world. According to the Ministry of Agriculture, Brazil is the ninth largest producer in the world and harvested 11.26 million tons in 2009/2010. The rice production is distributed in the states of Rio Grande do Sul, Santa Catarina, and Mato Grosso (MAPA, 2013). Rice bran is the waste of the rice (the outer layer of the grain) produced during rice polishing process. The bran contains very significative amounts of carbohydrates, proteins, lipids, insoluble fibers, vitamins, and minerals (LACERDA et al., 2010). It is intended for animals feeding such as pigs, horses, cows, goats, and birds. It is also used as snacks ingredients distributed to low-income families (SILVA et al., 2006). LACERDA et al., 2009 demonstrated that cookies made with up to 50% extruded rice bran have better nutritional quality. In addition, the bran benefits health because of its antioxidant compounds and its dietary fibers. Antioxidant agents such as gamma oryzanol, tocopherol, tocotrienol and phytosterol can be used to lower cholesterol, treat menopausal symptoms, and act as an anti-aging agent, while the benefit of dietary fibers is to prevent cardiovascular disease (NAGENDRA, et al., 2011).

However, until recently, consumption of rice bran by humans was limited because of the enzyme lipase that causes instability of its properties. This enzyme hydrolyzes the bran oil into glycerol and free fatty acids that produce a rancid odor, bitter taste, and inedible (GLUSHENKOVA et al., 1998). Rice bran commercially stabilized by heat treatment is mainly used to feed animals.

Fermentation is the process that converts carbohydrates into alcohols and CO_2 involving activities of microorganisms under anaerobic conditions. According to Steinkraus (1997, 1995), fermentation of foods has five main objectives, such as: 1) enriching the diet through the development of a diversity of flavors, aromas and textures in food substrates; 2) preservation of substantial amounts of food through fermentation of lactic acid, alcohol, acetic acid, and alkaline fermentation; 3) enrichment of food substrates with protein, essential amino acids, vitamins; 4) elimination of anti-nutrients (phytic acid in rice bran) and 5) reduction of cooking time.

Penteado Feltrin and colleagues (2014) revealed in their research that the fermentation of rice bran with *Rhizopus oryzae* increased the availability of proteins by 150% (from 10 to 25% per 100 g) after 96 hours. Meanwhile, rice bran fermentation using microbial *Aspergillus niger* increased its nutritional value and reduced the phytic acid content (HARDINI, 2010). Phytic acid is the anti-nutritional factor that interferes with the digestibility and absorption of nutrients if ingested in high concentrations.

The efficiency of protein conversion by yeast depends on factors such as: temperature and availability of nutrients. The average time to double the protein content is five hours in batch fermentation systems (BURROWS, 1970). According to Park and Ramirez (1989), baking yeasts *Saccharomyces cerevisiae* are attractive organisms for the commercial production of protein due to their easy spread and not having a pathogenic relationship with man. The objective of this work was to analyze the organoleptic and nutritional characteristics of rice bran through fermentation using the yeast of *S. cerevisiae*.

MATERIAL AND METHODS

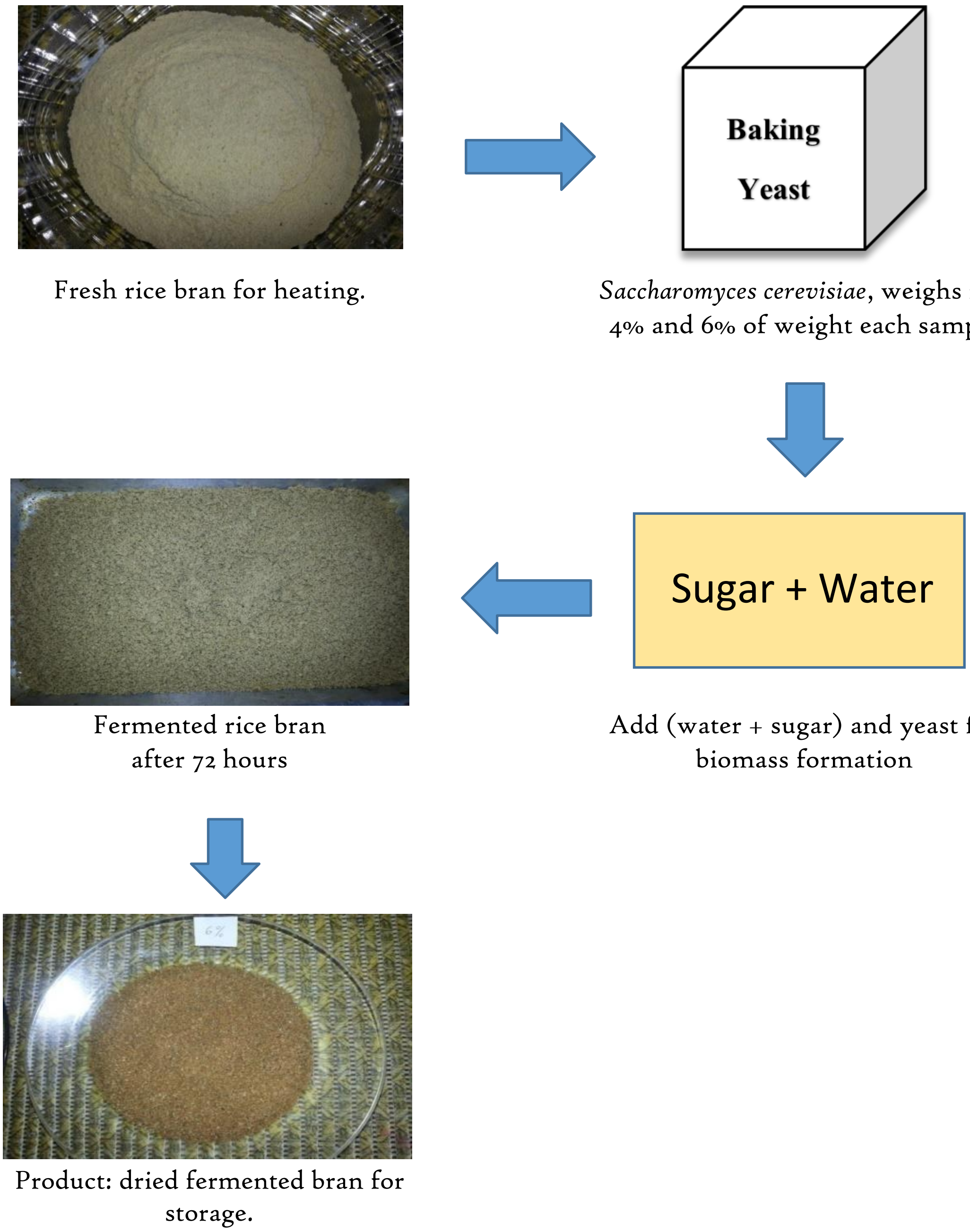
FERMENTATION PROCESS

The rice bran was obtained from the company *Arroz Cristal*, Aparecida de Goiânia. Before the fermentation process, the samples were preheated for sterilization. For the generation of biomass, water with a 1: 1 composition was added. The added water is previously mixed with sugar (3% w/w). Then, the biomass was treated with 2%, 4% and 6% w/w of baking yeast *Saccharomyces cerevisiae* then incubated at 30 ° C for 72 hours. The fermented products were dried by heating for storage purpose. Figure 1 shows the flow of fermentation processes.

pH DETERMINATION

Homogenized samples (5 g) were solubilized in distilled water (20 ml) and the pH will be determined with pH meter. The pH meter will be calibrated using standard buffers at pH 4 and pH 7.

Figure 1: The flow of rice bran fermentation process



ORGANOLEPTIC ANALYSIS

Organoleptic analyzes of the fermented product include analyzes of texture, color, flavor and aroma. Then, the fermented bran was stored for four months to observe its properties changes.

PROTEIN ANALYSIS

Total proteins were analyzed using the Kjeldahl method of the Instituto Adolfo Lutz (BRASIL, 2005). Procedure: Weigh 1 g of the sample on tissue paper. Transfer to the Kjeldahl balloon (paper + sample). Add 25 mL of sulfuric acid and about 6 g of the catalytic mixture. Heat in an electric plate in the chapel until the solution becomes blue-green and free of undigested material (black dots). Heat for another hour. Let cool. Transfer the material from the flask quantitatively to the distillation flask. Add 10 drops of the phenolphthalein indicator and 1 g of powdered zinc (to aid in the cleavage of large protein molecules). Immediately connect the flask to the distillation set. Immerse the tapered end of the soda in 25 mL of 0.05 M sulfuric acid, contained in a 500 mL Erlenmeyer flask with 3 drops of the red methyl indicator. Add to the flask containing the digested sample, using a funnel with a tap, a 30% sodium hydroxide solution until a slight excess of base is guaranteed. Heat to a boil and distill until approximately (250-300) mL of distillate is obtained. Titrate the excess 0.05 M sulfuric acid with 0.1 M sodium hydroxide solution, using methyl red.

Calculation:

$$\text{Total protein (\%)} = \frac{V \times f \times 0{,}0014 \times 6{,}25 \times 100}{P(g)}$$

$$\text{Total protein (\%)} = \frac{V \times f \times 0{,}008755 \times 100}{P(g)}$$

Where:

V = spent volume of 0.1N acid
f = 0.1N acid factor
0,0014 = milliequivalent gram of nitrogen
6,25 = general conversion factor from nitrogen to protein
P = weight

RESULTS AND DISCUSSION

According to Pestana et al. (2008), the physical-chemical characteristics of rice bran depend on factors such as cultivar, grain treatment before beneficiation, beneficiation system employed and degree of polishing to which the grain was submitted. Brazilian legislation does not establish quality standards for rice bran.

The organoleptic properties of fresh unfermented bran (control) are light brown in color, the fibrous texture of finely granulated powder, bitter taste and sui generis aroma of rice. After treated with a fermentation process, the results (Table 1, Figure 2) showed that the addition of microorganisms contributed to the improvement of taste, aroma, and texture. The fermented bran produced a golden-brown color, granular texture, pleasant aroma and flavor. The color change can be caused by heating the bran before processing which is one of the drawbacks of traditional heating. High lipid content makes the taste and aroma rancid and unpleasant. Fermentation technology then decreases the lipid level. The microorganism *S. cerevisiae* utilized the lipid substrate as an energy source in the cell's metabolism and inactivated lipase enzyme. It produced a more pleasant taste. The yeast turned sugar into alcohol that contributed desirable aroma particularly by adding 4% and 6% of yeast of w/w. The textural properties refer to the increase in particle size gradually by 6%, 4% and the largest particles were in a product treated with 2% yeast.

Table 1: Organoleptic characteristics of fermented rice bran after 72 hours

Caracteristics	*S. cerevisiae* Treatment			
	Control	2%	4%	6%
Texture	Fibrous, finely granulated powder	Agglutinated granules (granulated after heating)	Agglutinated granules (granulated after heating)	Agglutinated granules (granulated after heating)
Color	Light brown	Marrom escuro	Dark brown	Dark brown
Flavour	Bitter	Less bitter and slightly alcoholic	Pleasant, alcoholic	Pleasant, alcoholic
Aroma	Sui generis of rice	Slightly alcoholic	Alcoholic	Alcoholic

Dry product with 2% treatment. Dry product with 4% treatment. Dry product with 6% treatment.

Figure 2: Results of fermented rice bran with different treatments

The pH decreased during the fermentation process and consequently increased the acidity. According to Panda et al. (2007), pH is a fundamental factor in the development of food flavor and aroma. Coote and Kirshop (1976) revealed that the pH declines rapidly at the beginning of the fermentation process and changes more slowly in the final stage. The decrease in the speed of change in pH during active fermentation reflects the nature of the logarithmic scale originating from the conversion of the hydrogen ion concentration to its negative logarithm, which is pH. Comparing unfermented bran with the fermented bran, the pH decreased from 5.9 to pH 5.4 for bran with 2% treatment, and, pH 5.1 and 4.9 for 4% and 6% treatments respectively. A similar result was reported by Feddern et al. (2007) that the bran treated with 3% yeast of *S. cerevisiae* reduced the pH from 6.5 to 5.8 for six hours.

The changes of carbohydrates in proteins by species of microorganisms have been explored in several areas. Bioconversion of agro-industrial raw material is a potential process to produce proteins, mainly for nutritional supplementation in animal and human food (KUPSKI et al., 2012). Compared to fungi, algae and bacteria, yeasts have advantages such as easy to inoculate, high lysine content and the ability to grow at acidic pH. Among the yeasts most used in fermentation is *S. cerevisiae*. There was a significant increase in protein content for fermented bran. Unfermented bran contains 13% protein per 100g, while fermented bran contain protein as follow: 15.5% for bran treated with 2% yeast, 19.4% for 4% treatment and 21% for bran treated with 6%. Penteado-Feltrin et al. (2014) confirmed that the increase in protein solubility is also important to favor the digestibility of rice bran, as cellulose and lignin break down, which hinder the action of proteolytic enzymes. Chinma et al. (2014) reported that the rice bran fermentation using 3% of the same yeast increased the protein from 10% to 23% per 100 g of the bran after 17 hours. Meanwhile, the protein content did not significantly increase in fermentation after six hours, from 12 to 13% per 100 g (FEDDERN et al., 2007). It is worth mentioning that the composition of rice bran varies depending on the type of the rice and the milling techniques used. In addition, the fermentation time was a necessary factor for protein synthesis, and these compared results reflect this.

Fermentation also resulted in rice bran stability with a longer shelf life. After storage for four months, the properties of the fermented bran did not show significant changes.

CONCLUSION

The fermentation technology has produced organoleptically acceptable products in which the treatment of 4% and 6% generate the most desirable qualities. The pH decreased during the fermentation process. The protein content of 2% yeast was 15.5, while the treatments with 4% and 6% yeast produced 19.4% and 21% respectively, compared to the control bran (13%). In addition, the fermentation process improved the bran stability with longer shelf life.

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