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Quality assessment of japonica brown rice J02 (*Oryza sativa* L. J02) and its hydrolysis ability for the production of a rice-based drink containing an abundance of dextrin

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Abstract:

Plant-based beverages have become very popular in recent emerging markets. Brown rice milk offers several important health benefits because of its various nutritional ingredients such as proteins, carbohydrates, gamma-aminobutyric acid (GABA), and essential vitamins and minerals. Brown rice milk is also low in fats and cholesterol and free of lactose. In this study, japonica brown rice J02 from Thanh Hoa Province presented high nutrition with high protein (8.6% dm) and lipid (2.2% dm) contents as calculated from dry matter and GABA contents of 32 mg/100 g, which is suitable for the plant-based milk processing. J02 rice is also capable of hydrolysis to obtain high molecular sugar in the liquor with slow digestion ability (dextrose equivalent - DE<40) and high hydrolysis efficiency (up to more than 90%). The results obtained from HPLC analysis indicated that the hydrolysis conditions for the rice drink at 75°C and 150 g/l starch produced Spezyme alpha concentrations at 0.024%w/w and low content in low molecular oligosaccharide (DP<10) in the solution. The results indicated that Japonica rice J02 is a good candidate for the production of a rice-based drink.

Keywords: **degree of polymerization, dextrose equivalent, hydrolysis, Japonica rice, rice-based drink.**

Classification numbers: **3.1, 3.5**

1. Introduction

Rice is the most important foodstuff in Vietnam and many Asian countries. Cultivated Asian rice (*Oryza sativa* L.) makes up about 90% of the world's rice and provides about 20% of the world's dietary energy demand [1]. There are two species of cultivated rice in the world, namely, *Oryza sativa* in Asia and *Oryza glaberrima* in Africa. Among them, *Oryza sativa* is the most cultivated species and represents almost all rice products around the world [2].

Plant-based drinks and beverages have become an emerging beverage market all over the world. The plant-based beverage market was valued at $13.56 billion in 2018 and is estimated to reach $22.45 billion by 2026 while registering a Compound Annual Growth Rate (CAGR) of 6.7% from 2019 to 2026. Because they contain essential minerals and vitamins with low fat content and no cholesterol, plant-based beverages may be used as a substitute for dairy products. Besides, without lactose, plant-based beverages are also suitable for those that are lactose intolerant. Thus, plant-based drinks are considered healthy products [3]. The vegan trend in the food industry is rising with the global vegan food market expanding from $26.16 billion in 2021 to $61.35 billion in 2028 at a CAGR of 12.95% from 2021 to 2028. In recent years, plant-based drinks based on oat, soy, almond, coconut, rice, and cashew have been launched on global markets by various food industries worldwide and rice milk is one of the most popular dairy-free alternatives in Asian countries [4]. Rice milk is considered one of the best plant-based drinks. Naturally, rice milk provides various health benefits and other nutritional ingredients, which further drives the expansion of the global rice milk market. Rice and brown rice containing unsaturated fatty acids help reduce cholesterol in the blood. Vitamin B6 obtained from rice is also well-known to help maintain heart health. Thus, rice milk contains plenty of heart-healthy nutrients with lots

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of antioxidants such as tocopherol selenium, which aids in the prevention of all types of infections and many other heart diseases. In addition to these factors, the global rice milk market is expected to witness substantial growth at a CAGR of 9% over the forecast period of 2020-2030 [4].

Rice is an essential product of Vietnamese people. The System of Rice Intensification (SRI) is known as a farming methodology that is low water and labour-intensive that uses younger seedlings individually spaced and typically hand weeded with special tools. This method has been applied in Thieu Hoa, Thanh Hoa and was aimed at increasing the yield of rice produced in farming as well as product quality. The production of organic rice according to SRI and SRI2 methods creates a high level of protein products and a low level of carbon emission to produce clean and safe rice guarantees for health and safety. The local company tends to associate with the community and nearby cultivators to develop organic Japonica rice (*Oryza sativa japonica var*. J02) grown in an area that helps decline greenhouse emissions, create an environmentally friendly product, provide a source of clean materials for cultivators, and increase income as well as product price. This method is a point of strength for the company as their rice product is an optimal raw material for producing a domestic rice drink in Vietnam.

Brown rice contains more nutrients than white rice such as digestible fiber and antioxidant compounds like selenium, manganese, tocopherol, and GABA from brown rice germ and endosperm, which are all known to promote beneficial effects on human health [5]. Recent epidemiological studies have shown that the consumption of whole grains can reduce the risk of metabolic disorders, cardiovascular diseases, and some types of cancer [6]. Thus, brown rice is an ideal raw material for rice drink processing. Our previous study was successful at obtaining hydrolysis conditions for rice powder by Spezyme Alpha to apply to rice drink processes [6]. In this study, the organic Japonica rice (*Oryza sativa japonica* var. J02), produced by SRP (Sustainable rice production) farming methodology, was assessed for its quality. The hydrolysis ability was also investigated for the further development of rice milk technology with high benefit and nutrition.

2. Materials and methods

2.1. Materials

Japonica brown rice J02 (*Oryza sativa japonica var*. J02) was obtained from Lam Son JSC, Trieu Hoa, Thanh Hoa. The paddy was farmed by the System of Rice Intensification (SRI) farming methodology, and, after harvest, the paddy was passed through a rice hulling machine system (SH 100, China) according to factory processing standards after many steps such as drying, cleaning, sifting, gravity grading, and magnetic separation. The length of the obtained brown rice grains was not longer than 6 mm; the impurity content was less than 0.2 %; and damaged grain accounted for less than 0.15%. From a sensory aspect, the rice grains had a typical rice scent with no strange odour and were short and slender in shape.

The rice was ground with a superfine, two-stage grinding system (60B, SH CO, China) with the practical size from 20-120 mesh. Spezyme Alpha was kindly supplied from DuPont, Wilmington, Delaware, USA [7].

2.2. Methods

Physiochemical analysis: Protein content was determined according to the method of TCVN 8133-1-09. The fiber, ash, lipid, amylose, moisture, and carbohydrate contents were analysed according to TCVN 4329:2007, TCVN 9939:2013, TCVN 10730:2015, TCVN 5716-1:2008, TCVN 1643:2008, and TCVN 4074:09, respectively. GABA analysis was carried out according to the method described by C.T.T. Quynh, et al. (2013) [8]. The safety of the rice was confirmed by the determination of lead, cadmium, total aflatoxin and aflatoxin B1, carbaryl residue, chlorantraniliprole residue, flutolanil residue, and total mould and yeast according to TCVN 7602:2007; TCVN 7603:2007; TCVN 7569:75962007; TC 86/69 TL; TCCS 10/2010 BVTV; TCVN 7082:2002; TC 96/98-CL; TCVN 8101:2009; and TCVN 8275-1:2009, respectively.

The dextrose equivalent was determined as the percentage (in dry basis) of glucose to the total sugar in the supernatant of the hydrolysed syrup. The hydrolysis yield was expressed as the ratio of obtained reducing sugar to the initial starch content. The glucose and total reducing sugar were determined by the DNS method [9].

Determination of oligosaccharide: Oligosaccharide determination was carried out using HPLC with a refractive index detector (RID). Water (H_2O) (100%) was used as a mobile phase with the flow rate of 0.1 ml/min and sample injection volume of 20 µl [10]. The RID high-pressure liquid chromatography system HPLC 1290 - Agilent was used to analyse the sugar and oligosaccharide contents in the hydrolyzation product. The following instruments: HyperRez XP analysis column, Carbohydrate Na^+, (7.7x300 mm) with guard column HyperRez XP, Carbohydrate Na^+, (7.7x50 mm) (Thermo Fisher Scientific, UK) were used for this study. Standard linear oligosaccharide standards include glucose, maltose, maltotriose (DP3), maltotetraose (DP4), maltopentaose (DP5), maltohexaose (DP6), maltoheptaose (DP7), maltooctaose (DP8), maltononaose (DP9), maltodecaose (DP10), and linear maltooligosacharide (DP=10-40) [10].

2.3. Hydrolysis of brown rice J02

The hydrolysis of brown rice J02 by Spezyme Alpha (α-amylase) was carried out according to the previous study [6]. Spezyme Alpha is a thermostable starch that hydrolyses α-amylase produced from a strain of genetically modified bacteria *Bacillus licheniformis* that catalyses the hydrolysis of α - 1,4 glucoside bonds inside the polysaccharide chain, thereby converting starch into soluble dextrin, which reduces the viscosity of a starch slurry. The Spezyme alpha enzyme is recommended to be used at a temperature of 83-85°C at an enzyme concentration 0.02-0.025%(w/w) and pH 5.7-5.8 [7].

To apply Japonica rice J02 for plant-based drink processing on an industrial scale, the hydrolysis ability of J02 rice by alpha amylase was carried out at 150 g/l in dry matter with the hydrolysing condition of 75ºC for 60 min with the enzyme concentration of 0.024%w/w without pH adjustment according to a previous study [6]. After partial saccharification, the syrup is heated for enzyme deactivation and to prevent further glucose formation. The obtained rice liquor was identified as reducing sugar content, starch content, hydrolysis yield, and the dextrose equivalent (DE) index. During the hydrolysis duration, the samples were collected at 15 min, 30 min, 45 min, and 60 min and the starch residue, hydrolysis efficiency, and the DE index were analysed.

The oligosaccharide compositions of the obtained rice liquid were also determined by the method described above. All the experiments were carried out in triplicate and the results were present as the average of the experimental results.

3. Results and discussion

3.1. Composition of Japonica brown rice J02

The nutritional value of brown rice J02 was identified by its moisture, starch, total carbohydrate, protein, lipid, cellulose, ash, and GABA contents. The compositions of J02 are presented in Table 1. Brown rice contains 83% grain dry matter whereas proteins account for around 8.6%. The lipid content was 2.2% in brown rice J02 and the cellulose and ash contents were 1.1 and 14%, respectively. Besides these main substances, brown rice J02 also contains GABA with a content of 32 mg/100 g. This brown rice has protein and lipid contents much higher than those in white rice with 6.7 and 0.4% dry matter, respectively [5], because of the remaining rice bran in brown rice. Thus, the rice-based drink obtained from brown rice should provide more nutritional value than normal rice milk.

Table 1. Chemical composition of Japonica rice J02.

Chemical elements	Unit	Content
Moisture	%	14.5±0.2
Starch	% dry matter	83.7±0.7
Carbohydrate	% dry matter	3.3±0.1
Protein	% dry matter	8.6±0.1
Lipid	% dry matter	2.2±0
GABA	mg/100 g	32±1.4
Cellulose	% dry matter	1.1±0.1
Ash	% dry matter	1.4±0

Compared with brown rice nutrients reported in a previous study [5], the protein and lipid obtained from J02 was higher at 15.7 and 14.67%, respectively. The GABA present in the peripheral nervous system is well known as the main inhibitory neurotransmitter in the central nervous system [11]. Brown rice J02 contained 32 mg of GABA per 100 g of rice. Compared with the report obtained by D. Karladeea, et al. (2012) [12], the GABA content in J02 was approximately 10 times higher than Chieng Mai brown rice. Compared with the analysis results of brown rice from Bac Huong (Vietnam) for rice milk production, we find that the nutritional content of the brown rice of the Japanese variety J02 is higher in terms of nutritional content, for example, the protein content was 7.52±0.12 g/100 g while other components were similar [6]. This is the advantage of J02 rice as it can provide products processed from J02 with higher protein content, lipid content, and high concentrations of GABA making it a valuable and suitable raw material for a plant-based drink.

3.2. Food safety of Japonica brown rice J02

Common heavy metal pesticide residues and mycotoxins are presented in Table 2. The results show that all indicators were not detected or at levels much lower than the allowable limit. This confirms the safety of the protein-rich rice J02 when it used to produce a brown rice drink on an industrial scale.

Table 2. Residues of pesticides, heavy metals, and mycotoxins of Japonica rice J02.

Chemical elements	Unit	Content
Lead	mg/kg	0.072
Arsenic	mg/kg	Non detected
Cadmium	mg/kg	Non detected
Total aflatoxin	µg/kg	Non detected
Aflatoxin B1	µg/kg	Non detected
Carbaryl	mg/kg	Non detected
Chlorantraniliprole	mg/kg	Non detected
Chlordane	mg/kg	Non detected
Flutolanil	mg/kg	Non detected
Sulfuryl fluoride	mg/kg	Non detected
Total mould and yeast	CFU/g	Non detected

3.3. The hydrolysis of Japonica rice using enzymes

DE is calculated by the amount of reducing sugars in a total sugar product. Therefore, the dextrose equivalent describes the degree of conversion of starch to dextrose. The purpose of rice hydrolysis is to obtain rice milk with an abundance of long chain oligosaccharides (e.g., starch-derived low digestible oligosaccharides), but not final products of starch hydrolysis such as glucose or fructose, which might increase the glycemic index (GI). The preferred hydrolysis condition gives rice milk with a low DE index (less than 40) and high hydrolysis yield (or high in the ratio of obtained reducing sugar to the initial starch content).

The result obtained in Table 3 indicates that the hydrolysis efficiency and DE values increased with heating time. The DE value was quickly attained at 25 after 15 min of enzymatic reaction. After that, the DE value slowly increased to 32.48 after 30 min of heating and the maximum of DE value was obtained after 60 min of heating with a value of 35.2. The hydrolysis efficiency attained approximately 60% after 15 min of heating. After 60 min of heating, the hydrolysis efficiency was more than 90% in value, higher than the results obtained in a previous study on Bac Huong rice [6]. The results indicate that Japonica brown rice J02 should be considered a prospective raw material for rice drink processing.

Table 3. Effect of hydrolysis time on the composition of oligosaccharides in Japonica rice hydrolysate by Spezyme-α.

Sample	15 min	30 min	45 min	60 min
Starch (g/l)	50±1.4	46.9±0.4	15±0	10±0.7
Degree of polymerization DP <10 (g/l)	43.8±0.2	43.5±0.6	45.5±0.2	42±0.1
Dextrose equivalent (DE)	25	32.48	34	35.2
Hydrolysis efficiency (%)	60.1	62.6	88.05	92.6

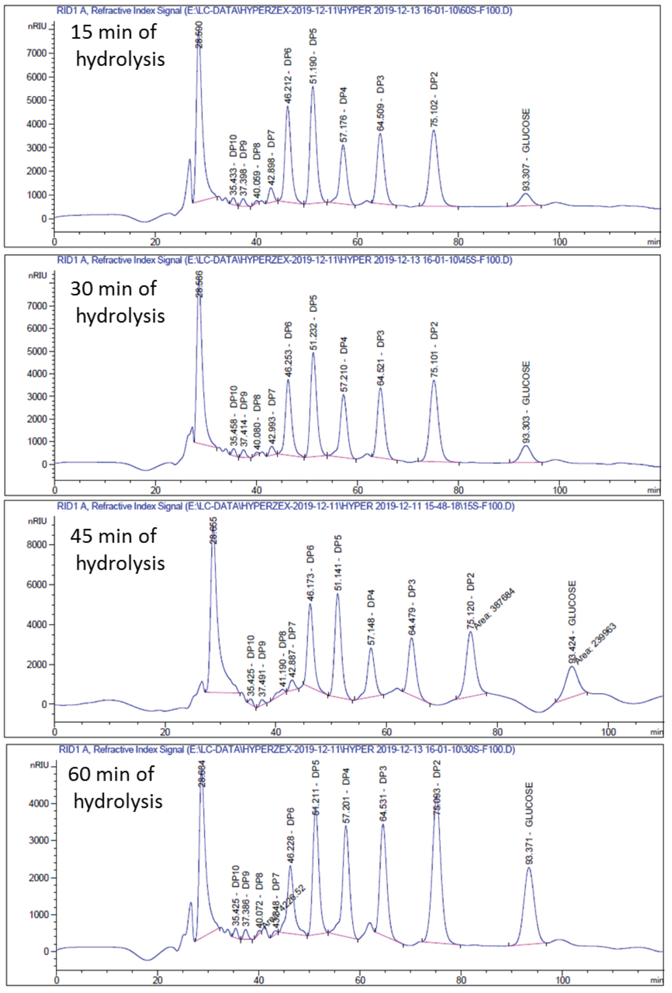

Fig. 1. High-performance liquid chromatography chromatograms of oligosaccharides in Japonica rice hydrolysate by Spezyme-*α*.

Starch is a polysaccharide with 10,000 monosaccharide or dextrose units. By enzyme hydrolysation, the starch molecule is broken up at α1-4 linkage glycosidic bonds to form dextrin with long chains of glucose and oligosaccharides with high or low molecular chain length (DP) of 2-10 shorter molecules and glucose.

In an attempt to indicate the starch hydrolysation, the DE is used. Rice hydrolysis products may contain glucose, maltose, dextrin, oligosaccharides, polysaccharides, etc. Lower DE values and higher in degrees of polymerization DP in the obtained hydrolysis rice products will promote lower digestion energy of rice-based drinks. Thus, this kind of product would be very beneficial for customers with chronic diseases such as diabetes or high blood sugar. The purpose of rice hydrolysis is to obtain rice milk with an abundance of long chain oligosaccharides, which are known as starch-derived low digestible oligosaccharide components. The preferred hydrolysis condition should produce rice milk with a low DE index and high hydrolysis yield. To evaluate the quality of J02 rice gelatinisation and liquefaction by enzyme, the DP amount of obtained hydrolysis products were analysed by HPLC. The results

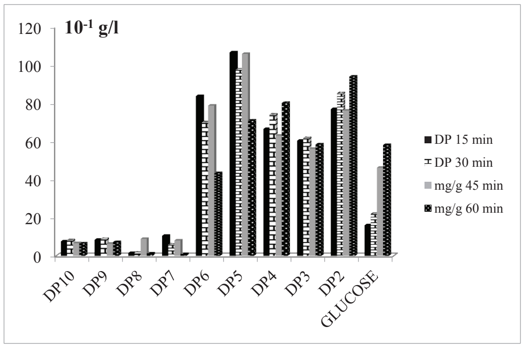

Fig. 2. Effect of hydrolysis time on the composition of oligosaccharides in Japonica rice hydrolysate by Spezyme-*α*.

are presented in Figs. 1, 2, and Table 3. The results indicated that prolonging the heating process did not greatly increase the formation of DP<10 in the solution but did increase the hydrolysis efficiency of rice starch or the formation of DP>10. C.T. Luyen and H.T. Binh (2015) [13]carried out the hydrolysis of several An Giang rice types for rice milk processing. However, the final products contained very high glucose content. This means that the products might quickly increase blood sugar. By using only alpha amylase with the control of hydrolysis conditions in the liquefaction of J02 brown rice, liquid rice products with low DE and low DP components should be achievable for the production of a healthy rice-based drink. It should be concluded that Japonica rice J02 from Lasuco showed the capacity for hydrolysis by enzyme to produce rice liquid containing high molecular sugar, which is very valuable for the production of a rice-based drink.

4. Conclusions

Brown Japonica rice J02 presented high nutritional value with high protein (8.6% dm), lipid (2.2% dm), and GABA contents (32 mg/100 g) and is suitable for plant-based drink processing. The hydrolysis of brown Japonica rice J02 led to the formation of high molecular sugar in the liquor with slow digestion ability (DE<40) and high hydrolysis efficiency (up to more than 90%). The results obtained from HPLC analysis indicated that the hydrolysis condition for a rice drink at 75ºC, 150 g/l starch, and Spezyme alpha concentrations at 0.024% w/w produce low molecular oligosaccharide content (DP<10) in the solution. These results indicate that Japonica rice J02 is a good candidate to produce a rice-based drink.

CRediT author statement

Thu Trang Vu: Conceptualisation, Methodology, Investigation, Draft preparation; Thi Trang Nguyen, Hong Quan Duong, Van Hung Nguyen: Data curation and Analysis; Viet Hung Le, Viet Hung Nguyen, Xuan Lam Nguyen, Van Tan Le: Editing; Giang Hoang, Quoc Tuan Hoang, Thi Thao Nguyen, Tien Cuong Nguyen, Ngoc Hung Pham, Hong Son Vu, Thi Hanh Nguyen: Data analysis; Ky Son Chu: Validation.

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COMPETING INTERESTS

The authors declare that there is no conflict of interest regarding the publication of this article.

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