Chemical Compositions and Physico-Chemical Properties of Malted Sorghum Flour and Characteristics of Gluten Free Bread

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Abstract—This study investigated the effect of germination on chemical compositions, physico-chemical properties of malted (germinated) red sorghum flours and evaluated characteristics of gluten free breads from sorghum flour. Results showed that germinated sorghum flour had higher amylase activity, swelling power and solubility at 95°C, but lower in the peak, break down, final and set back viscosities than ungerminated sample (p≤0.05). Five gluten free breads made from sorghum flour blends, with different ratios of ungerminated and germinated sorghum flour, were compared for the physical properties with those made from wheat flour. Crumb hardness, cohesiveness, gumminess and chewiness of sorghum breads were found significantly higher than those of wheat bread. With increasing of ungerminated flour proportion, the bread hardness increased while the cohesiveness declined. Sorghum breads appeared red to human eyes with a values of 10.41-15.77. Their crust and crumb colors differed significantly from those of wheat bread.

Keywords—Flour, Germination, Gluten free bread, Sorghum

I. INTRODUCTION

Bread is an important staple food for several countries. Wheat flour (Triticum aestivum) is more popular than other cereal grains for bread making. Its popularity has stemmed from the gluten and its mild, nutty flavor. Gluten is an essential structure-forming protein which contributes to the elastic characteristics of dough and good appearance of bread [1]. However, a number of people have celiac disease (CD) which is defined as an inflammatory response in the small intestinal mucosa exacerbated by prolamin proteins in the cereal grains i.e. wheat (gluten), rye (secalin), and barley (hordein) [2]. As a result, there has been a great interest in development of gluten free bread. Part of this interest gets involved with the replacement of wheat flour with other flour. Among the other grain cereals, sorghum (Sorghum bicolor) is a rich source of various phytochemicals, including tannins, phenolic acids, anthocyanins, phytosterols and policosanols [3], the physico-chemical properties of sorghum flour are also found similar to those of wheat flour. Thus, sorghum flour is likely to have the potential to replace wheat flour for those allergic to gluten [4], [5]. However, the absence of gluten in sorghum flour may cause a liquid batter and baked bread with quality defects post-baking e.g. poor color and crumbling texture [5]. A number of studies have focused on improving the quality of cereal-based flour for bread making. Reference [6] applied the fermentation to decrease the pH of sorghum flour from 6.2 to 3.4, to reduce total starch and water-soluble proteins, and to increase enzyme-susceptible starch and total protein. Consequently, blending fermented sorghum flour with wheat flour was able to increase volume of bread loaf, weight of bread, and reduced crumb firmness. In the studies of [7], bread was made from pregelatinized cassava starch and sorghum flour. It was found that crumb firmness and chewiness declined with increasing pregelatinized starch concentration whereas crumb adhesiveness increased with increasing the starch content. In addition, Enzyme combinations e.g. trans-glutaminase, alpha-amylase, xylanase and protease were alternative methods to improve dough rheology, bread quality and bread shelf-life [8]. The process of germination has been used successfully to improve the nutritional properties of legume seeds by removing several antinutrients (phytates and trypsin inhibitor), increasing oligosaccharides, and improving digestibility of starches and proteins in legumes. The results of studies by [9] indicated that germination improved the functional properties of sorghum and it would be possible to design new foods, gluten free bread, using germinated sorghum. The technique of germinating legumes before consumption is a common practice to produce a natural product. In order to further expand the use of this grain, the effect of grain germination on physical and physico-chemical properties of red sorghum flour was investigated and its application to make gluten free bread was also evaluated through several aspects of physico-chemical and physical properties compared with those made from ungerminated sorghum flour and wheat flour (as reference sample). In view of the current increasing incidence of celiac disease, we approach the use of sorghum flour on a replacement basis for wheat flour in gluten-free bread for alterations.

II. MATERIALS AND METHODS

A. Sorghum Sample

Sorghum grains of the red variety (Sorghum bicolor), used in this study, were purchased from the local market in Petchaboon province, Thailand. The samples were packed in plastic container and kept at room temperature until further use. Red variety of sorghum was chosen due to its

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concentrated pigmentation. Also, this variety is widely grown in Thailand for animal feeds.

B. Sorghum Germination
Sorghum seeds were germinated using the method of [9]. The sorghum grains (2,000 g) were soaked in tap water for 24 h at room temperature with two changes of water to remove dirt and husk. Later, the soaked grain was spread out thinly on a jute bag saturated with water, covered with another jute bag and allowed to germinate in the dark for two days at room temperature (27°C) and water spraying on the grains was needed during germination process to control the grain’s moisture. At the end of the process, the germinated grains were tray-dried at 40°C to obtain final moisture content of 5%, approximately. The root portions were manually removed. The dried, ungerminated and germinated sorghum seeds were separately ground to a fine homogeneous powder in an electric blender and passed through a 100 mesh screen. The milled samples of ungerminated sorghum flour (USF) and germinated sorghum flour (GSF) were packed in sealed plastic bags and stored at 4°C until use. Commercial wheat flour (WF) was also used as a reference sample.

C. Determination of Physico-Chemical Properties of Flour
1. Proximate Analysis
All flour samples were analyzed for moisture, ash, protein and fat contents, following AOAC method [10]. The conversion factor (N x 6.25) was applied to convert nitrogen to crude protein content. All measurements were carried out in triplicate.

2. Alpha-Amylase Activity
Alpha-amylase activity of wheat flour, germinated and ungerminated sorghum flours was determined using the Megazyme α-amylase assay kit (Megazyme International Ltd., Bray, Ireland).

3. Swelling Power (SP) and Solubility
Swelling power and solubility of all tested flours were determined in triplicates at a test temperature range between 55 to 95°C according to [11].

4. Pasting Profiles
A Rapid Visco Analyser (RVA 4, Newport Scientific, Narrebeen, Australia) was used to study the pasting properties of wheat and sorghum flours. Using a method of [6], flour (3g) was weighted directly into an aluminum RVA container and 25 ml of distilled water was added. Sample was heated from 25 to 90°C in 8-9 min, held at 90°C for 2-3 min and then cooled to 50°C in 2 min. The RVA parameters measured were pasting temperature (temperature at which paste viscosity starts to increase), peak viscosity (maximum hot paste viscosity), holding strength (the trough at minimum hot paste viscosity), and final viscosity (viscosity after cooling to 50°C and holding the temperature).

D. Bread Making
Six bread formulations were prepared using a modified method described by [12]. There was one formula made from 100% of wheat flour to serve as the control whereas the other five were prepared by replacing wheat flour with 50% of sorghum flour and 50% of cassava flour. In the portion of sorghum flour, different ratios of ungerminated to germinated sorghum flours were blended, including 100:0; 75:25; 50:50; 25:75 and 0:100 (w/w). The other baking ingredients, weighed on flour weight basis, were 75% water, 6.7% sugar 3.5% shortening and 1.7% salt. To make bread, the dry ingredients were manually mixed in a wide bowl and then added to mixing bowl containing water and shortening. The components were thoroughly mixed with electric mixer on medium speed for 20 min. The batters were placed in baking pans, sprayed with water at the time of loading and baked in an oven at 200°C for 60 min. Subsequently, the loaves of bread were deplanned and cooled at room temperature for 2 h, packed in unperforated low density polythene bags. All samples were stored at 27°C prior to physical analysis within 24 h.

E. Physical Evaluation of Bread
1. Measurement of Bread Color
As bread crust and crumb differ widely, their colors were examined separately using method of [13]. Color values of bread measured using a chroma Meter (Minolta CR-300, Osaka, Japan) were reported in L* a* b* system. Crust color was firstly measured at six positions on top of the bread. The bread was then sliced transversely using bread knife to obtain three uniform slices of 20 mm thickness. The measurement of crumb color was done in the middle on both sides of each slice.

2. Measurement of Bread Crumb Texture
The instrumental texture measurements were made on fresh prepared bread samples with a TA-XT2 texture analyzer (Stable Microsystems Ltd., Surrey, London) as described by [14]. One cm of the edges of the bread samples was removed from all sides. Approximately, a 20 mm piece was punched out, placed on the flat stage, and the texture determined. Texture profile analysis (TPA) was performed with a 25-mm circular probe. The parameter settings were as follows: pre-test speed, 2 mm/s; post-test speed, 1 mm/s; rupture test distance, 1%; distance 30% strain; force, 0.10 kg; time, 1.0 s; (auto) trigger force, 0.020 kg and contact area 230 mm². The above procedure was repeated for each determination. Real-time data acquisition was accomplished by following the TAXT2 User Guide. The software was used to calculate hardness (kg), springiness (%), cohesiveness and chewiness (N), gumminess (N), adhesiveness (N.s) values of the bread samples.

F. Determination of Total Phenolic Content
The Folin–Ciocalteu method as described by [15] was used to determine the total phenolic contents of flours and bread samples. Prior to analysis, fresh prepared bread samples were
air dried at ambient temperature, fined into homogeneous powder using an electric blender and the passed through a 20 mesh screen. Briefly, 50 mg of the tested samples was dissolved in 2.5 ml of 95% ethanol. The sample was vortex-mixed and centrifuged at 9,000 rpm for 10 min. A 1 ml of the supernatant was then transferred to a test tube. After that, 1 ml of 95% ethanol; 5 ml of distilled water and 0.5 ml of Folin-Ciocalteu phenol reagent was added, respectively. After an incubation period of 5 min at room temperature, 1 ml of 5% Na2CO3 was included, mixed well and kept in the dark for an hour. The absorbance of the solution was measured at 725 nm using a UV spectrophotometer (Shimadzu, USA). Gallic acid was used as a standard and the results were calculated as mg gallic acid equivalents of the sample (mg GAE/g).

G. Statistical Analysis
Data were subjected to statistical analysis of variance (ANOVA) using SPSS for Windows. Results were reported as average values ± standard deviation (SD). The difference in means was determined by Duncan Multiple Range Test (DMRT). Statistical significance was set at 95% confidence level.

III. RESULTS AND DISCUSSION

A. Proximate Compositions and α-Amylase Activity
The chemical compositions of two laboratory prepared sorghum flours and commercial wheat flour are presented in Table I. The WF had a moisture content of 9.93%, a protein content of 15.33%, a fat content of 0.86% and ash of 0.45%, which differed significantly from those of sorghum flours (p≤0.05). Also, the results were slightly different from literature values i.e. 14.20-15.22% for moisture content, 9.75-11.20% for protein content (N×5.7) and 0.64-0.74% for ash content [16]. In our study, the GSF contained 9.03% protein, 3.39% fat and 1.01% ash. These findings were similar to that of [17] who reported the composition of GSF of ten cultivars, comprising 10.08-16.45% protein, 2.22-5.36% fat and 1.06-5.24% ash.

GSF had the lowest moisture content among the three flours. This could be explained by the subsequent drying after germination in order to prevent the growth of microorganisms. The protein content of two sorghum flours was significantly lower than that of wheat flour (8.38-9.03% compared to 15.33%). These values were similar to those reported by [5], i.e. 14.89% for hard wheat flour, 11.24% for soft wheat flour, 6.28-9.47% for sorghum flour. However, there is no significant difference in protein content between germinated and USF (p>0.05). The two sorghum flours contained more fat than commercial WF (0.86%) whereas the fat content of GSF and USF was statistically similar (3.39 and 3.20%). In the studies of [5], only 0.15 and 0.32% fat was found in flours of two sorghum cultivars. The different fat content between the two studies could be due to botanical properties and the effect of processing method.

The activity of α-amylase extracted from wheat and sorghum flours are shown in Table I. As expected, GSF had the higher α-amylase activity (32.6243 CU/g) than USF and WF (0.0013 and 0.0155 CU/g). The reason is because germination triggers the enzyme system of sprouting seeds [9]. For α-amylase, its activity leads to the breakdown of starch into maltose [18]. In sorghum, the embryo is the major site for the synthesis of many hydrolytic enzymes. It has been reported that during germination of sorghum, the activities of amylase increased with increasing time, and reached a maximum on the 3rd day [9]. After 72 h of germination period, there was no significant change in amylase activity [18]. In the work done by [19], 39-135 U/g of α-amylase was found in sorghum germinated at 30°C. The difference from our results may be caused by the individual natures of the sorghum cultivars used and germination process adopted.

B. Physico-Chemical Properties of Flour

1. Swelling Power and Solubility
Figs. 1 and 2 show the swelling power (SP) and solubility of WF, GSF and USF at the temperature of 55-95°C. The SP (g/g dry flour) of all samples increased with temperature. Nearly four times increase in SP was observed in all three flours at 95°C as compared to those at 55°C.

When heated in the presence of water at 55°C, the SP of USF was similar to WF (ca. 0.05 g/g dry flour). GSF had a lower SP than that of WF and USF until the temperature at 75°C. Rapid increases in SP of GS occurred between 75 and 95°C. At the elevated temperature at 95°C, SP of GSF was higher than the other flours (0.2 compared to 0.17-0.18 g/g dry flour). This result agreed with [5] who observed the higher SP of two cultivars sorghum flours than those of hard and soft WF after the temperature at 75°C. The differences in SP of the three flours studied could be attributed to the chemical components as evidenced by the previous studies. Reference [20] reported that flours of three bean cultivars (Majesty, dark red kidney bean; Red Kanner, light red kidney bean; AC Nautica, navy bean) had lower SP than those of bean starch isolated from bean flour. This was because of the presences of higher protein, lipid, fat and fiber contents and larger amount of amylase-lipid complex in flour that could inhibit the swelling of starch granules.

When starch dispersions are heated, the swelling of granules and starch polymer solubilization also occur. Starch solubility is considered as an indicator of the degree of molecule in starch granules dispersion after cooking. SP can be positively related to the amount of soluble solids (e.g. amylose) leached outside the granules. However, for some starch types, including potato, tapioca and waxy corn starch, SP decreases when more solids leached out during cooking at higher temperatures. In the present study, the degree of solubility (%) of three flours at temperatures between 55 and 95°C is presented in Fig. 2. The results illustrated that solubility increased with temperature for all the flours tested. However, it seemed that there was no correlation between the
swelling power and the solubility of flours. GSF had a higher percentage of solubility than USF and WF at all temperatures tested. As can be seen, the solubility of GSF showed a continuous increase from 14.4% at 55°C to 48.53% at 95°C while the solubility of the other two flours increased slowly and reached a maximum value of ca. 30% at 95°C.

2. Pasting Properties

Table II summarizes the pasting profiles of WF, GSF and USF. The RVA parameters of all samples were significant different (p≤0.05). WF exhibited the highest peak viscosity of 154.46 RVU, followed by USF (137.28 RVU) and GSF (29.44 RVU). Among the three flours studied, USF had the highest final viscosity, followed by WF and GSF (256.6, 168.13, 24.47 RVU, respectively). The final viscosity indicated the re-association of starch granules especially amylose during cooling time after gelatinization and the formation of gel network [5]. A lowest breakdown for GSF as compared to WF and USF (6.02, 31.58 and 59.96 RVU, respectively) suggested that the GSF could withstand to heat and high mechanical shear conditions and less prone to loss the viscosity up shearing and holding. During the re-association process, the final viscosity could increase which is known as setback. The setback is also correlated to retrogradation of starch molecules. The highest setback was observed for USF (213.69 RVU), whereas setback value of GSF was lowest (1.06 RVU). These results indicated that GSF was possibly having the lowest rate of starch retrogradation and less syneresis, and vice versa for USF. The pasting temperature of WF was higher than that of USF (83.95 and 81.53°C respectively). GSF which had the lower swelling power did not show pasting temperature. In the studies of [6], sorghum flour showed the pasting temperature of 71°C, the peak viscosity of 129-143 RVA, trough viscosity of 84-85 RVA, and final viscosity of 213-238 RVA. These values were similar to pasting properties of USF in our study.

C. Physical Properties of Bread

1. Bread Color

Color is one of the most important indicators of bread quality. The desirable crust and color of bread should be golden brown and creamy white, respectively. The crust and crumb colors of sorghum breads and wheat breads (control) showed significant differences as presented in Table III. Crust and crumb colors of sorghum breads and wheat breads (control) showed significant differences as presented in Table III. The results suggested that with increasing levels of USF incorporation in sorghum flour blends, L* values of crust and
crumb continued to increase significantly. As can be seen in breads made with 100:0 of GSF:USF, it had the darkest crust (45.09) and crumb (29.88), whereas one made from ratio of 0:100 (GSF:USF) were lighter in color (59.60 for crust and 40.85 for crumb). The $a^*$ values indicated the red color in bread crust and crumb while the $b^*$ values indicated the yellow color. Bread crusts of control (10.61) had slightly higher of $a^*$ values than those of all sorghum breads (8.22-9.29). It is important to highlight that crumbs of all breads made with sorghum blends had a significant higher value of $a^*$ (10.41-15.77) than that of control (1.89).

### TABLE II

| PASTING PROPERTIES OF WHEAT, UNGERMINATED AND GERMINATED SORGHUM FLOURS |
|-------------------|------------------|------------------|
| Viscosity (RVU)   | Wheat flour      | Ungerminated sorghum flour | Germinated sorghum flour |
| Peak              | 154.46±2.41      | 137.28±4.22      | 29.44±0.54               |
| Trough            | 94.5±1.34        | 105.69±5.13      | 23.42±0.14               |
| Final             | 168.13±2.38      | 256.61±8.11      | 24.47±0.46               |
| Break down (RVU)  | 59.96±1.10       | 31.58±1.80       | 6.02±0.61                |
| Setback (RVU)     | 73.62±1.06       | 150.92±8.59      | 1.06±0.42                |
| Peak time (min)   | 5.49±0.03        | 5.15±0.04        | 3.49±0.03                |
| Pasting temperature (°C) | 83.95±1.14      | 81.53±0.85      | ND*                      |

Means in each row with different superscripts are significantly different ($p \leq 0.05$) by DMRT.

**GSF: Germinated sorghum flour, USF: Ungerminated sorghum flour**

### TABLE III

| CRUST AND CRUMB COLORS OF CONTROL AND GLUTEN FREE BREADS |
|-------------------|------------------|------------------|
| Bread              | GSF: USF         | crust            | crumb            |
| (control)          | Wheat flour      | $L^*$            | $a^*$            | $b^*$            |
| Wheat              | 57.61±1.00b      | 10.61±0.45a      | 19.77±0.34a      | 72.40±1.98a      | -1.89±0.27a       | 15.92±0.27a      |
| 100:0              | 45.09±2.67        | 9.29±0.42b       | 22.23±1.28b      | 29.88±2.34b      | 10.41±0.12d       | 3.50±0.14d       |
| 75:25              | 46.91±3.16        | 8.90±0.59b       | 22.09±1.26b      | 33.34±2.05b      | 15.31±0.86b       | 15.37±1.05b      |
| 50:50              | 51.10±1.5        | 8.22±0.43c       | 24.82±0.68c      | 35.58±0.68c      | 14.99±0.81f       | 17.23±0.65c      |
| 25:75              | 55.58±0.74       | 8.55±0.43c       | 24.89±0.74c      | 36.50±0.83c      | 15.77±0.26c       | 14.01±0.70c      |
| 0:100              | 59.60±1.27       | 8.70±0.78c       | 24.93±0.41c      | 40.85±0.07b      | 14.66±0.25c       | 15.40±0.24b      |

Means in each column with different superscripts are significantly different ($p \leq 0.05$) by DMRT.

**GSF: Germinated sorghum flour, USF: Ungerminated sorghum flour**

This was due to the red variety of sorghum employed. Crust of the control bread showed the lowest $b^*$ value of 19.77 ($p<0.05$). The increasing USF level in sorghum bread formulation resulted in the increasing $b^*$ value of bread crumb. Bread made with 100:0 (GSF:USF) exhibited the lowest yellowness intensity of crust (3.50) whereas the ratio of 50:50 gave the highest $b^*$ value (17.23).

### 2. Bread Crumb Texture

The texture profile analysis (TPA) of fresh experimental gluten-free bread crumbs are presented in Table IV The wheat flour bread was also prepared and used as control. Significant differences ($p \leq 0.05$) were observed between the TPA parameters of five gluten-free breads and the control, except springiness. The analysis of hardness indicated that the gluten free breads made with sorghum flour blends were considerably harder in comparison with the control (14.19-43.71 compared to 2.39 N). When the substitution level of USF increased, the hardness of bread crumb seemed to increase, except at GSF to USF (25:75) ratio. Bread with the highest hardness was made with GSF to USF (0:100) ratio. The replacement of whole wheat flour with sorghum flour blends decreased adhesiveness, and increased the gumminess and cohesiveness in the majority of bread crumb samples. Using only USF in bread formulation resulted in breads with harder crumb and less cohesiveness as compared to using only GSF. This finding was agreed with [6] who reported that the increase in crude fiber and water-soluble pentosans caused by the germinating grain root and shoot growth and the hydrolysis of non-starch polysaccharides during germination, resulted in increased water-holding capacity and dough viscosity, and consequently decreasing dryness and the crumb-firming rate of bread.

### D. Total Phenolic Contents

Table V shows the contents of total phenolic in the sorghum and wheat flours. In the flour obtained from USF, total phenolic contents were significantly higher than that of WF (1.87 compared to 0.83 mg GAE/g). The variation of total phenolic contents in sorghum can be due to a number of...
factors such as cultivar, growing location, and growing system [21]. The total phenolic contents of control and gluten free breads are shown in Table VI. It was found that wheat flour bread contained the lowest amount of total phenolic compounds (0.95 mg GAE/g). Five formulations of gluten free breads had total phenolic content ranged from 1.00 to 1.20 mg GAE/g. ANOVA showed significant differences ($p \leq 0.05$) in total phenolic content among the gluten-free breads. The highest total phenolic content was found in breads made with GSF: USF ratio of 50:50 (1.20 mg GAE/g), followed by those of 75:25 (1.16 mg GAE/g). In this study, the loss of phenolic compounds could occur during baking at 200 °C for 60 min. In the studies of [22], the level of total phenol in cooked, extruded sorghum (both whole and decorticated grains) was reduced significantly as compared to unprocessed grains. This could be due to the extrusion treatments e.g. steam, heat and extruder screw speed, that resulting in the decomposition of phenolic compounds.

### IV Conclusions

Red variety of sorghum is an attractive raw material for gluten free products due to the color and low allergenicity. Germination of sorghum grains could be one way to modify the chemical and functional properties of sorghum. The results suggested that there was significant difference in chemical compositions of GSF and USF. The significant differences were also observed in pasting properties of the flour, including peak, break down, final and set back viscosity as well as pasting temperature. Breads containing germinated sorghum had better texture (hardness) than those of ungerminated sorghum bread. Moreover some physical properties of malted sorghum bread were comparable to those of wheat flour bread such as adhesiveness and springiness whereas the malted sorghum bread contained higher level of total phenolic compounds. Overall conclusions, germination of sorghum seed, prior to use as bread flour to make gluten free bread, could improve chemical compositions and physicochemical properties of flour. The sorghum flour blended with the ratios between 50:50 to 100:0 (GSF:USF) had potential to be applied to prepared gluten free bread. However, the crust and crumbs of gluten free breads made with sorghum blend were harder and less elastic than those of wheat bread; therefore, some modifications are still needed such as an addition of some hydrocolloids and emulsifiers to obtain the best possible characteristics of gluten free bread from sorghum.

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