Gluten free brownies made with composite rice flour

P Penjumras\textsuperscript{1,*}, P Thongfathamrong\textsuperscript{1}, S Umnat\textsuperscript{1}, P Chokeprasert\textsuperscript{1}, I Wattananapakasem\textsuperscript{1} and A Phaiphan\textsuperscript{2}

\textsuperscript{1}Program of Food Technology, Maejo University-Phrae Campus, 54140 Rongkwang, Phrae, Thailand.
\textsuperscript{2}Program of Food Science and Technology, Faculty of Agriculture Ubon Ratchathani Rajabhat University, 34000 Ubon Ratchathani, Thailand

*E-mail: p_atp@hotmail.com

Abstract. This study was conducted to determine the effect of composite rice (Riceberry and Red jasmine rice) flour ratio and germination process on the physical and chemical properties and sensory characteristics of brownies. The ratios of Riceberry and Red jasmine rice flour at 75:25, 50:50, and 25:75 were investigated. As the amount of red jasmine rice flour increased, affected physical properties by increasing in the specific volume and L*, a*, b* values but decreased the hardness. However, there was no influence on chemical composition. The sensory characteristics were found to increase with increasing ratio of red jasmine rice flour. However, there were no significance (p>0.05) different on sensory characteristics except the taste attribute score. Thus, the selected ratio was 50:50 Riceberry and Red jasmine rice flour. The effect of germination was found to increase specific volume, and L*, a*, b* values but decreased reducing sugar. There was no significance (p>0.05) different of sensory characteristics between brownies made from raw and germinated rice flours. Therefore, rice grains should be germinated before utilization of rice flour as substitution of wheat flour to produce gluten free product.

1. Introduction
Wheat flour is the principal component of nearly all bakery products, such as bread, cake and cookie, all of which, contain gluten. Nevertheless, many people suffer from an allergy or intolerance to a substance called gluten. Celiac disease, sometimes called celiac sprue or gluten-sensitive enteropathy, is known as gluten intolerance to certain amino acid sequences found in the prolamin fraction of wheat (gliadin), rye (secalin) and barley (hordein) [1]. Due to the presence of multiple proline and glutamine residues, making them resistance to gastrointestinal digestion, therefore, the ingestion of these proteins leads to the inflammation, atrophy, and hyperplasia of the small-intestinal crypts of the celiac patient [2]. The functional properties of wheat are generally ascribed to the visco-elastic properties of its gluten proteins. While monomeric gluten proteins (gliadin) show viscous behavior, polymeric gluten proteins (glutenin) are elastic [3]. Specifically, the wheat gluten proteins (gliadins and glutenins), in the presence of water and under mechanical work, form a continuous phase named gluten network [4]. It is responsible of the extensible and cohesive properties of the dough while reducing its stickiness. In recent years, there has been increasing interest in substituting wheat flour with non-gluten-containing sources available in bakery formulation include pseudo cereals (buckwheat, quinoa and amaranth), minor cereals (fonio, teff, millet and job’s tears), cereals (rice, corn and sorghum), and other cereals.
Some of these crops are also nutrient dense, thus, incorporation of them could also improve its nutritional quality (Moreno et al., 2014). Additionally, replacement of wheat flour with other crop has gain attention because of economic reasons, especially in Asian country where cultivating conditions are not suitable for wheat growth [6]. Rice (Oryza sativa L.) is major cereal crop in Asian country as Thailand, China, India, Indonesia, Burma, Vietnam, Japan and the Philippines [7].

Several bakery products have been studied on the usage of rice flour as replacement for wheat flour as Table 1. such as cake made from composite rice of Jasmine and Yellow 11 [8], mixed Hom-Mali and glutinous rice [9], black glutinous rice (Khaw Niaw Dam) [1, 6], chiffon cake prepared with black rice [10], yellow cake prepared from rice flour and peanut paste [11], white bread made from rice flour mixed with corn starch and cassava starch [12] and our previous study of cream puff made from red jasmine rice flour [13]. Unfortunately, rice protein cannot generate a visco-elastic network like gluten in wheat which retains carbon dioxide during dough fermentation [14].

To overcome this problem, hydrocolloids were incorporated in gluten-free flour to mimic the viscoelastic properties of gluten [13, 15, 16]. However, brownie requires a lower specific volume compared to other bakery products such as bread and cake which are type of air leavened products. Thus, substituting wheat flour with rice flour could be suitable for brownie. Rice can be classified by their colours which are white rice, brown rice, red rice and black rice. Nowadays, pigmented rice has been receiving more attention in commerce in Thailand due to consumer trend of healthy awareness. They are good sources of basic nutrients, fiber, minerals and phytochemicals [17]. During the milling process, broken is inevitably generated huge amounts broken rice and is sold by Thai farmer as raw material for animal feed at low price whereas this broken rice contains high nutrient. It would be beneficial to develop an alternative product that makes a better utilization of broken rice. In addition, germination or malting of cereals grains may result in biochemical activities. Many studies have been reported its advantages and health benefit Germination rice contain numerous nutrients especially γ-amminobutyric acid (GABA) [18]. Therefore, the purpose of this research was to determine the effect of composite rice flour ratio (Riceberry and Red jasmine rice) and germination on physical and chemical properties and sensory acceptability of brownies.

2. Materials and Methods

2.1. Materials
Red jasmine (red rice) and Riceberry rice (black rice) were purchased from the local market in Phrae province, Thailand, butter (Allowri brand), dark chocolate (Tulip brand, Freyabadi Co. Ltd.), white sugar (Mitr Phol Co., Ltd.) and fresh whole eggs (~50 g/piece) were used in this study.

Rice was ground using blender (Model HR 2115, Philips, Indonesia), and screened through a 150-mesh sieve. The rice powder was contained in airtight box and then kept at room temperature (∼25 °C) until the moment of preparation within a week.

2.2. Brownies preparation
A total of three brownies formulations (Table 1) were prepared. First, the flour was sieved through a 150-mesh sifter before adding it into a bow of mixer. Butter and chocolate chips were melted using double boiler method at 100 °C for 10 min and cooled to room temperature for 10 min. Then, sugar, eggs, and flour were added into the bowl. The final mixture was placed in a 12*12*1 inch baking tray and was oven-baked at 180°C for 30 min following our preliminary experiment. After baking, it was removed from the baking tray and left to cool for 1 hour at room temperature. Physical properties and sensory evaluation were carried out on the fresh brownies. The rest of cooled brownies were kept in an airtight box and put in a freezer at 8 °C until used for further chemical analysis. The selected formulation was used for further study on the effect of germination.
### Table 1. Formulation of brownies.

| Ingredients (g) | Ratio of Riceberry: Red jasmine flour |
|----------------|---------------------------------------|
|                | 75:25       | 50:50       | 25:75       |
| Riceberry flour| 112.5       | 75          | 37.5        |
| Red jasmine flour| 37.5       | 75          | 112.5       |
| Chocolate chip | 200         | 200         | 200         |
| Butter         | 175         | 175         | 175         |
| White sugar    | 175         | 175         | 175         |
| Egg            | 165         | 165         | 165         |

2.3. Germination of rice samples
The rice grains of Riceberry and Red jasmine rice were dehulled and germinated according to Chokeprasert and Penjumras [19]. The grains were washed and soaked in tap water at room temperature for 6 hours. Water was drained off and grains were washed again with tap water and were then left to germinate in a plastic container with dark condition at room temperature for 18 hours. After germination, the grains were steamed for 10 min and were dried at 50 °C to maintain moisture content ≤ 13%. The dried grains were ground and kept in an air tight box until the moment of brownie preparation.

2.4. Physical properties analysis
The physical characteristics of brownie, including specific volume, colour and hardness. The specific volume (mL g⁻¹) of brownies was obtained by the ratio between the apparent volume (mL) and the mass (g) after baking. The mass was obtained by weighing the brownies on a precision scale (Ohaus, Pioneer PA214, USA). The apparent volume of the brownies was determined by the method of displacement of sesame seeds [10]. The colour of the brownies was measured using a colorimeter (Color Flex 500, Hunter Lab, USA). Results were expressed in CIE colour values; L* = lightness (0=black, 100=white), a* (-a* = greenness, +a* = redness) and b* (-b* = blueness, +b* = yellowness). The hardness of brownies was measured using a Pressure tester (Model FT 327). All the analyses were performed in triplicates.

2.5. Analysis of chemical composition
Ash content (muffle furnace at 550-600 °C), lipid (Soxhlet method), and reducing sugar (Lane & Eynon method) were evaluated according to the official methods of the AOAC (2012) [20]. All the analyses were performed in triplicates.

2.6. Sensory evaluation
The degree of overall preference for brownies were determined by hedonic scale test. The untrained panels were recruited from the students and staff at Maejo University-Phrae Campus. All untrained panels were informed how to evaluate brownies. The 30 panels received samples and were asked to rate them based on degree of preference on a 9-point hedonic scale (1 = dislike extremely, 5 = neither like nor dislike, and 9 = like extremely) to evaluate the product attributes (appearance, color, flavor, taste, texture, and overall acceptance). Panels evaluated the samples in a testing area and were instructed to rinse their mouths with water between samples to minimize any residual effect.

2.7. Statistical analysis
Data were subjected to Analysis of Variance (ANOVA) using SPSS for Window version 24. In case of any differences in mean, multiple comparisons were performed using Duncan’s Multiple Range Test (DMRT) at 5% level of significance (P≤0.05).
3. Results and Discussion

3.1 The effect of composite flour ratio on quality of brownie

The effect of composite rice flour ratios on physical and chemical properties and sensory characteristics of brownies were investigated. The results are presented in Table 2 and Table 3.

### Table 2. Effect of raw rice flour composite ratios on physical and chemical properties of brownies.

| Properties                      | Ratio of Riceberry: Red jasmine flour |
|---------------------------------|---------------------------------------|
|                                 | 75:25   | 50:50   | 25:75   |
| **Physical properties**         |         |         |         |
| Specific volume (mLg⁻¹)         | 0.78±0.13ᵃ | 0.95±0.05ᵇ | 1.05±0.01ᵇ |
| Colour L*                       | 7.56±0.04ᵃ | 7.56±0.04ᵃ | 10.59±0.23ᵇ |
| a*                             | 0.44±0.38ᵃ | 0.27±0.06ᵇ | 6.50±0.32ᵇ |
| b*                             | 5.55±0.43ᶜ | 6.50±0.32ᵇ | 8.33±0.06ᵇ |
| Hardness (N)                    | 10.75±0.64ᶜ | 7.89±0.96ᵇ | 5.83±0.64ᵃ |
| **Chemical properties**         |         |         |         |
| Ash content (%)[^ns]            | 0.96±0.10 | 0.94±0.10 | 0.89±0.10 |
| Fat (%)[^ns]                    | 19.74±0.99 | 21.33±3.37 | 20.23±1.86 |
| Reducing sugar (%)[^ns]         | 5.49±0.20 | 5.44±0.03 | 5.35±0.08 |

Mean ± standard deviation values followed by a different letter within the same row are significantly different (p<0.05) by Duncan’s multiple range test.
[^ns]: not significant (p>0.05) different within the same row by Duncan’s multiple range test.

From Table 2, ANOVA showed significantly differences in physical properties of specific volume with different ratios of rice flour. The specific volume of brownies was found to increase with increasing of Red jasmine rice. This could be related to amylase content. The Riceberry flour contains 12.37% amylase content [21] meanwhile Red jasmine rice flour contains 17.93% amylase content [22]. The amylase content is an important component for providing desirable qualities by three-dimensional network. The higher value of amylase content contributes to good capability of rice starch granules to engage water molecules and structure setting must be expanded by water vapor during oven-baking [22, 23]. Thus, the resulting brownie is highly aerated and gives higher specific volume. Therefore, increasing of Red jasmine rice affected the decreasing of hardness of brownies. In addition, increase in ratio of Red jasmine rice resulting dominant of L*, a* and b* values. Abdel-Aal et al. [24] reported that cyanidin-3-glucoside and peonidin-3-glucoside were identified as two major anthocyanins which attribute to dark purple colour in pigmented rice [25], especially black rice show higher value of anthocyanin compared to red rice [26]. Therefore, the formulation with higher ratio of Riceberry provided lower L* value. The positive a* values or redness were the higher for the brownies made from higher ration of red rice varieties due to the reddish coloured external layers [27]. Sensory characteristics were tested to evaluate acceptance by the untrained panel as shown in Table 3.

### Table 3. Effect of raw rice flour composite ratios on sensory characteristics of brownies.

| Properties          | Ratio of Riceberry: Red jasmine flour |
|---------------------|---------------------------------------|
|                     | 75:25   | 50:50   | 25:75   |
| Appearance[^ns]     | 6.38±1.10 | 6.62±1.01 | 6.64±1.01 |
| Colour[^ns]         | 6.66±1.10 | 6.72±1.13 | 6.94±0.84 |
| Flavour[^ns]        | 6.78±1.09 | 6.86±1.01 | 6.86±1.20 |
| Taste[^ns]          | 6.48±1.42ᵃ | 6.66±1.47ᵇ | 7.02±1.20ᵇ |
| Texture[^ns]        | 6.26±1.71 | 6.50±1.56 | 6.80±1.29 |
| Overall acceptance[^ns] | 6.60±1.28 | 6.80±1.23 | 6.98±1.00 |
Mean ± standard deviation values followed by a different letter within the same row are significantly different (p≤0.05) by Duncan’s multiple range test.

The sensory characteristics were found to increase with increasing ratio of Red jasmine rice flour. However, there were no significant (p>0.05) different in ratio of rice flour except the acceptance of taste characteristic was found significantly different (p≤0.05). It was observed that the brownie made from ratio of Riceberry: Red jasmine flour of 25:75 gave the highest taste score. Observing the overall characteristics, it can be stated that there was no significant (p>0.05) different score between formulation. All sensory characteristics had scored higher than 5, this means that panels accepted brownies made from composite rice flour. Therefore, the brownie made from composite of 50:50 ratio of Riceberry and Red jasmine rice flour should be selected formulation for further step.

The effect of germination on properties of brownie was evaluated using formulation as shown in Table 1. The results were represented in Table 4 and 5.

Table 4. Effect of germinated on physical and chemical properties of brownies.

| Properties                | Raw          | Germinated   |
|---------------------------|--------------|--------------|
| Specific volume (mLg⁻¹)   | 0.98±0.08ᵃ   | 1.15±0.25ᵇ   |
| Colour L*                 | 7.16±0.04ᵃ   | 8.29±0.04ᵇ   |
| a*                        | 1.54±0.31ᵃ   | 1.78±0.29ᵇ   |
| b*                        | 5.57±0.41ᵃ   | 6.47±0.11ᵇ   |
| Ash content (%)ns         | 0.96±0.01    | 0.98±0.03    |
| Fat (%)ns                 | 20.74±0.99   | 21.05±1.37   |
| Reducing sugar (%)        | 5.55±1.04ᵇ   | 5.12±0.13ᵃ   |

*ns = not significant (p>0.05) different within the same row

Mean ± standard deviation values followed by a different letter within the same row are significantly different (p≤0.05)

Table 5. Effect of germination on sensory characteristics of brownies.

| Characteristics | Raw          | Germinated   |
|-----------------|--------------|--------------|
| Appearanceⁿˢ    | 7.30±1.02    | 7.43±0.94    |
| Colourⁿˢ        | 6.90±1.24    | 7.63±0.96    |
| Flavourⁿˢ       | 7.17±1.02    | 7.40±1.04    |
| Tasteⁿˢ         | 6.73±1.57    | 7.43±1.04    |
| Textureⁿˢ       | 6.63±1.73    | 7.20±1.03    |
| Overall acceptanceⁿˢ | 6.70±1.42 | 7.40±0.77 |

*ns = not significant (p>0.05) different within the same row

The results were found that germination process affected increase on specific volume and L*, a* b* values but decrease in reducing sugar (Table 4). In a germination process, rice has been softened during the soaking step and makes rice flour increase in water uptake during mixing of ingredient and expansion during oven baking thus the brownie made from germinated rice flour provided the higher specific volume. Although during germination process germinated rice may loss anthocyanin, the germinated rice contains numerous nutrients especially γ-amminobutyric acid (GABA) [18]. There was no significant different on sensory characteristic scores (Table 5). Germination significantly decreased reducing sugar in brownie made from germinated rice flour may be due to germination process increase in alpha-amylase activity which had broken down complex carbohydrates into simpler sugar.
then dissolve in water during soaking process [14] and led less reducing sugar. Therefore, the germination process of grains before utilization for making product could be alternative process to provide gluten free product.

4. Conclusion
As the amount of Red jasmine rice flour increased, affected physical properties by increasing in specific volume and Hunter color values without resulted chemical composition. The sensory characteristics were found to increase with increasing ratio of Red jasmine rice flour. However, there were no significant (p>0.05) different except the taste score. Thus, the selected ratio was 50:50 Riceberry and Red jasmine rice flour. The effect of germination was found to increase specific volume, and Hunter colour values but decreased reducing sugar and there were no significant (p>0.05) different of sensory characteristics between raw and germinated rice flour recipe. Therefore, rice grains should be germinated before utilization of rice flour as substitution of wheat flour. This product supposes to be an alternative healthy gluten free product.

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