EFFECTS OF SUBSTITUTION OF HEAT-MOISTURE TREATED UNPOLISHED RED RICE ON PHYSICOCHEMICAL PROPERTIES OF COMPOSITE FLOUR, *IN VITRO* DIGESTIBILITY AND QUALITY OF BREAD

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SUMMARY

Heat-moisture treatment (HMT) has been reported to increase an amount of resistant starch (RS) in rice flour, which plays an important role in enhancing nutritional values of unpolished red rice (URR) by reducing the risk of obesity and diabetes. This study was carried out to investigate changes in chemical compositions (ash, lipid, protein, and carbohydrate contents), physicochemical properties (pasting properties, swelling index, and water absorption) of composite flour, and *in vitro* digestibility, quality properties (specific volume, textural properties), and sensory evaluation of bread containing the unpolished red rice flour (URRF) after heat-moisture treating with 30% moisture content and 100°C. HMT did not cause significant changes in the chemical composition of URR. However, the heat-moisture treated unpolished red rice flour (HMTURRF) had slight decrease in the swelling index and increase in the water absorption, gently increase in pasting temperature and decrease in peak and final viscosities, setback, and breakdown as compared with the control one. In addition, quality properties of bread with substitution of the HMTURRF were significant changes with decrease in specific volume and springiness, increase in hardness and gumminess. Breads partially substituted with HMTURRF had a lower content of rapidly digestible starch (RDS) (57.06 – 63.99%) and a higher content of resistant starch (RS) (16.85 – 27.66%) compared to completely wheat-based ones (65.05% and 13.70%, respectively). The sensory evaluation had no notable changes in color, appearance, texture, odor, and flavor but there was a slight difference in the overall acceptability. As a result, HMTURRF can be substituted for 20% of wheat four in producing low glycemic index (GI) breads.

Keywords: Heat-moisture treatment, low glycemic index, resistant starch, unpolished red rice.

INTRODUCTION

Unpolished red rice (URR) is the whole red rice grain with an intact bran layer along with pericarp, seed coat, embryo, and endosperm. Red rice is a treasure of health because of the outer bran layer, in which there is a lot of fiber in the bran, germ, and endosperm. Red rice consumption can inhibit the proliferation of breast cancer cells (Ghasemzadeh *et al.,* 2018) and limit the invasiveness of cancer cells in a dose-dependent manner (Pintha *et al.,* 2014). The major component of rice is starch, which gives the main energy for human daily activity and efficient digestion of food. Compared to other rice, red rice gives the consumers a feeling of fullness, which helps consumers regulate their carbohydrate intake. As the main component in rice, starch digestibility is an important consideration in nutrition, especially in people whose background diseases such as diabetes, obesity, etc. The total starch content includes slowly digestible starch (SDS), rapidly digestibility starch (RDS), and resistance starch (RS). A low water content results in a slow digestibility of starch in bread, leading to lower blood glucose levels, thus preventing obesity, diabetes, and cardiovascular diseases (Martínez *et al.,* 2018). Heat-moisture treatment (HMT) is applied to improve the physical and functional characteristics of starch in high efficiency, safety, and environmental protection without rupturing the granular structure. The modification during HMT is discovered to be dependent on the kind of starch source, moisture level, incubation time, and temperature (Gunaratne et., 2018). The hydrothermal treatment improved rice starch's mechanical and thermal stability and encouraged link strengthening, resulting in a more stable structure (Arns *et al.,* 2015). The URR that has been treated with HMT has several health benefits, including lowering the risk of obesity and diabetes. Therefore, this study investigated the effects of different mixing ratios between heat-moisture treated URR and wheat flour on chemical composition and physicochemical properties of composite flour, *in vitro* digestibility, quality, and sensory of bread substituted with heat-moisture treated URR.

MATERIAL AND METHODS

Material

Unpolished red rice (*Oryza punctata*) was purchased from Ben Tre granary. It is grown at Cuu Long Delta and verified by National Institute for Food Control (3962/PKN – VKNQG). Dry baker's yeast (Mauripan, Vietnam) was purchased in Coop-mart supermarket. Alpha-amylase from *Asperillus niger* (28.75U/mg) and Amyglucosidase from *Aspergillus oryzae* (300U/ml) used in this study were purchased from Sigma – Aldric Co. (St. Louis, Mo, USA). Other chemcials were purchased from Merck Chemical Co.

Methods

Preparation of heat-moisture treated unpolished red rice flour (HMTURRF)

The HMTURRF was prepared following the method of Hung *et al*., (2020). The moisture content of the grains was adjusted to reach 30% before HMT by immersion of URR grains in cold distilled water (4^oC) to avoid the growth of mold. The grains were collected and transferred into a glass flask after their moisture content reaches 30%. After equilibrium at 4^oC for 24h, the grains were heated at 100^oC for 6h and dried in the oven at 50^oC to obtain 10 – 11% moisture content. Finally, the dried grains were ground into fine flour and stored until use.

Composite flour preparation and bread-making

The composite flours were prepared by carefully mixing the fine HMTURRF with wheat flour at different ratios. The dough preparation followed the method of Tien *et al*., (2018). The composite flour (300g) was contained in a bowl with 18g of sugar, 4.5g of table salt, 6g of dry baker's yeasts, and 187.8mL of water. They were mixed well in a mixer for 15min and was put into a cabinet at 30^oC for 90min for fermentation. The mixed dough was punched every 30min. The fermented dough was cut into unit pieces with a weight of 130g each. Each piece was blenched in 15min, then punched, rolled, placed in the pan, and proofed at 38^oC for 33min. Afterward, the pans containing proofed dough were transferred into the oven and baked at 180^oC for 20min. Finally, the bread was stored for further analysis.

Determination of chemical composition of composite flour

The proximate analysis of composite four including moisture, ash, lipid, and protein contents was measured based on the AACC Approved Methods 44-01.01, 08-01.01, 30-10.01, 46-10.01, respectively. Total carbohydrate content was determined according to the equation: $\%\text{Carbohydrate content}_{db} = 100\% - [\%\text{moisture} + \%\text{ash}_{db} + \%\text{protein}_{db} + \%\text{lipid}_{db}]$

Determination of pasting properties, swelling power, and water absorption of composite flour

Pasting properties of composite flour were determined based on Tien *et al*., (2018) by using a micro visco-amylograph (Brabender® GmbH & Co. KG, Germany). Swelling index was determined according to Hung *et al.* (2020). The flour was vortexed with 20 mL distilled water in 1 min and boiled in a water bath at 90^oC with shaking of 200 rpm for 30 min. Then, the suspension was cooled under running tap water in 30s and 10 min in ice bath. The suspension was centrifuged at 4500×g for 10 min and equilibrated for 5 min at room temperature. The swelling index was calculated by dividing the mass of residue after centrifuge (g) and the mass of the flour sample (g). Water absorption of the composite flour was determined according to Hung *et al*., (2020). Flour was vortexed with 40 mL distilled water in 10 min at ambient temperature and centrifuged at 1000×g for 15 min. The water absorption was calculated by dividing the mass of water existed in the remaining sample (g) and the mass of flour sample (g).

Determination of in vitro digestiblity of bread substituted with HMTURRF

In vitro digestibility was determined following the method of Tien *et al.,* (2018). Samples were mixed with 20 mL of sodium acetate buffer (pH 6.0) and boiled in a water bath at 90^oC for 30 min, put in the oven at 37^oC for 15 min to equilibrate. After that, 5 mL enzyme solution of α-amylase (1,400 UmL^{-1}) and amyloglucosidase (13 $AGUmL^{-1}$) was added and incubated with shaking at 37^oC. Next, 0.5 mL of hydrolysate was removed and determined for total glucose concentrations (G20 and G120, respectively) after 20 min and 120 min of incubation by using the phenol-sulfuric acid method. The remaining solution after hydrolysis after 120 min was digested with amyloglucosidae (50 UmL^{-1}) to determine the total glucose content release (TG). G20, G120, and TG were used to calculate the content of RDS, SDS, and RS.

Determination of loaf volume

Loaf volume, the ratio of the loaf volume was measured at 50 min after loaves were removed from the oven by using the rapeseed displacement method (Tien *et al.,* 2018).

Determination of texture properties

A cube slide (3×3×3 cm^3) of bread was used to determine the firmness, springiness, and toughness by using Zwitt/Roell Textural analyzer following the method of Tien *et al*., (2018). The textural analysis was set up at a

P30C cylinder probe (30 mm in diameter), 2 mm/s for all the speeds (pretest speed, test speed, post speed), and 50% compression distance.

Sensory evaluation

The sensory evaluation of bread substituted with the HMTURRF was carried out according to the method of Inglett *et al*., (2005). The color, odor, flavor, texture, appearance, and overall acceptability of bread were valued by 30 untrained panelists at the International University for the hedonic test. Each of them was served with four slides of different kinds of substituted bread and one cup of water. Each slice of bread was coded with a three-digit random number in a randomized and balanced order and assessed at room temperature. The panelists were asked to evaluate samples and indicate the degree of liking for the characteristics of the bread on the nine-point hedonic scale on a response sheet.

RESULTS AND DISCUSSION

Chemical composition of composite flour

Table 1. Chemical contents (%, dry basis) of composite flours

Sample	Protein	Lipid	Ash	Carbohydrate
C0	13.85±0.06^{a}	1.94±0.03^{a}	0.49±0.02^{c}	70.41±0.12^{d}
C10	13.37±0.15^{b}	1.92±0.03^{a}	0.53±0.02^{c}	71.70±0.19^{c}
C20	12.89±0.12^{c}	1.89±0.03^{a}	0.57±0.01^{b}	72.69±0.08^{b}
C30	12.42±0.06^{d}	1.86±0.06^{a}	0.67±0.01^{a}	73.62±0.22^{a}

C0, wheat flour; C10, 90% wheat flour and 10% HMTURRF; C20, 80% wheat flour and 20% HMTURRF; C30, 70% wheat flour and 30% HMTURRF. Data followed by the same superscript letter in the same column are not significantly different (P > 0.05).

The effect of HMT on chemical composition of wheat flour and treated URR flour at different ratios are presented in Table 1. Significant differences were observed in protein and carbohydrate contents between the control sample and treated samples. The protein contents of treated samples were notably reduced in comparison with untreated sample. The moisture and protein contents of treated samples decreased from the lowest HMTURRF substitution to the highest HMTURRF substitution. The lipid content was also decreased following the higher percentage of HMTURRF but not remarkable. In comparison with ash content of 100% wheat flour sample (0.49% ash), that of C10 slightly increased (0.53%), while those of C20 and C30 significantly increased (0.57% and 0.67%, respectively). The carbohydrate content significantly increased with incresing the substitution concentration. According to the research of Hung *et al*. (2020), the macronutrient concentrations of URR were 9.11% protein content, 1.83% lipid content, 1.59% ash content, and 87.5% carbohydrate content, it means the protein, lipid contents of URR were lower than that of wheat flour, while ash and carbohydrate contents of URR were higher than that of wheat flour.

Pasting properties, swelling power, and water absroption of composite flour

Table 2. Pasting properties of composite flour

Parameters	C0	C10	C20	C30
Pasting temperature [^{o}C]	60.97±0.31^{c}	61.87±0.21^{bc}	62.8±0.1^{ab}	63.13±0.81^{a}
Peak viscosity [BU]	837±10.69^{a}	745±27^{b}	679±2.08^{c}	604±6.03^{d}
Final viscosity [BU]	1054±8.62^{a}	935±17.8^{b}	839±21.5^{c}	801±18^{c}
Breakdown [BU]	394±13.86^{a}	287±27.7^{b}	206±7.57^{c}	89±4^{d}
Setback [BU]	435±8.19^{a}	431±9.08^{a}	420±2.52^{a}	415±11.53^{a}
Swelling index [g/g]	5.31±0.11^{a}	4.86±0.33^{ab}	4.67±0.24^{b}	4.65±0.14^{b}
Water absorption capacity [g/g]	0.85±0.03^{b}	0.91±0.02^{b}	1.03±0.01^{a}	1.08±0.03^{a}

C0, wheat flour; C10, 90% wheat flour and 10% HMTURRF; C20, 80% wheat flour and 20% HMTURRF; C30, 70% wheat flour and 30% HMTURRF. Data followed by the same superscript letter in the same row are not significantly different (P > 0.05).

The physicochemical properties including pasting properties, swelling power, and water absorption of the composite flours with different ratios of wheat flour and HMTURRF are given in Table 2. HMT induced notable differences in some pasting properties of flour of rice grain and has also been proven by some previous research. While breakdown, peak viscosity, and final viscosity considerably reduced with increasing the ratio of HMTURRF, the value of pasting temperature of the composite flour significantly increased. There was no notable decrease in the setback of four samples. The final viscosity values differences were significant between all samples, except

C20 and C30. Regarding swelling index and water absorption, HMT was proved to cause a reduction in swelling power (Hung *et al.,* 2020), and the water absorption index of untreated flour was lower in comparison with HMT flour. The higher substituted HMTURRF flour needs a higher temperature to start gelatinization, thus it took a long time to gelatinize. In contrast with swelling index, the higher modified samples had the higher water absorption capacity values. Amylose was reported to have a stronger ability to bind water than native starch by Thilagavathi (2015). Overall, in this study, HMT affected pasting temperature, peak viscosity, final viscosity, breakdown, swelling index, and water absorption capacity, but not for setback. Therefore, the higher HMTURRF substitution took a higher temperature to start gelatinization, had lower paste strength, lower potential to form a gel after processing, and higher heating and shear stress resistance. The gel stability and retrogradation potential did not considerably reduce under HMT.

***In vitro* digestibility of bread loaves**

Table 3. Starch fraction (RDS, SDS, RS) of breads made from composite flours

Sample	Starch fraction (% w/w, db)		
	RDS	SDS	RS
B0	65.05±0.61^{a}	21.25±0.54^{a}	13.70±0.40^{d}
B10	63.99±0.81^{a}	19.16±0.42^{b}	16.85±0.83^{c}
B20	58.56±1.26^{b}	17.91±0.58^{b}	23.53±0.68^{b}
B30	57.06±0.72^{b}	15.28±0.38^{c}	27.66±0.39^{a}

B0, 100% wheat flour bread; B10, 90% wheat flour and 10% HMTURRF bread; B20, 80% wheat flour and 20% HMTURRF bread; B30, 70% wheat flour and 30% HMTURRF bread. RDS, rapidly digestible starch; SDS, slowly digestible starch; RS, resistant starch. Data followed by the same superscript letter in the same column are not significantly different (P > 0.05).

The amounts of RDS, SDS, and RS of breads made from different ratios of composite flours are shown in Table 3. B0, which was made from 100% wheat flour, had the highest RDS. SDS value of B0 was also significantly higher than SDS values of the bread samples substituted with HMTURRF. RS was the only one that significantly increased in each sample with higher amount of HMTURRF substitution (13.7%, 16.85%, 23.53%, and 27.66% w/w for B0, B10, B20, and B30, respectively). In the research of Tien *et al*. (2018), the RDS and SDS of bread made from composite flour, which had HMT, also went down and rose up for RS value in comparison with the bread made from 100% wheat flour. Similar results were reported by Hung *et a*l. (2020) for URR grains. The rise in thermo-stable RS indicated the connections between wheat starch and proteins or lipids or both developed during HMT, thus partially limiting the accessibility of starch chains to be hydrolyzed by enzymes (Chung *et al.,* 2009).

Specific volume and textual properties bread loaves

Table 4.Specific volume and textureal properties of breads made from wheat and composite flours

Sample	Specific volume [cm^3/g]	Textural properties		
		Hardness [N]	Springiness [mm]	Gumminess [N]
B0	5.25±0.08^{a}	2.94±0.07^{c}	12.43±0.56^{a}	1.88±0.01^{d}
B10	4.55±0.09^{b}	5.13±0.17^{b}	11.66±0.48^{ab}	2.46±0.11^{c}
B20	3.82±0.10^{c}	6.80±0.27^{b}	11.12±0.09^{b}	4.41±0.17^{b}
B30	2.81±0.14^{d}	31.42±1.53^{a}	10.67±0.28^{b}	5.52±0.12^{a}

B0, 100% wheat flour bread; B10, 90% wheat flour and 10% HMTURRF bread; B20, 80% wheat flour and 20% HMTURRF bread; B30, 70% wheat flour and 30% HMTURRF bread. Data followed by the same superscript letter in the same column are not significantly different (P > 0.05).

Table 4 shows the results of the specific volume of bread loaves made from composite flour between wheat flour and HMTURRF. The values decreased notably depending on the ratios of substituted HMTURRF in bread. The reduction of specific volume was caused by the dilution of gluten content in substitution of composite flour, which leads to reduced elasticity and extensibility (Hung *et al.,* 2020). The hardness, springiness, and gumminess values in textural properties of bread loaves determined by Zwitt/Roell Textural analyzer were also illustrated in Table 4. The hardness value of B0 was 2.94 N, significantly lower than treated samples. The hardness of bread was significantly increased along with increasing the HMTURRF ratio. According to Hung *et al.,* (2020), the greater proportions of short-chain molecules and solubilized amylose in these starches easily degraded after baking caused the increase in the hardness of breadcrumb with the substitution of the HMTURRF. Regarding springiness, B0 had the highest value (12.43 mm). There was no significantly different in the group substituted with treated bread. Additionally, there were significant increases in the gumminess results of four samples. It means that that HMT not only made the breadcrumbs harder but also demanded more energy to swallow them before they reached a stable condition for consumption, reduced the physically springs back after the bread had been deformed during the first compression.

Evaluation of sensory profile of bread samples

Table 5. Mean sensory score of breads

Sensory attribute	B0	B10	B20	B30
Color	7.30 ± 0.16^{a}	6.87 ± 0.14^{a}	7.27 ± 0.14^{a}	7.37 ± 0.15^{a}
Appearance	7.47 ± 0.12^{a}	7.20 ± 0.14^{a}	7.67 ± 0.09^{a}	7.07 ± 0.13^{a}
Texture	7.33 ± 0.13^{a}	7.13 ± 0.1^{a}	7.40 ± 0.12^{a}	6.80 ± 0.14^{a}
Odor	7.10 ± 0.15^{a}	7.33 ± 0.08^{a}	7.33 ± 0.12^{a}	7.10 ± 0.14^{a}
Flavor	6.73 ± 0.11^{a}	6.80 ± 0.14^{a}	6.93 ± 0.11^{a}	6.43 ± 0.15^{a}
Overall Acceptability	7.20 ± 0.1^{ab}	7.13 ± 0.07^{ab}	7.50 ± 0.07^{a}	6.67 ± 0.14^{b}

B0, 100% wheat flour bread; B10, 90% wheat flour and 10% HMTURRF bread; B20, 80% wheat flour and 20% HMTURRF bread; B30, 70% wheat flour and 30% HMTURRF bread. Data followed by the same superscript letter in the same row are not significantly different ($P > 0.05$).

The evaluation of bread substitution at different ratios between wheat flour and HMTURRF was illustrated in Table 5. It shows that there were no significant differences in all samples in color, appearance, texture, odor, and flavor tests. In the overall acceptability, the bread with 20% substituted HMTURRF, which was the highest in rating (7.50 point), was not siginficantly different from the second rating sample which 100% bread made from wheat flour (B0 with 7.20 point) and the third rating bread with 10% substitution of HMTURRF (B10 with 7.13 point).

CONCLUSION

In conclusion, 30% moisture and 100°C of HMT application on URR caused significant differences in pasting properties, swelling index, and water absorption capacity of composite flour between wheat flour and HMTURRF at different ratios. Starch fraction and quality of bread substituted with different ratios of HMTURRF and wheat flour were also considerably changed, which were the increase of RS contents and decrease of RDS contents. As a result, the bread with 20% substituted HMTURRF could be produced industrially and commercially. This research may be a premise for further research to develop nutritional products that apply HMT with certain conditions to not only increase resistant starch but also keep the constant quality of other components.

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NGHIÊN CỨU SỰ ẢNH HƯỞNG CỦA VIỆC BỔ SUNG GẠO LỨT ĐỎ ĐƯỢC XỬ LÝ NHIỆT-ẨM ĐẾN TÍNH CHẤT LÝ-HÓA CỦA BỘT, KHẢ NĂNG TIÊU HÓA *IN VITRO* VÀ CHẤT LƯỢNG BÁNH MÌ

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TÓM TẮT

Xử lý nhiệt-ẩm được biết có khả năng làm tăng thành phần tinh bột kháng (RS) của gạo, đóng vai trò quan trọng trong việc nâng cao giá trị dinh dưỡng của gạo lứt đỏ (URR) nhằm giảm nguy cơ béo phì và tiểu đường. Nghiên cứu này được thực hiện để khảo sát sự biến đổi của các thành phần hóa hóa học (hàm lượng tro, hàm lượng lipid, hàm lượng protein, và hàm lượng carbohydrate), tính chất lý-hóa (đặc tính hồ hóa, độ trương nở, độ hấp thụ nước) của bột phối trộn, khả năng tiêu hóa *in vitro*, chất lượng sản phẩm (thể tích, kết cấu) và đánh giá cảm quan của bánh mì có chứa bột gạo lứt đỏ sau khi xử lý nhiệt-ẩm với độ ẩm 30% và 100°C. Xử lý nhiệt ẩm không gây ra những thay đổi đáng kể trong thành phần hóa học của URR. Tuy nhiên, có sự khác biệt đáng kể về tính chất lý-hóa của bột (độ trương nở giảm nhẹ và độ hấp thụ nước tăng), đặc tính hồ hóa (nhiệt độ hồ hóa tăng, giảm độ nhớt cực đại, độ nhớt cuối cùng, độ nhớt giảm, và độ nhớt rung) so với mẫu đối chứng. Ngoài ra, chất lượng của bánh mì có bổ sung bột gạo lứt đỏ đã được xử lý nhiệt-ẩm (HMTURRF) cũng có những thay đổi đáng kể như thể tích và độ đàn hồi giảm xuống, trong khi độ cứng và độ dẻo tăng lên. Bánh mì được thay thế một phần bằng HMTURRF có hàm lượng tinh bột tiêu hóa nhanh (RDS) thấp hơn (57.06 – 63.99%) và hàm lượng tinh bột kháng (RS) cao hơn (16.85 – 27.66%) so với các loại bánh làm hoàn toàn từ lúa mì (lần lượt là 65.05% và 13.70%). Màu sắc, hình thức, kết cấu, mùi và hương vị không có sự khác biệt đáng kể khi khảo sát đánh giá cảm quan, nhưng có một chút khác biệt về mức độ chấp nhận chung. Kết quả cho thấy, bột gạo lứt đỏ sau khi xử lý nhiệt-ẩm có thể thay thế 20% bột mì trong sản xuất sản phẩm bánh mì sinh đường thấp.

Từ khóa: Gạo lứt đỏ, tinh bột kháng tiêu hóa, sản phẩm sinh đường thấp, xử lý nhiệt-ẩm.

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