**Technical paper**

The Effects of Processing Steps on γ-Oryzanol Retention in Rice Bran Added Bagels and Doughnuts

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Rice bran is a rich, natural source of γ-oryzanol, which has been reported to possess important health-promoting properties. The goal of this study was to incorporate this healthy ingredient into bakery products, and the effects of processing techniques on the retention of γ-oryzanol were investigated. Doughnuts and bagels were prepared with 10% rice bran added to high-protein formula flour. The products were sampled at various intervals during processing. Total γ-oryzanol was extracted and analyzed using high performance liquid chromatography. Sensory attributes were measured for the control and supplemented bakery products. The results showed that the total γ-oryzanol content decreased by 22% and 12% in doughnuts and bagels, respectively. The manufacturing steps causing significant losses (P < 0.05) of γ-oryzanol were the following: proofing (~19.2%) in doughnut processing and boiling (~10%) in bagel production. Substitution of 10% rice bran significantly (P < 0.05) increased the darkness of the products. Sensory evaluation results indicated that doughnuts and bagels with 10% rice bran were considered more acceptable than the control (P < 0.05).

Keywords: rice bran, doughnut, bagel, γ-oryzanol

**Introduction**

Rice bran, a by-product of the rice milling process, constitutes approximately 10% of rough rice grain and is composed of the aleuronic layer of the kernel and some of the endosperm and germ. Rice bran is particularly rich in dietary fiber (21.0%) and lipids (20.9%) and contains significant quantities of protein (13.4%). It is also a good source of B vitamins and minerals (Saunders, 1985). Unrefined rice bran oil consists of 20% saturated, 47% monounsaturated, and 33% polyunsaturated fatty acids (Sayre and Saunders, 1990). Studies in animals have shown a hypolipidemic effect with stabilized, full-fat rice bran, but not defatted rice bran (Hundemer et al., 1991; Kahlon et al., 1992; Marsono et al., 1993; Newman et al., 1992). Stabilized, full-fat rice bran added to the prudent diet of hyperlipidemic adults reduced cholesterol and LDL-C and improved lipid ratios in 78% subjects, and there were no significant differences in the lipid-lowering effects of oat bran and rice bran (Gerhardt and Gallo, 1998). Rice bran contains less total dietary fiber (6 – 14.4 vs. 15 – 22 g/100 g), and less soluble fiber (1.8 – 2.7 vs. 5.3 – 8.4 g/100 g) than oat bran (Saunders, 1985). It has suggested that the oil is the active component. Compared to other edible plants, rice bran oil contains high unsaponifiable matters (5.0 – 6.9%), which include several nutritionally important fat-soluble nutraceuticals that could potentially prevent chronic diseases. These nutraceuticals are γ-oryzanol, tocopherols, tocotrienols, phytosterols, and phospholipids (Gopala Krishna, 2002; Gopala Krishna et al., 2003; Yu et al., 2007). γ-Oryzanol, comprised of a mixture of ferulate esters of triterpene alcohol and sterol, is merely found in rice bran and at levels of 1.0 to 3.0% in oil (Khatoon and Gopala Krishna, 2004). γ-Oryzanol has been reported to possess multiple, beneficial
health properties; for example, it acts as an antioxidant (Xu et al., 2001), reduces cholesterol levels (Rong et al., 1997; Wilson et al., 2007), inhibits platelet aggregation (Kim et al., 1995), and shows anti-inflammatory (Islam et al., 2008) and anti-proliferation activities (Hou et al., 2004). Increased awareness of the link between rice bran γ-oryzanol and health has resulted in the development of a series of γ-oryzanol-containing functional foods and cosmetics (Noboru and Yusho, 1970; Lloyd et al., 2000; Tecson-Mendoza, 2007; Lerma-Garcia et al., 2009).

Due to its beneficial attributes, the nutritional and technological aspects of rice bran have been studied for application in bakery products, especially bread and cookies. Rice bran supplementation in wheat flour has improved the protein, fat, lysine, and dietary fiber contents in bread and cookies proportionally with the supplementation level (Sharma and Chauhan, 2002). Rice bran can be incorporated successfully up to a level of 15% without affecting the loaf weight, height, or volume in yeast bread (Sharp and Kitchen, 1990; Lima et al., 2002). Breads containing up to 10% of either defatted or full-fat rice bran were still considered acceptable (Sekhon et al., 1997). Stabilized full-fat rice bran up to a level of 20% and unstabilized full fat or stabilized defatted rice bran up to 10% have been found to be suitable in various food products (Singh et al., 1995). Bread making is a complex physicochemical process involving several steps such as mixing, yeast fermentation (proofing), and baking. For other types of bakery foods, boiling and frying may also be required. It is essential to understand the effects of processing on the retention of γ-oryzanol when developing rice bran-containing bakery foods. This study focused on the quality of two bakery products (bagels and doughnuts) supplemented with 10% rice bran and measured the changes in γ-oryzanol at each major step in the processing of those products.

Material and Methods

Rice bran samples Rice bran from the Taiken 9 variety of rice was kindly supplied by the Union Rice Factory (Changhua County, Taiwan). Bran samples were ground to pass through 1-mm sieves and then subjected to stabilization with a rotary cooker at 120°C for 40 minutes. The bran was removed from the cooker, cooled to 25°C, vacuum packaged in plastic bags and stored in a freezer for further use.

Proximate analysis of bran samples The chemical composition including the moisture content and crude protein, crude fat and crude ash levels, was determined according to the AACC (2003) method. Moisture was determined using a hot air oven at 130°C (AACC 44-15A). Protein content (% N x 5.95) was determined by the Kjeldahl method (AACC 46-13). The crude fat measurement was performed based on the Soxhlet extraction method using petroleum ether (AACC 30-20). The ash content was measured by the direct ashing method (590°C, 8 h) (AACC 08-01). All analyses were performed in triplicate. The chemical components of the bran sample were 0.86% moisture, 12.84% crude protein, 13.12% crude lipid, and 5.94% crude ash.

Baking procedure Two products, doughnuts and bagels, were made. The product sampling protocol was followed to pinpoint changes in γ-oryzanol content during bakery product making. The sampled products were extracted immediately with an appropriate solvent, and the γ-oryzanol content was immediately determined using high-performance liquid chromatography.

All of the ingredients, except for the rice bran, in the baked products were purchased from a local supermarket. Doughnuts were produced using the straight dough method following an in-house formulation as presented in Table 1. In this study, rice bran was substituted for 10% of the formulated flour. All ingredients

<table>
<thead>
<tr>
<th>Table 1.</th>
<th>Formulation (Baker’s %) of the doughnut for a straight-dough baking procedure</th>
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</thead>
<tbody>
<tr>
<td>Ingredient</td>
<td>Dough (%)</td>
</tr>
<tr>
<td>High-protein flour</td>
<td>90.0</td>
</tr>
<tr>
<td>Rice bran</td>
<td>10.0</td>
</tr>
<tr>
<td>Sugar</td>
<td>10.0</td>
</tr>
<tr>
<td>Salt</td>
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<table>
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<tr>
<th>Table 2.</th>
<th>Formulation (Baker’s %) of the bagel for a sponge-dough baking procedure.</th>
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</thead>
<tbody>
<tr>
<td>Ingredient</td>
<td>Sponge (%)</td>
</tr>
<tr>
<td>Flour</td>
<td>65.0</td>
</tr>
<tr>
<td>Rice Bran</td>
<td>--</td>
</tr>
<tr>
<td>Yeast</td>
<td>1.4</td>
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<tr>
<td>Water</td>
<td>40.0</td>
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<tr>
<td>Salt</td>
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<tr>
<td>Sugar</td>
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<tr>
<td>Vegetable Oil</td>
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were placed in a mixing bowl (MIWE, Arnstein, Germany). Mixing was performed for 2 min with a paddle tool at a slow/medium speed (level 2 out of 6). Then, the dough was kneaded until uniform, smoothed, and allowed to stand for 30 min at 28°C and 75% relative humidity (RH) in a fermentation cabinet (National, Lincoln, NE, USA). After resting, the dough was weighed and divided into 40 g dough balls, which were placed on a flour-dusted canvas cloth and proofed for 40 min at 32–35°C and 85% RH. The dough was fried at 160°C until golden brown on both sides. The total frying time was approximately 5 minutes. Samples were taken after all of the ingredients were mixed, at the end of dough proofing, and after frying.

The bagel was prepared using the sponge and dough method (Cauvain, 1998) with slight modifications. In the formulation (Table 2), 65% of the total flour was incorporated at the sponge stage, and 35% was incorporated at the dough stage at which point bran was added to replace 10% of the formulated flour. Sponges were prepared by mixing flour (65%, 510 g), yeast (1.4%, 10 g), and water (40.0%, 330 g) and were set at 28°C for 2 hours. Following the sponge stage, the pre-ferment was remixed with the remaining ingredients: 25% flour (220 g), 10% rice bran (82 g), 20% water (108 g), 1.3% salt (9 g), 5.8% sugar (41 g), and 2.5% vegetable oil (18 g) and kneaded until the dough was uniform and smooth. The dough was allowed to rest in a fermentation cabinet held at 28°C at 75% RH for 20 min. The dough was divided into equal pieces, formed into bagel shapes, and allowed to proof at 32–35°C at 85% RH. After 25 min, the shaped dough was boiled in water on one side for 2 minutes, turned over, and boiled on the second side for 1½ minutes. The bagels were removed from the water and drained on a rack for 1 minute. The boiled bagels were placed on a greased cookie sheet and baked with a top heat of 190°C at 85% RH. After 25 min, the shaped dough was boiled in water and drained on a rack for 1 minute. The boiled bagels were placed on a greased cookie sheet and baked with a top heat of 180°C and a bottom heat of 190°C for 14 min in a deck oven (MIWE, Arnstein, Germany). Samples were taken after mixing all ingredients, at the end of dough proofing, after boiling, and after baking and cooling.

γ-Oryzanol extraction and analysis Extraction of γ-oryzanol was performed according to AACC Method 30-10 (2003) and the method of Heinemann et al. (2008) with a slight modification. Extraction reagents of analytical grade were obtained from Wako Pure Chemical Industries Ltd. (Osaka, Japan), and those of HPLC grade were obtained from Sigma Chemicals, St. (Louis, Mo, USA).

Four grams of the wet bakery sample were placed in 50-mL beaker, 8 mL of 95% ethyl alcohol were added, and the mixture was stirred to moisten all of the particles. Twenty milliliters of 4 N HCl were added, and the mixture was mixed well, placed in a beaker, and heated in a water bath at 70–80°C for 90 minutes. Then, the mixture was cooled, added to 20 mL of 95% ethyl alcohol, and transferred to a stopped flask. Petroleum ether (25 mL) was added, and the flask was shaken vigorously for 1 minute. The mixture was allowed to stand until the upper liquid layer was mostly clear. The supernatant was isolated by centrifugation at 10,000 × g for 20 min. The above steps were repeated twice. The supernatants were combined and evaporated to dryness. The dried material was dissolved in 2 mL of HPLC-grade hexane. An aliquot of the sample was filtered through a PTFE syringe filter (0.45-μm, 13-mm nylon membrane; PALL Cooperation).

γ-Oryzanol was analyzed using a KNAUER Smartline (Advanced Scientific, Germany) HPLC series 1000 equipped with a UV-VIS detector (Jasco Spark Holland BT 9510, Japan) and a 250 × 4.6 mm Hypersil silica-gel column (Thermal scientific, Wilmington, NC) at 30°C. Detection was accomplished by measuring the absorbance at 330 nm. The mobile phase was ethyl acetate/acetic acid/n-hexane (isocratic at 1.8:1.8:98.4 (v/v/v)), and a flow rate of 1.5 mL/min was utilized. The γ-oryzanol contents were quantified by comparison of the peak areas with that of a γ-oryzanol standard curve.

Color determination The color of the outer crust and the inner crumb from all bakery samples was measured using a Hunter Lab colorimeter (ColorFlex 45/0, Hunter associates laboratory Inc., Reston, Virginia, USA) based on the CIE L’ a’ b’ color system. The color values were expressed as L’ (whiteness), a’ (redness/greenness) and b’ (yellowness/blueness). The instrument was calibrated using white (X = 78.25, Y = 83.01, Z = 89.21) and black reference tiles. The analysis was performed in triplicate.

Sensory evaluation The two composite product samples and the control were served to 20 semi-trained panelists from the staff and students of Chinese Culture University who were familiar with the sensory attributes. A 9-point hedonic scale was designed to measure the degree of preference of the samples, including taste, aroma, texture (hardness and chewing), color, and overall preference. The samples were presented on identical plates that were coded with 3-digit random numbers and served simultaneously. The categories were converted to numerical scores ranging from 1 to 9, with 9 as the highest and 1 as the lowest preference. The control group was set to 5 as a benchmark to assess the differences between the control and the trials. Necessary precautions were taken to prevent carry-over flavor during the tasting by ensuring that the panelists rinsed their mouths with water after each stage of the sensory evaluation.

Statistical analysis All sample analyses were run in triplicate. Statistical analysis was performed using the SAS package (version 9.0). Analyses of variance using Analysis of Variance (ANOVA) were conducted. The differences between the sample means were analyzed by Duncan’s multiple range test at α = 0.05.

Results and Discussion The γ-oryzanol concentrations in the mixed starting ingredients, proofed dough, and final doughnut are shown in Table 3. γ-Oryzanol in the starting ingredients was recovered at 78% from the bran doughnut. The proofing caused a significant loss (−19.2%) of γ-oryzanol (P < 0.05), and frying decreased the amount of
The production of bagels in this study resulted in a significant 12% loss of total γ-oryzanol content in the steps between the starting material and the bagel (Table 4). No significant change in γ-oryzanol content was observed during proofing and boiling. Baking was the primary source of γ-oryzanol loss (P < 0.05) in bagel production.

Watanabe et al. (2004) reported that γ-oryzanol was unstable in bread (straight dough method, baking at 200°C for 20 min); ~92% of the content was lost in 30% brown rice-substituted bread. This study had examined the effects of individual processing steps on the retention of γ-oryzanol through mass balance studies during the preparation of rice bran baked products. The results showed that the proofing stage of the current baking processes caused decreases in γ-oryzanol content. However, with doughnuts, proofing showed a greater impact on γ-oryzanol retention compared with that for bagels. Comparison of two proofing processes, it suggested that γ-oryzanol was less stable in straight dough method used for doughnut, on the contrary, remained more stable in the sponge and dough process used for bagel. Regarding the thermal treatment, the frying step (160°C, 5 min) during doughnut production did not change the γ-oryzanol content significantly. During bagel production, the level of γ-oryzanol was influenced significantly by baking (180–190°C, 14 min) but not by boiling (100°C, 3.5 min). It seems that the thermal conditions during processing, particularly dry heat at extended treatment time, had an impact on the γ-oryzanol content.

Table 5 shows the average color ranges for the outer crust and inner crumb regions from the control and rice bran-supplemented doughnut and bagel. Lower L values indicate that both the crust and crumb of the doughnut and bagel were darker with the addition of bran. In addition to darkening, the presence of bran resulted in increased redness (a values) in both the crust and crumb regions, but yellowness (b values) increased only for the crumb. The rice bran after stabilization had a lower L value and higher a and b values than the bran before stabilization (data not shown), which indicated a darker shade and that the powder was a deeper red and yellow compared to its non-stabilized form. A similar observation was reported by Lima et al. (2002) when they examined the functional properties of bread made with processed full-fat and defatted bran. The results of the sensory evaluation are shown in Figure 1. Doughnuts and bagels prepared from wheat flour blended with 10% rice bran were rated as harder (P < 0.05), more odorous (P < 0.05) and more satisfying (P < 0.05) than the standard baked products. This might be result from the roasted flavor of the stabilized bran.

The rice bran used in this study was stabilized and full-fat. Stabilization processing denatures the naturally occurring anti-nutritional factors and thus increases the nutritional value of rice bran and related products. No adverse stabilization effect was observed for the chemical composition of rice bran (Khan et al., 2009). Because the fat was not removed, the bran could bring quality fatty acids into the baked products. Moreover, full-fat rice bran resulted in better textural characteristics than defatted bran for making bread (Lima et al., 2002). Our study suggested that processing can affect the retention of γ-oryzanol in baked food. Therefore, to reduce the loss of γ-oryzanol from rice bran-added products, optimization of the processing conditions is necessary. Other technologies can be used to develop a variety of rice bran-containing products. Evaluation of the effects of processing methods on the γ-oryzanol contents would be interesting.
Table 5. Color determination for rice bran-containing baked products.¹

| Parameter | Doughnut | | | Bagel | | |
|-----------|----------|----------|----------|----------|----------|
|           | Control  | Rice bran | Control  | Rice bran |
| Crust     |          |          |          |          |
| L         | 39.40 ± 0.20 | 34.32 ± 0.06 | 60.88 ± 0.16 | 55.05 ± 0.16 |
| a         | 18.09 ± 0.17 | 18.06 ± 0.09 | 8.46 ± 0.16 | 11.37 ± 0.20 |
| b         | 27.04 ± 0.21 | 22.44 ± 0.21 | 29.94 ± 0.12 | 27.63 ± 0.20 |
| Crumb     |          |          |          |          |
| L         | 56.58 ± 0.13 | 50.92 ± 0.16 | 71.74 ± 0.25 | 63.38 ± 0.28 |
| a         | 8.50 ± 0.02 | 9.19 ± 0.11 | 3.58 ± 0.13 | 4.65 ± 0.07 |
| b         | 20.94 ± 0.13 | 23.07 ± 0.01 | 22.67 ± 0.04 | 23.20 ± 0.12 |

¹Data from three replications.
²Significant differences between the means of the control and the rice bran groups (P < 0.05).

Fig. 1. The results of the sensory evaluation of bran-added doughnut and bagel compared to the control. ¹Significant differences between the means of the control and the rice bran groups (P < 0.05)
References


URL cited