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Original Article

Effect of calcium chloride and pH on the nutritive values and *in vitro* starch digestion of germinated brown rice (GBR), texture and sensory evaluation of rice jelly

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Abstract

This study aimed to determine the nutritive values of germinated brown rice (GBR) after soaking in calcium chloride and citrate buffer solutions. The GBR was used to produce germinated brown rice powder (GBRP) for application in a rice jelly product. Results showed that calcium chloride with acidification provided of nutritive values of GBR. It was found that the free GABA and total phenolic content were significantly increased. In contrast, the total starch and estimated glycemic index of the GBR were all significantly decreased. Texture properties of the rice jelly from GBRP were found to have decreased compared with the non-GBR. The sensory evaluation showed that consumers had a preference for the rice jelly produced from the GBRP. It was concluded that GBRP rice jelly could be produced to provide a nutritious low-medium GI product, which could be used as a functional food alternative for diabetics

Keywords: germinated brown rice, free GABA, total phenolic, *in vitro* starch digestion, rice jelly

1. Introduction

Germinated brown rice (GBR) is a functional ingredient, which is produced by soaking brown rice grains in water to promote germination (Komatsuzaki *et al.*, 2007). GBR has gained much attention in the literature, with research conducted to study its nutritional composition and bioactivity (Cornejo, Caceres, Martinez, Rosell & Frias, 2015). During this process of germination, the biochemical activity produces essential compounds which drastically change the chemical composition of the rice; resulting in higher amounts of nutrients, such as, γ-aminobutyric acid (GABA), phenolic compounds and γ-oryzanol, compared with brown rice (BR) (Caceres, Martinez, Amigo & Frias, 2014; Patil & Khan, 2011). The nutritional and bioactive compounds of GBR are influenced by the nature of the raw material, steeping time, soaking solutions, and germination conditions (Charoenthaikij *et al.*, 2009; Komatsuzaki *et al.*, 2007). However, modification of these factors may also affect GBR's nutrient bioavailability and utilization. The germination of brown rice is activated and this decomposes large molecular substances such as starch, non-starch polysaccharides and proteins, and converts them into small molecular compounds. The process results in the germinated seeds containing more simple sugars, peptides and amino acids (Moongngarm & Saetung, 2010). In addition, the germination process significantly affects starch degradation related to pasting properties of GBR flour (Moongngarm, Moontree & Padtong, 2014; Charoenthaikij, Jangchud, Jangchud, Prinyawiwatkul & Tungtrakul, 2010). This is useful to improve the texture quality of existing or new products; particularly as brown rice has a chewy texture and is not easily digested. Indeed, it has previously been observed (Jiamyanyuen & Ooraikul, 2008) that germination improves the textural and sensory properties of cooked rice. Similarly, Kayahara & Tsukahara (2000) reported GBR to possess a softer texture compared with normal brown rice, making it both easier to cook and digest. Moreover, the germination conditions may change the physical structure of the starch, influencing the glycemic index (GI) of the rice.

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Brown rice is reported to have a higher GI (96) compared with white rice (83) and freshly cooked rice (64–93), respectively (Miller, Pang & Bramall, 1992). In order to control the rice starch digestion and glycemic response, various methods, such as heat-moisture treatment (Wang, Lui, Chen, Li & Xie, 2018) and the recrystallization of debranched starches (Kiatponglarp, Tongta, Rolland & Buleon, 2015) have been used. It has been reported the structural modification of starch can be changed digestibility. It can be changed via structural modification and complexation with other food-derived ingredients, particularly polysaccharides (Chen, Xie, Zhao, Qiao & Liu, 2017), proteins (Chi, Li, Zhang &Chen, 2018) and phenolic compounds (Koh, Wong, Loo, Kasapis & Huang, 2010). Since the consumption of GBR is associated with improved human health, the development of nutritional innovative GBR products have become of interest to the food industry. GBR is often used as an enhanced nutrient flour substitute in foods such as bread (Cornejo, Caceres, Martinez, Rosell & Frias, 2015), cookies (Chung, Cho & Lim, 2014), noodles (Gong *et al.*, 2017) and porridge (Khoirina, Wardhani, Murtini, Kusnadi & Yuwono, 2019). From the above review of literature, it was observed that the products from GBR contains rich bioactive components, partial substituted with GBR flour (30–70 g/100 g, solids basis) and poor edible quality. This is because during germination, degradation of starch by amylase affects the pasting viscosity, negatively influencing the textural characteristics and sensory properties of the product. Consequently, GBR should only be applied for softening the texture of products.

The Jellies are usually made by cooking fruit juice with sugar (Shinwari & Rao, *2018*). However, to our knowledge the addition of GBR to jelly has not been explored. In this study, we would be likely to study the probability of jelly products using GBR. Germinated brown rice powder (GBRP) can be used to produce jelly by exposing the materials to spray drying. Therefore, the development of this type of jelly may help expand the range of products available to the consumer. Accordingly, the aim of this study was to determine the effects of calcium chloride and pH buffers on GBR nutritive values (GABA and phenolic compounds) and *in vitro* starch digestion. The GBR was used to produce GBRP and evaluated in a rice jelly product.

2. Materials and Methods

2.1 Materials and preparation of brown rice

Khao Banna 432 (*Oryza sativa* L. cv. PCRC92001-432) paddy rice was provided by the Department of Agriculture, Prachinburi province, Thailand. Brown rice was produced by mechanically removing the husk of paddy rice. It was packed in plastic bag made of liner low density polyethylene (LLDPE) and stored at 10 ºC prior to the experiment.

2.2 Germination condition and preparation of germinated brown rice powder (GBRP)

Brown rice seeds were sterilized with 0.1% sodium hypochlorite solution (1:3 w/v) for 30 minute and then rinsed with distilled water. Afterwards, brown rice seeds were soaked in solutions (1:3 w/v: seeds: solution ratio). The solution conditions were 0.5 and 1.0 mM of $CaCl_2$ in citrate buffer adjust pH 3 and pH 5. Soaked was carried out at 35±2 ºC for 48 hrs and then rinsed with distilled water. The obtained GBR (GBRC5P3, GBRC5P5, GBRC10P3 and GBRC10P5) were collected, packed in metallized bags and stored at 4±2 ºC until analysis. After germination, seeds were produced to germinated brown rice powder (GBRP) with spay dry process. The GBR sample was mixed with water ratio of 1:12 (w/v) and then blended for *3* minute (Philips, EM-*44*A, Astute Electrics Thailand Co., Ltd.). It was pasteurized at 80±2 ºC for two minutes and mixed with 15g/100g of maltodextrin (DE10) (Thai Food and Chemical Co., Ltd.), total soluble solid (45±2 ºBrix). It was fed into a spray dryer (JCM Engineering Concept., Thailand, SD-06) and the flow rate was maintained at 25 mL/min. The inlet air temperature of the spray dryer was operated at 130 ºC.

2.3 Rice jelly produces

The rice jelly form GBRP was prepared using the recipe consist of GBRP (10%), gelatin powder 240 bloom (5%), 10% of skim milk powder/100 formula (Thai Food and Chemical Co., Ltd.) and 5% of sugar /100g formula) (Mitr Phol Sugar Corp., Ltd.,Thailand), based on weight basis. The all ingredients were mixed with water (*1*: 2.8 w/v) and heat to 80 ºC on hot plate for two minutes to dissolve. Then, it was poured into a 30 ml plastic cup (2.75 cm diameter) and cooling down to room temperature, the cup was stored at 4±2ºC in a refrigerator for 24 hrs before analysis. The rice jelly products were made from GBRP coded (RJ1, RJ2, RJ3 and RJ4). The control was non-germinated powder (RJP).

2.4 Determination of free GABA content

Free GABA content was determined in triplicate according to the method of Cohen & Michaud (1993), using HPLC (Agilent 1100 Series, Agilent Technologies, Calif., USA) equipped with a column (Supelcosil™ LC-DABS, 4.6 mm I.D.x150 mm, Sigma-Aldrich Co. LLC, St. Luis, Mo., USA). Acetonitrile-acetate buffer pH 6.8 (20:80, v/v) was used as the mobile phase with a flow rate of 1.0 mL min^{-1} and an injection volume of 10.0 µL. The column temperature was 40 ºC and UV detector was set at 315 nm.

2.5 Determination to total phenolic content (TPC)

The total phenolic content of the sample was determined by the Folin–Ciocalteu reagent, according to method of Lai, Li, Lu & Chen (2009).The sample 0.1 ml of the extract solution (*1* mg/ml distilled water) was transferred into a test tube containing 0.1 ml of 50% Folin–Ciocalteu reagent and mixed thoroughly with 2 ml of 2% (w/v) sodium carbonate. The mixture was allowed to stand for a further 30 min in the dark and absorbance was measured at 750 nm. The total phenolic content was calculated from the calibration curve, and the results were expressed as mg of gallic acid equivalent (GAE) per g dry weight.

2.6 *In vitro* starch digestion and expected glycemic index

In vitro starch digestion of sample was determined following the method by Goni, Alonso & Calixto (1997). The glucose concentration was measured using glucose oxidase-peroxidase kit GOPOD reagent (Megazyme International Ireland, Ltd.). The rate of starch digestion was expressed as a percentage of the total starch hydrolyzed at different times (30, 60, 90, 120, 150, and 180 min). A non-linear model established by Goni, Alonso & Calixto (1997) was applied to describe the kinetics of starch hydrolysis. The first order equation has the form:

$$C = C_{\infty} (1 - e^{-kt})$$

where C corresponds to the percentage of starch hydrolyzed at time t, C_{∞} is the kinetic constant and t is time (min). Expected GI was estimated using the model

$$GI = 39.71 + (0.549 \times HI)$$

2.7 Total starch content (TS)

The TS content was measured according to the AOAC International method 996.11 (AOAC, 1998) by using Megazyme assay kit (Megazyme International Ireland, Ltd.)

2.8 Texture properties of rice jelly

The texture analysis was carried out following Lee, Yeom, Ha & Bae (2010) by using the TA-XT plus Texture Analyzer (Stable Micro System, Texture Technologies Crop. NY, USA. The texture parameters (hardness, cohesiveness, springiness and chewiness) of rice jelly were determined at 25 $\pm$ 2 ºC in texture profile analyzed mode (TPA) with cylinder probe P/0.5R, 70% of compression degree and 20 mm/s of test speed.

2.9 Sensory evaluation

Consumer testing was conducted at King Mongkut's University of Technology North Bangkok Prachinburi Campus, Prachinburi, Thailand. Criteria for recruiting consumer (N=100) was not allergic to ingredients used in test product. Sensory characteristics of the rice jelly consist of color, rice flavor, texture and overall liking by using a 9-point hedonic scale (1=dislike extremely, 9= like extremely) (Lawless & Heymann, 1998).

2.10 Statistical analysis

All the data were analyzed using SPSS for Windows software (SPSS, version 18.0; Technical Data and Computer Software, Chicago, IL., USA). The difference between the treatments was analyzed using the analysis of variance (ANOVA) with Duncan's multiple-range test (DMRT). Statistical significant difference was established at ($p \leq 0.05$).

3. Results and Discussion

3.1 Moisture content and nutritive value of GBR

The moisture content of GBR and non-GBR no significant difference ($p>0.05$) were ranging from 6.67 to 7.2% dry basis. Germination by steeping brown rice seeds in calcium chloride and citrate buffer solution were a significant effect on the increasing the rate of GABA. In the study, the free GABA content was increased from 3.07 mg/100g of dry sample (non-GBR) to 115.79 mg/100g of dry sample, respectively (Table 1). The highest of free GABA content was observed for the GBRC10P5, due to GABA in rice grains is synthesized from glutamic acid by glutamate decarboxylase (GAD) (Shelp *et al.*, 2012). It is apparent that under stress conditions, GAD activity has been linked to increase in cytosolic Ca^{2+} concentrations to contain Ca^{2+}/ Calmodulin (CaM) binding (Cholewa, Cholewinski, Shelp, Snedden & Bown, 1997) response to GABA shunt pathway, and that it promotes GABA accumulation (Yin, Yang & Gu, 2014). Moreover, in other stress conditions elevated GAD activity has been associated with cytosolic acidification stress (Bown, Macgregor & Shelp, 2006). Thus, a reduction in intracellular pH from normal physiological values could elevate GABA levels (Snedden, Chung, Pauls & Brown, 1992). So, this has been demonstrated in various studies, which have examined the cytosolic stress effect on free GABA content by combining Ca^{2+} and pH buffers.

We also observed total phenolic content to be similar to GABA content, with GBR significantly higher than non-GBR ($p \leq 0.05$). The total phenolic content increased with calcium ion concentrations and pH buffer during the soaking process. The GBRC10P5 showed the highest total phenolic content (*328.89* mg GAE/*100* g). Our results are in agreement with previous studies, which have examined grain germination (Caceres, Martinez, Amigo & Frias, 2014; Moongngarm & Saetung, 2010) and related the induction of enzymes involved in the phenylpropanoid pathway with the degradation of cell wall polysaccharides and proteins causing the release of bound phenolic (He, Han, Yao, Shen &Yang, 2011).

Table 1. Moisture content, free GABA content, and total phenolic of GBR

Treatment	Moisture (g/*100*g)^{ns}	Free GABA content (mg/100 g)	Total phenolic (mg GAE/100 g)
non-GBR	7.02±0.01	*3.07*±0.08 ^{d}	60.35±1.12 ^{e}
GBRC5P3	6.79±0.03	*22.28*±0.45 ^{c}	165.*66*±*0.92* ^{d}
GBRC5P5	6.67±0.03	*107.04*±1.60 ^{b}	216.16±*1.02* ^{b}
GBRC10P3	7.02±0.03	*21.52*±0.24 ^{c}	207.*14*±*1.18* ^{c}
GBRC10P5	6.78±0.08	*115.79*±2.46 ^{a}	328.89±*1.77* ^{a}

Each value represents the mean of three determination ± standard deviation. Value with different letter in the same column are significantly different ($p \leq 0.05$).

3.2 Total starch content of GBR

The total starch content of GBR was showed in Table 2. The highest total starch content found in non-GBR (81.14 g/100 g dry sample), was observed to significantly decrease ($p \leq 0.05$) after soaking in the solutions (range: 60.66-75.34 g/100 g dry sample). In line with our findings, Qi *et al.* (2019) reported GBR total starch content to decrease, and sugar to significantly increase in the germination treatment. We also observed GBRC10P3 to contain the lowest amount of total starch, possibly due to the hydrolysis of starch from acidic solution and α-amylase activity. During soaking in

Table 2. Total starch, kinetic parameters of the *in vitro* starch digestibility, and estimated glycemic index of GBR.

Treatment	Total starch (g/100 g dry sample)	C_∞ (g/100g)	k (min $^{-1}$)	eGI
non-GBR	81.14±0.92^{a}	62.12±1.25^{a}	0.086±0.002^{a}	94.61±2.2^{a}
GBRC5P3	67.88±0.74^{d}	16.77±0.69^{d}	0.029±0.001^{cd}	54.71±1.7^{d}
GBRC5P5	75.34±0.61^{b}	38.21±0.78^{b}	0.046±0.001^{b}	62.27±3.2^{bc}
GBRC10P3	60.66±0.55^{e}	15.52±0.97^{d}	0.021±0.002^{d}	53.16±1.5^{d}
GBRC10P5	70.11±1.10^{c}	31.14±1.44^{c}	0.035±0.001^{c}	65.70±2.4^{b}

C_∞: equilibrium concentration of starch hydrolyzed after 180 min, k: kinetic constant and eGI: estimated glycemic index. Each value represents the mean of three determination ± standard deviation. Value with different letter in the same column are significantly different ($p \leq 0.05$).

solution, or germination the activity of α-amylase rapidly increases (Lorenzo, Yamaguchi, Pierdomenico & Amedeo, 1995) with calcium chloride activating α-amylase in the presence of calcium metal ions molecules for enzymatic activity (Hill, Macdonald & Land, 1997). The α-amylase subsequently plays an important role in hydrolyzing the endosperm starch so that it can be metabolized (Sugimoto, Takeda, Nagata &Yamaguchi, 1998). Similarly, Siddhuraju & Becker (2001) suggested that total starch content decreases with germination, due to starch breaking down into oligosaccharides and a reduction in phytate content. Moreover, Charoenthaikij *et al.* (2009) reported that germination increases the activity of amylase, resulting in a reduction in brown rice sugars.

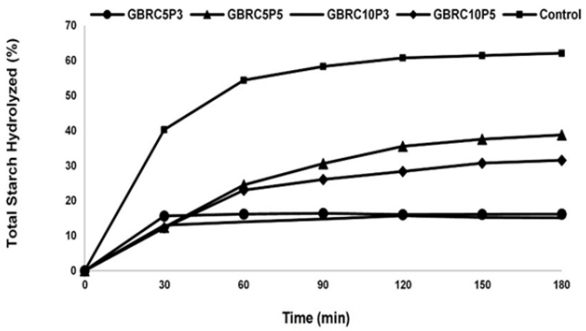

Figure 1. *In vitro* starch hydrolysis rate of germinated brown rice

3.3 *In vitro* starch digestibility and estimated glycemic index (eGI) of GBR

The *in vitro* starch digestibility curves and regression curves of GBR from the different soaking solutions were showed in Figure 1 and Table 2, respectively. The highest rate (62.12 g/100g) of starch hydrolysis after 180 min (C_∞) was found in non-GBR, with a significant reduction in C_∞ values observed in the soaking solutions (range: 15.52-38.21 g/100g). However, a non-significant ($p>0.05$) difference in C_∞ values were observed between GBRC5P3 (15.14%) and GBRC10P3 (16.21 g/100g), respectively. Presumably, acidification had an effect on hydrolyzing the starch granules and calcium ion activation of α-amylase, as it has been demonstrated (Sirisoontaralak, Limboon, Jatuwong & Chavanalikit, 2016) that soaking rice in an acidified calcium solution produces a high total soluble solid. The reduction in starch digestion is due to the α-amylase enzyme being hydrolyzed in the amorphous region, making the rice easier to digest (Dura, Błaszczak & Rosell, 2014). In addition, it is likely that annealing led to less accessible germination or more resistant starch granules during soaking. This result agrees with Cornejo, Caceres, Martinez, Rosell & Frias (2015) was studied in GBR flour for bread. The result of k value indicated that kinetic constant of starch hydrolysis. It was significantly ($p \leq 0.05$) decreased after soaking in a solution, which was related to the starch digestion. The estimated glycemic index (eGI) of GBR were significantly reduced ($p \leq 0.05$) after being soaked in the different solutions, suggesting that solution acidity had a marked effect on eGI. Our results demonstrated that the high acidic solutions (pH3) of GBRC5P3 and GBRC10P3 produced the lowest eGI values, which could be classified in a low GI group ($GI \leq 55$). In contrast, soaking the sample in the acidic solution (pH5) in GBRC5P5, GBRC10P5 produced a medium GI classification ($GI = 56\text{-}69$), whereas the non-GBR was in the high GI group ($GI > 70$). The significant reduction of eGI induced by the rice germination may be associated with the internal changes in the starch granules during germination.

3.4 Texture properties of rice jelly products

The texture characteristics of the rice jelly products were prepared using germinated brown rice powder (GBRP) showed in Figure 2 (RJ1, RJ2, RJ3 and RJ4). It was found that the rice jelly, which was prepared using non-GBR (RJP) had the highest of hardness (102.56), chewiness (36.11), springiness (0.74) and cohesiveness (0.89) texture characteristics. These texture parameters were decreased in the GBRP samples; however, there was no significant difference in the springiness of any GBRP formulation. The texture parameters in RJ3 had lowest values compared to their sample (43.77, 6.44, 0.21 and 0.60, respectively). The texture parameters of rice jelly were dependent on the different GBRP, which was influenced by the GBR soaking solutions. It is likely that all the sample texture properties were mainly related to their starch contents, as calcium and acidity may have cooperatively affected the enzymatic degradation of starch into smaller molecules after being steeped in the solutions (Kim, Oh & Chung, 2017; Wu, Yang, Toure, Jin & Xu, 2013). According to the literature, acidified starches resulted in softer gels because of the low number of high molecular due to hydrolysis (Wang, Truong & Wang, 2003). This can affect the pasting viscosity and gel texture; an important textural characteristic that reduces hardness and flexibility.

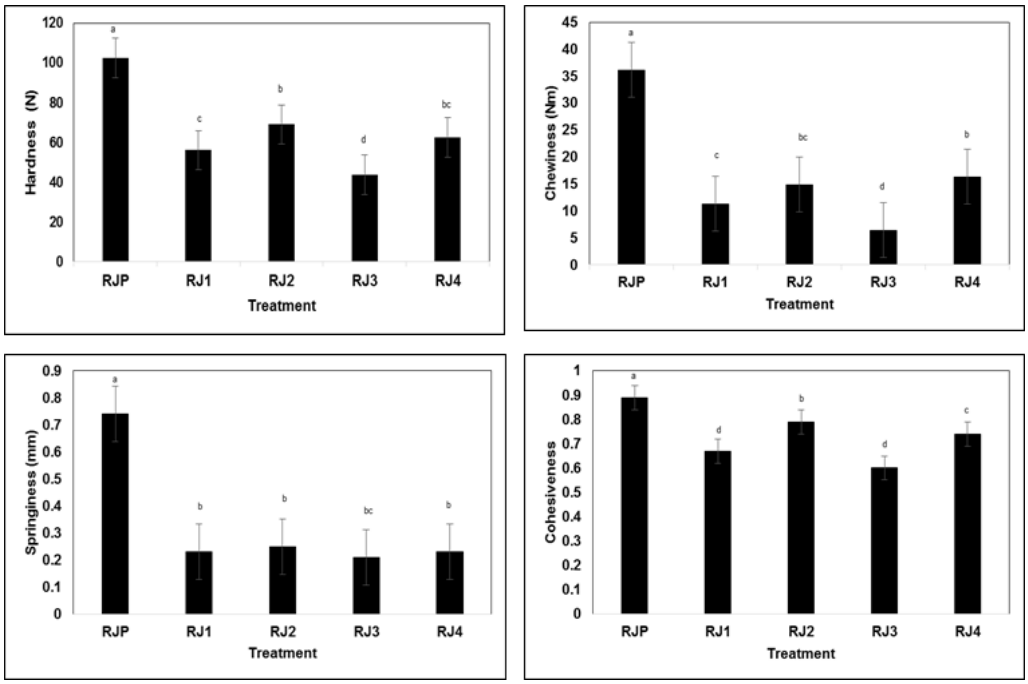

Figure 2. Texture profiles of rice jelly products prepared using GBRP, RJP*:* rice jelly powder made with non-GBR. Each value represents the mean of three determination ± standard deviation.

3.5 Sensory evaluation of rice jelly products

The sensory evaluation results of the rice jelly were presented in Figure 3. In comparison with the rice jelly from RJP, the panelists rated the softness, sweetness and overall acceptability attributes of RJ1- RJ4 significantly higher ($p \leq 0.05$). The RJ4 achieved the highest score for softness (7.2), and was rated as *''*like moderately*''*. This may have been due to the texture properties (hardness and chewiness) being the major texture attributes determining the sensory characteristics of the rice gels. It is possible that using rice as a jelly ingredient may not be necessary, as the high amylose content in the starch has a negative effect on the consumer acceptance of the jelly texture. On the contrary, germination least affected the color and rice flavor in the RJ4 sample showed the lowest scores.

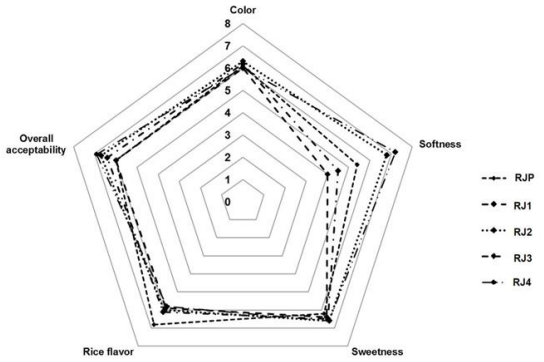

Figure 3. Sensory scores of quality attributes of rice jelly products prepared using GBRP. RJP*:* rice jelly powder made with non-GBR. Each value represents the mean of three determination ± standard deviation. Value with different letter are significantly different ($p \leq 0.05$).

4. Conclusions

The germination of brown rice, using calcium chloride with an acidification solution, was successful at increasing the contained bioactive compounds (GABA and total phenolic content). The lower level of starch content during germination, suggested that the GI of brown rice could be decreased. Moreover, GBRP potentially can be used as an ingredient in rice jelly formulations to improve textural characteristics and meet consumer preferences. Therefore, GBRP rice jelly could be produced to provide a low-medium GI product, which could be used as a functional food alternative for diabetics.

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