Effects of Germination Conditions on Physicochemical Properties of Germinated Brown Rice Flour

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Abstract

This study was conducted to determine effects of germination conditions on selected physicochemical properties of germinated brown rice flour (GBRF). A 3x3 full factorial in CRD was used: 3 levels of steeping water (pH3, pH5 and pH7) and 3 levels of steeping time (24, 48 and 72 h). The non-germinated brown rice flour served as the control. The result showed that protein content of GBRF ranged from 5.51 to 8.02% on a dry basis. Germination conditions significantly affected \( P<0.05 \) gamma-aminobutyric acid (GABA), \( \alpha \)-amylase activity as well as pasting profiles of GBRF. As the germination time increased, GABA content and \( \alpha \)-amylase activity of GBRF considerably increased \( P<0.05 \), compared to the control. The pasting curve pattern of GBRF differed markedly from that of the control. Therefore, steeping of brown rice until small bud development which has been observed may offer a new approach to modify properties of GBRF.

Keywords: germination; brown rice; GABA; physicochemical properties

Introduction

Brown rice (\textit{Oryza sativa} L.) has become popular, particularly among health conscious people. Brown rice contains more nutritional components, such as dietary fibers and vitamins than the ordinary milled white rice. According to its dark appearance as well as hard texture, brown rice is not considered as table rice (Ohtsubo \textit{et al.}, 2005). The digestion of protein and absorption of minerals such as calcium, magnesium, iron and zinc are disturbed by phytic acid which is one of acids contained in husked rice, consequently, the nutrition troubles may be occurred (Anonymous, 2003). Phytate is soluble in an acid solution which was eliminated from foods by soaking or enhancing fermentation to create phytate hydrolysis products having weak mineral binding properties (Harland and Morris, 1995). In the meantime, there was a marked decrease in phytate accompanying germination according to phatase activity (Kikunaga \textit{et al.}, 1991). In addition, germination causes the breakdown of macromolecules increasing the rate of starch and protein digestibility. The breakdown of the high molecular weight polymers during germination also leads to the generation of bio-functional substances including gamma-aminobutyric acid (GABA). GABA in rice grains is an important non-protein amino acid which is synthesized from glutamic acid by glutamate decarboxylase (Ohtsubo \textit{et al.}, 2005). It is a representative depressive neurotransmitter in the sympathetic nervous system and has been proved to be effective for decreasing blood pressure of the animals and humans. GABA-enriched food could also be used as a dietary supplement to help treat sleeplessness, depression and autonomic disorder (Zhang \textit{et al.}, 2006).

Recently, germinated brown rice has attracted public attention, especially in Japan. Generally, supplementary foods made from germinated flours have low viscosity, high nutrition, and properties acceptable to various foods such as weaning foods (Capanzana and Buckle, 1997) as well as bakery products (Watanabe \textit{et al.}, 2004). However, the qualities of the
germinated brown rice flours (GBRF) are influenced by several factors such as varieties and germination conditions affecting product qualities. Therefore, the aim of the present study was to determine effects of germination conditions on the physicochemical properties of GBRF.

Materials and methods

Preparation of Brown Rice

Brown rice grains, Khao Dawk Mali 105 (*Oryza sativa* L. cv. KDML 105) were purchased from Department of Agriculture, Thailand. It was packed under vacuum condition in plastic bag made of Nylon (15 µm thickness) and linear low density polyethylene (120 µm thickness) having a water vapor transmission rate (WVTR) of 5.1 g/(m² d) and oxygen gas transmission rate (OGTR) of 73 cc/(m² d bar) placed in containers and stored at -16 °C before the experiment was carried out.

Germination procedure

A 3x3 full factorial in CRD was used in this experiment. Three levels of steeping water (pH3, pH5 and pH7) and three levels of steeping time (24, 48 and 72 h) were investigated. The experiment was carried out by steeping 200 g of brown rice grains in 80 ppm of Tsunami 100® (Peroxy acetic acid) for 15 min and washed with distilled water to obtain neutral pH. Subsequently, grains were steeped in water using grain-to-water at the ratio of 1:5 w/v. The pH of steeping water was adjusted to pH3 and pH5 with citrate buffer and to pH7 with phosphate buffer. Germinations were carried out at 35 °C with changing of steeping water every 6 h and kept in dark place until small bud development have been observed. Gminated grains were sampled after 24, 48 and 72 h of steeping time. The grains germinated in the required period were dried at 55 °C for 10 h and finely ground by using ultra centrifugal mill with 0.25 mm screener to produce uniform-size flours. The flours were packed in plastic bags made of linear low density polyethylene and stored at 4 °C until further analyses.

Chemical properties

Moisture content was determined by air oven method followed the method of AOAC (2000). Crude protein was determined by combustion method using protein analyzer (model dumatherm, Gerhardt, Germany). Percentage of protein was calculated by multiplying % N with a factor of 5.95 (Capanzana and Buckle, 1997). GABA was determined using HPLC method according to the method of Varanyanond et al. (2005). Alpha-amylase activity was analyzed using falling number apparatus (model 1500, Perten Instruments, Huddinge, Sweden) according to AACC method 56-81B (AACC, 2000).

Pasting properties

GBRF passed through 80 mesh sieve were analyzed in triplicate for pasting characteristics using a Rapid Visco Analyzer (4D, Newport Scientific Pty. Ltd., Australia) followed AACC method 61-20 (AACC, 2000).

Statistical analyses

Physicochemical data was analyzed by analysis of variance (ANOVA). Duncan’s Multiple Range Test (DMRT) was performed for post-hoc multiple comparison. Statistical significant difference was established at p<0.05.
Results and discussion

**Chemical properties of GBRF**

Table 1 shows the changes in chemical characteristics of GBRF compared to the control. Steeping brown rice grains in controlled conditions led to change in selected physicochemical properties of GBRF. All GBRF contained moisture content in the range of 7.74-9.29%. In general, malting slightly increased protein content in red sorghum, millet and maize (Traoré et al., 2004). In present experiment, the steeping time increased from 24 to 72 h showed slightly change of protein content. The result indicated that protein content of GBRF obtained from steeping in buffer solution, pH5 and pH7 were ranged from 6.91-7.34% based on a dry basis. However, GBRF under condition of buffer solution, pH3 during 24 to 72 h of steeping time contained protein content in the range of 5.51-7.45% (dry basis) compared to that of the control (7.81% dry basis).

GABA was increased by steeping rice germs in water (Saikusa et al., 1994). It was synthesized from glutamic acid by glutamate decarboxylase (GAD) and the activity of GAD showed high correlation with the germination ratio. In this study, GABA content of the control was 4.8 mg/100g GBRF before germination. As the steeping time increased, GABA content significantly increased (P<0.05). GABA content of GBRF obtained under buffer solution, pH3 dramatically increased as steeping time increase, showing 32.70 and 67.00 mg/100g after 24 and 48 h, respectively. Regardless of pH of steeping water, increase of steeping time from 48 to 72 h had less effect on GABA content than that of steeping time from 24 to 48 h. Ohtsubo et al. (2005) also reported the similar result that GABA content of brown Koshihikari rice (Japanese rice) increased after steeping for 24 h. The highest content of GABA can be accumulated during steeping brown rice grain in buffer solution, pH3 for 48 h of steeping time. It may attribute to GABA synthesis increases rapidly in response to a variety of environmental signals, including acidosis condition (Scott-Taggart et al., 1999).

The alpha-amylase activity of GBRF was also monitored compared to the control shown in table 2. Amylases play a significant role in seed germination and are instrumental in starch digestion (Muralikrishna and Nirmala, 2005). Generally, activity of alpha-amylase was absent in dry seeds of rice, but its activity rapidly appeared and increased as the process of germination occurred. Falling number (FN) test measured the changes in the physical properties of the starch portion of the kernel caused by these enzymes during the experiment. The increase in enzyme activity was shown as the decrease in falling number. In the present study, the activity of alpha-amylase increased with steeping time. This also agrees with other reports regarding alpha-amylase production during germination in maize (Helland et al., 2002), high amylose rice (Capanzana and Buckle, 1997) and millet (Nirmala et al., 2000). Germination significantly increased (P<0.05) alpha-amylase activity which was relatively higher than that of the control (FN = 455). The alpha-amylase activity was markedly increased in the first stage (24 h) of steeping time (FN = 158) and was present the highest activity during steeping in buffer solution, pH3 for 72 h (FN = 61) compared to the control. The similar trend could be observed both GBRF obtained under the condition of buffer solution, pH5 and pH7.
Table 1 Changes in crude protein, GABA and alpha-amylase activity of brown rice during germination

<table>
<thead>
<tr>
<th>Germination condition</th>
<th>Crude protein (%dry basis)</th>
<th>GABA (mg/100g GBRF)</th>
<th>Falling number (14% moisture basis)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Steeping time (h)</td>
<td>pH of steeping water</td>
<td></td>
<td></td>
</tr>
<tr>
<td>24</td>
<td>3</td>
<td>7.45 ± 0.05c</td>
<td>32.70 ± 0.16e</td>
</tr>
<tr>
<td>48</td>
<td>3</td>
<td>6.54 ± 0.09d</td>
<td>67.00 ± 2.01a</td>
</tr>
<tr>
<td>72</td>
<td>3</td>
<td>5.51 ± 0.02e</td>
<td>63.24 ± 0.19b</td>
</tr>
<tr>
<td>24</td>
<td>5</td>
<td>7.87 ± 0.01b</td>
<td>16.17 ± 0.16e</td>
</tr>
<tr>
<td>48</td>
<td>5</td>
<td>7.88 ± 0.04b</td>
<td>21.14 ± 0.70d</td>
</tr>
<tr>
<td>72</td>
<td>5</td>
<td>7.96 ± 0.03b</td>
<td>21.61 ± 1.62d</td>
</tr>
<tr>
<td>24</td>
<td>7</td>
<td>7.80 ± 0.02b</td>
<td>8.50 ± 0.28g</td>
</tr>
<tr>
<td>48</td>
<td>7</td>
<td>7.84 ± 0.01b</td>
<td>12.28 ± 0.22f</td>
</tr>
<tr>
<td>72</td>
<td>7</td>
<td>7.48 ± 0.08c</td>
<td>16.82 ± 0.43e</td>
</tr>
<tr>
<td>-</td>
<td>-</td>
<td>7.81 ± 0.01b</td>
<td>4.80 ± 0.25h</td>
</tr>
</tbody>
</table>

Mean value ± SD (n = 3). Value followed by different letters in the same column are significantly different (P<0.05). (-) non-germinated brown rice (control).

Pasting properties of GBRF

Among the important practical properties of germinated flour is their pasting characteristics. With both RVA and falling instruments, alpha-amylase activity is quantified in terms of the reduction in viscosity of a flour paste brought about by the action of the enzyme (Raschke et al., 1995). In our study, it can be seen that, germination conditions remarkably affected pasting profiles of GBRF compared to those of the control (Figure 1). No significant differences were obtained between the pasting temperature of GBRF (72.4 to 74.1 °C) and the control (74.0 °C). In other characteristic values of RVA viscogram, there were significant differences (P<0.05) among samples with varied steeping time and pH of steeping water. As the steeping time increased, values of trough, breakdown, and final viscosity of GBRF decreased. However, the viscosity of GBRF obtained from buffer solution, pH3 exhibited the greatly decreased within only 24 h of steeping time compared to others. GBRF obtained from buffer solution, pH3 by steeping beyond 48 h showed the lowest viscosity compared to GBRF obtained under condition of buffer solution, pH5 and pH7. Moreover, the pasting profile of the GBRF obtained from buffer solution, pH5 exhibited the similar profile as of pH7 paste during 48 to 72 h of steeping time. The results showed a good correlation between viscosity and enzyme activity. When the higher amount of alpha-amylase activity occurred, the lower paste viscosities have been observed. The reduction in the viscosity also may be caused by alpha-amylase degrades starch granules and reduces their water binding capacity (Helland et al., 2002).
Figure 1  Pasting profile of GBRF obtained from steeping at 35 °C in buffer solution, pH3 (a), pH5 (b) and pH7 (c) for 24, 48 and 72 h compared to the control measured by RVA.

Conclusion

This study indicated that, as the steeping time increased, protein content was slightly changed while GABA as well as \textit{alpha}-amylase activity of GBRF were dramatically increased. According to pH of steeping water, the viscosity of GBRF was greatly decreased at the low pH of steeping water. Therefore, germination was an important technique for enhancing bio-functional properties and reducing the viscosity of GBRF in such a way which may possible to use as food ingredient for products requiring lower viscosity and high nutritional enrichment.

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References