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Effect of alpha-amylase hydrolysis on reducing sugar and viscosity of two brown rice flours

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ABSTRACT

Brown rice flour is very attractive as an ingredient for functional food. The high viscosity of starch component can be reduced by enzymatic hydrolysis. However, there is limited information about enzyme treatment on brown rice flour. The objective was to investigate the effect of alpha-amylase hydrolysis on two varieties of brown rice flours (Sinlek rice and Jasmine rice). Both of them contain the same level of amylose content. In addition, Sinlek rice (a crossbred of Jasmine rice and Hom Nil rice) is nutritious and presents high amount of iron. Brown rice flour was prepared by milling with pin mill and then sieving (14 mesh sieve). Proximate composition and pasting profile measured by Rapid Visco Analyzer were determined to characterize the material. Alpha-amylase (Termamyl SC, 1.80 U/g and 4.40 U/g) was introduced in 20% (w/v) brown rice flour at a temperature of 90±2 ^{o}C and hold for 90 min. Reducing sugar and Brookfield viscosity of the hydrolyzed products were measured. The results showed that the unhydrolyzed Jasmine brown rice flour presented higher peak, breakdown and final viscosity than another sample. On the contrary, the hydrolyzed brown Jasmine rice exhibited less viscous when comparing at the same level of α-amylase. The viscosity of the hydrolyzed product was found to have a statistically significant difference between a couple of rice varieties as well as two levels of enzyme activity. In term of reducing sugar, it was only found a significant difference in enzyme level. The present study could provide guidance for food applications i.e. beverage and instant mixes.

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INTRODUCTION

Rice is the most important cereal crop in the world and the most staple food of over half the world's population (Zhai *et al*., 2001). It has been used in various food and industrial applications as an agent for thickening, gelling and filling, cosmetic dusting powder and photographic paper powder (Champagne, 1996). Brown rice has been becoming popular as its nutritional benefits and numerous health benefits including a reduced risk of coronary heart disease, diabetes, obesity and some forms of cancer (Mann and Cummings, 2009).

An approach to increase the valuable products of Sinlek and Jasmine brown rice flours is to use as a functional ingredient for beverage or instant mixes. However, with its viscosity, it cannot be prepared at high concentration. This limitation can be overcome by treating brown rice flour with an enzyme. Many factors, including composition, granular size and structure, type of crystal polymorph, amylose/amylopectin ratio, gelatinization properties, lipid-complexed amylose, and presence of non-carbohydrate component affect the qualities of rice flour (Lin *et al*., 2011; Yu *et al*,. 2012) and the susceptibility to enzymatic hydrolysis.

An understanding of the relationship between level of enzyme and viscosity of hydrolyzed product is very important for using in the food industries. The present study was designed to study the effect of alpha-amylase hydrolysis on reducing sugar and viscosity of Sinlek (SinBRF) and Jasmine (JasBRF) brown rice flours.

MATERIALS AND METHODS

Materials

SinBRF and JasBRF were obtained from Sininrice Co., Ltd. (Nakorn Pathom, Thailand). Thermostable alpha-amylase, Termamyl® SC, was provided from the Brenntag Ingredient (Thailand) Public Co., Ltd. and produced by a genetically modified strain of a Bacillus microorganism. This enzyme has optimum activity at a pH around 5.4 - 6.2 and at temperatures between 85 °C and 95 °C. The enzyme activity (4,444 units/mL) was measured according to the method of Bernfeld (1955). One unit of activity is defined as the amount of enzyme that catalyzed the liberation 1.0 mg of maltose from starch in 3 min at pH 6.9 at 20°C.

Chemical analysis

The moisture, protein, fat and ash contents of both brown rice flours were analyzed using AOAC methods (AOAC, 1990). Moisture content was determined by gravimetric method in a hot air oven at 105 °C until constant weight. Protein and fat contents were determined by using a standard Kjeldahl distillation and Soxhlet extraction method, respectively. Ash content was determined by weighing the resulting inorganic residue using a muffle furnace at 600 °C for 2 h to oxidize all organic matter.

Pasting properties

Pasting properties of brown rice flours were analyzed using a Rapid Visco-Analyser (RVA). Flour slurries (10% w/w; dry solid basis, 25 g of total weight) were equilibrated at 50 °C for 1 min, heated at 12 °C/min to 95 °C, maintained at 95 °C for 3.5 min, and cooled to 50 °C at the same rate. A constant rotation speed of the paddle at 160 rpm was used.

Enzyme hydrolysis

SinBRF and JasBRF were prepared by pin milling and sieving (14-mesh sieve). The suspension of brown rice flour prepared at 20% (w/v; dry solid basis) was heated to 90 ± 2 °C with a continuous stirring at 320 rpm in order to gelatinize starch. The gelatinized sample containing calcium chloride (200 ppm) was then hydrolyzed with alpha-amylase (Termamyl® SC) at 1.80 and 4.40 U/g solid. After 90 min the reaction was terminated by adjusting the pH to 3.0 with 1 N HCl at a temperature higher than 90 °C for 5 min The hydrolyzed samples were then neutralized to pH 6.5-7 with 1 N NaOH at 60 °C according to the method of Mc Pherson and Seib (1997).

The degree of hydrolysis was measured as an increase in the content of reducing sugars. It was measured using the dinitrosalicylic acid method (Miller, 1959). Glucose was used as a standard. The absorbance was measured at 540 nm by using a UV/visible spectrophotometer. The viscosity of hydrolyzed flour was measured by Brookfield Viscometer at 30 °C with a constant rotational speed of 30 rpm.

Statistical analysis

The data reported were the means of triplicate measurements. Statistically significant tests were performed using analysis of variance (ANOVA) at the 95% confidence level ($P < 0.05$). Means were separated using Duncan's multiple range test. All statistical analysis were evaluated using SPSS 10.0 software (SPSS, Inc., Chicago,IL, USA).

RESULTS AND DISCUSSION

The data presented in Table 1 revealed the proximate chemical composition of brown rice flour. JasBRF showed higher moisture, protein and fat contents than SinBRF (Table 1). Protein content of JasBRF and SinBRF used for enzymatic hydrolysis are 7.73% and 8.37%, respectively. Protein is highly concentrated on the surface of milled rice and decreases toward the center of the kernel (Champagne *et al*., 2006). Therefore, harder and stronger zones in the kernel would result in coarser particles.

Table 1 Proximate composition of Sinlek and Jasmine brown rice flours (percent dry weight).

Chemical composition	SinBRF	JasBRF
Moisture content	12.24 ± 1.29	13.65 ± 0.54
Protein	8.17 ± 0.11	8.41 ± 0.23
Fat	2.53 ± 0.05	2.73 ± 0.26
Ash	1.76 ± 0.01	1.83 ± 0.04
Carbohydrate	87.54 ± 0.13	87.03 ± 0.52

Both native SinBRF and JasBRF exhibited comparative pasting profiles (Table 2). JasBRF presented higher peak and final viscosities than SinBRF. The difference in peak viscosity could be due to the variation in water absorption rate and swelling of starch granules during heating as well as degree of starch damage. JasBRF showed high level of starch damage resulting in high peak viscosity (Sanni *et al*., 2001). In addition, JasBRF showed higher breakdown viscosity than SinBRF. Breakdown viscosity measures the susceptibility of the cooked starch to disintegration. The high breakdown viscosity indicated the lower ability of the starch to resist heating and shear stress during cooking (Adebowale *et al*., 2005).

Table 2 Pasting properties of Sinlek and Jasmine brown rice flours

Parameter	SinBRF	JasBRF
Pasting Temperature (°C)	87.50 ± 0.48[a]	86.17 ± 0.80[a]
Peak Viscosity (RVU)	182.75 ± 7.92[a]	231.00 ± 5.46[b]
Trough Viscosity (RVU)	146.64 ± 4.82[a]	153.42 ± 1.83[a]
Breakdown Viscosity (RVU)	36.11 ± 4.43[a]	77.58 ± 4.22[b]
Final Viscosity (RVU)	301.70 ± 7.71[a]	318.19 ± 8.14[a]
Setback Viscosity (RVU)	118.95 ± 1.34[b]	87.19 ± 2.80[a]

*Values with similar letters in the same row do not differ significantly ($P \leq 0.05$).

The degree of hydrolysis is expressed as reducing sugar content (Table 3). The reducing sugar of hydrolyzed SinBRF and JasBRF using 1.80 units of enzyme per gram of dry solid were 25% and 26 %, respectively. Treating the starch with greater amount of enzyme (4.40 U/g) enhanced the degree of hydrolysis of starch significantly ($P<0.05$). Therefore, the reducing sugar of hydrolyzed products increased. The hydrolysis of glycosidic bonds in gelatinized starch by amylase would result in the reduction of viscosity. The viscosity of hydrolyzed samples decreased as the amount of enzyme increased (from 88.2 cps to 59.8 cps for JasBRF and from 108.1 cps to 88.1 cps for SinBRF). The difference in viscosity reduction was due to both rice variety and level of enzyme activity. This indicated that enzyme treatment decreased the overall resistance of sample to flow such that the product can flow and spread easily.

Table 3 Reducing sugar and viscosity of hydrolyzed flours (20% flour, 90 ± 2 °C, 90 min)

Sample	Enzyme (U/g solid)	Reducing sugar (%)	Viscosity (cps.)
SinBRF	1.80	25 ± 1[a]	108.1 ± 10.3[b]
	4.40	46 ± 6[b]	88.1 ± 15.4[b]
JasBRF	1.80	26 ± 2[a]	88.2 ± 17.6[b]
	4.40	42 ± 4[b]	59.8 ± 10.4[a]

*Values with similar letters in the same column do not differ significantly ($P \leq 0.05$).

Although the unhydrolyzed JasBRF was higher pasting profile than SinBRF, the hydrolyzed JasBRF showed less viscous when comparing at the same level of α-amylase. The viscosity of brown rice flour is an overall viscosity. Therefore, it may be affected by other compositions i.e. non-starch polysaccharides or dietary fiber. Bonnand-Ducasse *et al*. (2010) reported that the presence of non-starch polysaccharides noticeably affects the pasting and rheological properties of starch or flour dispersions. Lai *et al*. (2011) found that an addition of dietary fiber (at 5% w/w on dry starch basis) caused significant increases in swelling power and pasting viscosities of 10% (w/w) rice starch dispersions. Furthermore, the dietary fiber improved the rheological parameters of the 10% (w/w) rice starch gel. Moreover, Tester and Sommerville (2003) studied the hydrolysis with α-amylase in starch-non starch polysaccharide systems and found that the reductions in the α-amylase hydrolysis were always greater as the concentration of non-starch polysaccharides increased and were more marked at high starch to water ratios. For decreasing amounts of water, the gelatinization process is restricted leading to a decrease in enzymatic hydrolysis.

CONCLUSION

Brown rice flour is a complex product. It presents of many compositions affecting the ability of enzyme hydrolysis. Level of enzyme activity had an influence on both reducing sugar content and viscosity of the hydrolyzed product. However, rice varieties (SinBRF and JasBRF) only affected on the viscosity of the sample. Therefore, the selection of hydrolyzed flour for each application should consider level of enzyme activity and rice variety as well as hydrolysis conditions (pH, temperature and time) in order to obtain a desirable reducing sugar content and product viscosity.

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