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Quality changes and *in vitro* digestibility of bread substituted with tuber starches modified by citric acid and heat-moisture treatment

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ABSTRACT

Heat-moisture treatment combined with citric acid favors the escalation of resistant starch that is antagonistic to the small intestinal hydrolysis and plays an important role in reducing diabetes and giving positive effects on human health. The aim of this research was to examine the qualities (specific volume, textural properties, *in vitro* digestibility, and sensory profiles measured by descriptive analysis) of bread substituted with 20% of the mixture of citric acid and heat-moisture treated tuber starches (sweet potato, potato, and cassava) and vital gluten (9:1, w/w). An incorporation of 20% of modified starches and gluten into wheat flour for bread-making resulted in a substantial enhancement on resistant starch content and hardness and gumminess values, but a momentous fall on specific volume and sensorial profiles as well as overall acceptability of composite breads. Among three kinds of supplemented baking-products, breads complemented with 20% of modified cassava starch and gluten displayed intermediate resistant starch content (32.0%), and hardness value (14.94 N), but highest specific volume (3.34 cm³/g), and score of overall acceptability (around 4.10/5.00) as compared to other modified starches.

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1 INTRODUCTION

Recently, the obesity rate has reached nearly one-third of the global population. Although there are various explanations for the development of obesity, the most crucial cause is especially diet (Chen *et al.*, 2010). Thus, the general recommendations for a healthy diet now are to reduce the intake of high cholesterol foods and to get more complex carbohydrates (Wolf *et al.*, 1999). However, the rate of starch digestion plays an important role in adjudicating the level of glycemic responses to dietary starches (O'Dea *et al.*, 1981). Therefore, a cost-effective dietary modification to lessen

pervasiveness of obesity could be to follow a low-carb diet by improving the intake of ingredients containing high resistant starch content.

Due to diverse ecological habitats, roots and tubers, especially sweet potato, potato and cassava, are acknowledged as the most important food crops after grains. They can form new openings in food chain supply, and make a significant contribution to sustainable development and food security. However, they are mainly characterized as underdeveloped, small-scale with almost no postharvest techniques applied in Vietnam (Kim *et al.*, 2001). Thus, to prevent the post-harvest losses

and take advantage of being a source of edible starch, there are more and more projects conducted on producing starches from roots and tubers.

Based on the rate of digestion in the human small intestine, starches are divided into three groups: rapidly digestible starch (RDS), slowly digestible starch (SDS), and resistant starch (RS) (Englyst *et al.*, 1992). RDS and SDS fractions are completely digested and absorbed in the human small intestine, whereas, small intestinal breakdown can be resisted by the total amount of starch that is defined as RS. RS plays an important role in reducing diabetes, lowering the risk of heart disease, and giving a positive effect on colonic health (Topping and Clifton, 2001; Sajilata *et al.*, 2006). Among four kinds of RS, RS type III occupies the most fraction and is easily produced by a hydrothermal method. Heat-moisture treatment combined with citric acid (CAHMT) can give a highest increase in the amount of SDS and RS content of the starches as compared to the native starch from sweet potato, yam, potato, or cassava and other treated starches (Hung *et al.*, 2014; Hung *et al.*, 2017).

The applications of RS in producing bakery are still extensively studying because the replacement of wheat in these products is a major technological challenge, and may give some remarkable effects on the specific volume, textural profiles and sensory qualities of the end-used products. In this research, 20% of composite flours of citric acid and heat-moisture treated sweet potato, potato, or cassava starch and vital gluten (9:1, w/w) was used to substitute for wheat flour in bread-making. In addition, effects of supplementation of composite flours for wheat flour on qualities (specific volume, textural properties, and sensory profiles) and *in vitro* digestibility capacity were also investigated.

2 MATERIALS AND METHODS

2.1 Materials

Potatoes (*Solanum tuberosum*) were grown in Da Lat city, Lam Dong province, and white sweet potatoes (*Ipomoea batatas* L.) were purchased at Hoa Tan village, Chau Thanh district, Dong Thap province. Cassava starch was produced and purchased from Hong Phat Cassava Processing Private Enterprise (Tay Ninh, Vietnam).

α -amylase from *Aspergillus niger* (28.75 U/mg) and amyloglucosidase from *Aspergillus oryzae* (300 U/mL) used in *in vitro* digestibility test were purchased from Sigma-Aldrich Company, while baking ingredients, VITEN wheat gluten, and other chemicals were purchased from local supplier in Ho Chi Minh City, Vietnam.

2.2 Methods

2.2.1 Starch isolation

Sweet potato or potato starch was isolated by repeated deposition method written by Lawal (2004). The starch released from the ground tubers was sieved through a series of sieves with aperture size of 0.25 mm and 0.125 mm. The final filtrate was settled down in 24 hours finally washed twice with tap water until the tailing fraction became negligible after settling. The isolated starch was dried in an oven at 40°C for 24 hours (moisture content < 10%).

2.2.2 Hydrothermal treatment of starches

RS was produced by the combination of citric acid and heat-moisture treatment that was based on the study of Hung *et al.* (2014). Starch was mixed well with citric acid to achieve moisture level of 30% and heated at 110°C for 8 hours. After heating, the mixture was neutralized with NaOH, settled and then centrifuged. Finally, the solid residue from centrifuging was dried at 40°C for 24 hours and grinding was applied to achieve modified starch.

2.2.3 Bread-making method

The formula and procedures from method 10-10B (AACC, 2000) with a slight modification were applied to bake bread. The dough was prepared from 300 g wheat flour with or without 20% of composite flours of modified sweet potato, potato, or cassava starch and vital gluten (9:1, w/w), 18 g sugar, 4.5 g salt, 6 g dry baker's yeasts, and 187.8 mL water. After mixing in 15 min, the dough was fermented at 30°C with humidity of 85% for 90 min, and punching was performed each 30 min. After 90 min of fermentation, the dough was divided into 3 pieces whose weight was around 130 g. Then, each piece was kneaded into a rounded shape for 15 min, and then it was laminated, rolled, cased off and placed in the pans and proofed at 38°C with humidity was 90% for 33 min. Finally, the dough was baked at 180°C for 20 min. After baking, the final product was formed. In order to determine *in vitro* digestibility, breadcrumb was dried at 50°C for 24 hours and then pulverized.

Bread made from wheat flour was coded as WFB, while breads with 20% of mixture of modified cassava, potato or sweet potato starch and vital gluten (9:1, w/w) supplementation were coded as 20CSB, 20PSB, or 20SPSB, respectively.

2.2.4 Evaluation of specific volume and texture properties of starch-substituted bread

Specific loaf volume (cm^3g^{-1}) was determined by dividing loaf volume, which was measured by the

rapeseed displacement method (Giami *et al.*, 2004), by its corresponding loaf weight.

Textural properties of breadcrumb prepared in a rectangle shape (2 cm × 1 cm × 1 cm) were measured using a Zwitt/Roell Textural analyzer followed the method of Ulziijargal *et al.* (2013).

2.2.5 Evaluation of in vitro digestibility of starch-substituted bread

A minor modification in the method of Englyst *et al.* (1992) was used to measure RDS, SDS and RS of starch-substituted bread. The enzyme solution containing α-amylase (1400 U/mL) and amyloglucosidase (13 U/mL) was used to digest starch for determining the glucose content released in 20 min (G20) and in 120 min (G120). The remained solution after hydrolysis for 120 min was digested with amyloglucosidase (50 U/mL) to determine the total glucose content release (TG).

G20, G120 and TG were used to calculate the content of RDS, SDS and RS.

2.2.6 Evaluation of sensory profiles of starch-substituted bread

Sensory qualities analysis was carried out based on the methods of Inglett *et al.* (2005) with moderate modification. The sensory tests were performed three times with an evaluation panel of 15 trained members. Testers were asked to score different kinds of breads in terms of crumb color, taste, aroma, appearance, texture, and overall acceptability by descriptive analysis (Table 1).

2.2.7 Statistical analysis

SPSS version 16 was used for one-way ANOVA of the results of qualities of breads. Tukey’s test with significance level at p < 0.05 was used to compare the means of the results.

Table 1: Summary table of sensory evaluation of bread sample

Score	Color	Appearance	Texture	Odor and taste
5	Uniform color; typical golden brown crust of bread with creamish white crumb inside	Crust of bread has smooth surface with fully uniform porosity of crumb	Fully crispy crust of bread and spongy crumb	Fully pleasant aroma characteristic for bread, harmonious taste and mild sweet aftertaste
4	Relatively uniform color; golden brown crust of bread with creamish crumb inside	Crust of bread has smooth surface with slightly uniform porosity of crumb	Relatively crispy crust of bread and relatively spongy crumb	Pleasant aroma characteristic for bread, harmonious taste, mild sweet aftertaste
3	Non uniform color; golden brown crust of bread with deep cream white crumb inside	Crust of bread has slightly rough surface, slightly uniform porosity of crumb, but bigger air cells	Slightly crispy crust of bread and slightly spongy crumb	Aroma slightly characteristic for bread, very mild sweet aftertaste
2	Slightly dark brown crust of bread with slightly greyish crumb inside	Crust of bread has rough surface; non uniform porosity of crumb with many large air cells occurring	Hard crust of bread and soft crumb	Yeasty odor, salty or sour taste and no sweet aftertaste
1	Dark brown crust of bread with grey crumb inside	Crust of bread has rough surface, non-uniform porosity of crumb with too many large air cells occurring	Too hard crust of bread and too soft crumb	Off flavor, strange odor, no sweet aftertaste

3 RESULTS AND DISCUSSIONS

3.1 Cross-sectional view of starch-substituted breads

Figure 1 exhibits the cross-sectional view of breadcrumbs supplemented with 20% of mixture of modified tuber starches and vital gluten (9:1, w/w). The addition of modified tuber starches under CAHMT and vital gluten made the crumb structure smaller and more regular than WFB whose

appearance had large and irregular gas cell. Generally, supplementation of modified starches containing high RS content did not have a remarkable depreciate impact on the external appearance and harmony of breads. Thus, these starches were suitable to supplement into wheat flour to make bread instead of fibers whose complementary addition could degenerate the color as well as appearance of breadcrumbs (Pomeranz *et al.*, 1977).

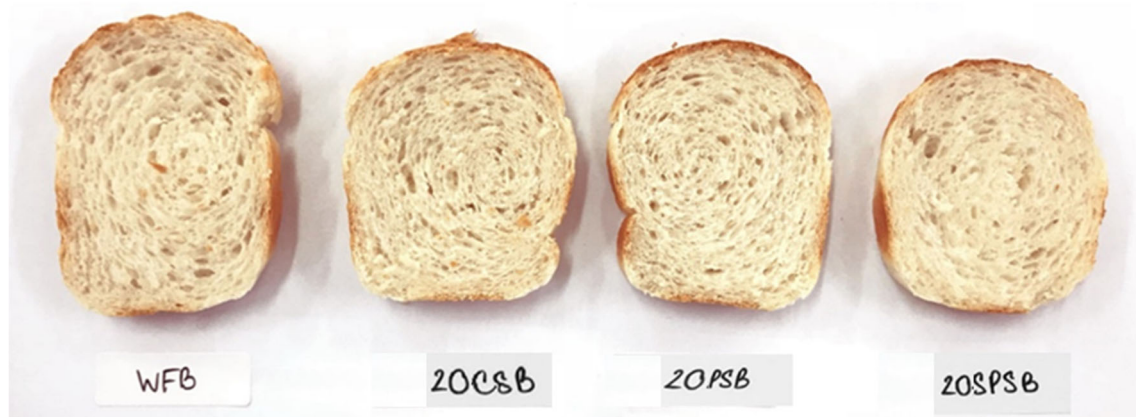


Fig. 1: Cross-sectional view of breadcrumbs supplemented with 20% of mixture of citric acid and heat-moisture treated cassava, potato or sweet potato starches and vital gluten (9:1, w/w).

WFB, bread made from wheat flour; *20CSB*, bread with 20% of mixture of modified cassava starch and vital gluten (9:1, w/w) supplementation; *20PSB*, bread with 20% of mixture of modified potato starch and vital gluten (9:1, w/w) supplementation; *20SPSB*, bread with 20% of mixture of modified sweet potato starch and vital gluten (9:1, w/w) supplementation.

3.2 Specific volume and textural profiles of starch-substituted breads

Table 2 demonstrates data of the specific volume of four kinds of bread samples supplemented with modified tuber starches and vital gluten (9:1, w/w). Specific volumes of the starch-substituted breads ranged from 2.41 to 3.34 cm³g⁻¹, which were lower than that of WFB (4.74 cm³g⁻¹). Thereupon, these outcomes implied that a supplementation of tuber starches under CAHMT and vital gluten (9:1, w/w) for bread-making ensured a significant devaluation on the specific volume of breads. Among three kinds of starch-substituted breads, 20CSB had the highest specific volume (3.34 cm³g⁻¹), while the lowest one belonged to 20PSB (2.41 cm³g⁻¹). This result was agreeable to the research published by Miyazaki and Morita (2005) who revealed that a partial substitution of heat-moisture treated maize starch considerably reduced specific volume of starch-substituted breads. The aforementioned reduction of the specific volume of loaves was due to adverse effect of dilution on gluten content in the composite flour. The reduction of gluten content gave the significant effect on the dough properties including less elasticity and smaller extensibility resulting in smaller loaf volume (Makinde and Akinoso, 2014). Miyazaki and Morita (2005) also reported that the substitution of heat-moisture treated maize starch to wheat flour decreased the elasticity of the dough because the modified starch did not bind readily with gluten to form elastic dough, resulting in low specific volume of loaves.

The textural profiles of breadcrumbs with modified tuber starches and vital gluten (9:1, w/w) supplementation expressed as hardness, springiness, and gumminess values are exhibited in Table 2. Hardness and gumminess values of the starch-substituted breadcrumbs ranged from 12.07 to 15.96 N and 5.95 to 7.71 Nmm, respectively, which were higher than those of WFB (7.55 N and 4.10 Nmm, respectively). Thus, an incorporation of tuber starches under CAHMT and vital gluten (9:1, w/w) for bread-making resulted in a substantial increment in the hardness and gumminess values of breadcrumbs. Nonetheless, no noticeable inconsistency in the springiness value of all breadcrumbs was recognized. Thus, citric acid and heat-moisture treated tuber starch and vital gluten (9:1, w/w) substitution did not affect the rubbery characteristic of breadcrumb although these breads had a lower protein and gluten contents than WFB. These aforementioned data corresponded with the previous projects which revealed that a supplementation of chemically modified cassava or commercial resistant starch into wheat flour gave the remarkable increment in the hardness value of breadcrumb (Ozturk *et al.*, 2009; Rodriguez-Sandoval *et al.*, 2016). The considerable increase in hardness and gumminess values might be due to the higher amounts of solubilized amylose and short-chain molecules in these starches which easily retrograded after baking. In addition, Hung *et al.* (2005) also reported that the breads baked from flours which contained low protein content and gluten quantity were harder than breads baked from flours had higher protein content.

Table 2: Specific volume and textural profiles of breads supplemented with 20% of mixture of citric acid and heat-moisture treated cassava, potato or sweet potato starches and vital gluten (9:1, w/w)

Sample	Specific volume (cm ³ g ⁻¹)	Textural profiles		
		Hardness (N)	Springiness(mm/mm)	Gumminess (Nmm)
WFB	4.74 ± 0.15 ^d	7.55 ± 0.46 ^a	0.91 ± 0.01 ^a	4.10 ± 0.34 ^a
20CSB	3.34 ± 0.19 ^c	14.94 ± 1.50 ^b	0.90 ± 0.01 ^a	6.92 ± 0.57 ^{bc}
20PSB	2.41 ± 0.15 ^a	12.07 ± 2.11 ^b	0.90 ± 0.02 ^a	5.95 ± 0.88 ^b
20SPSB	2.99 ± 0.04 ^b	15.96 ± 1.66 ^b	0.92 ± 0.01 ^a	7.71 ± 0.70 ^c

Data followed by the same superscript letter in the same column are not significantly different ($P < 0.05$).

3.3 In vitro digestibility of starch-substituted breads

Table 3 illustrates the percentages of RDS, SDS, and RS in bread supplemented with modified tuber starches and vital gluten (9:1, w/w). Both RDS and SDS of starch-substituted bread ranged from 51.6 – 53.1% and 14.9 – 16.4%, respectively, which was lower than those of WFB (62.0% and 25.7%, respectively). Therefore, the percentages of RDS and SDS reduced remarkably when 20% of mixture of modified cassava, sweet potato or potato starch and vital gluten was substituted. The RS content of 20CSB, 20PSB, and 20SPSB were 32.0, 31.3, and 33.3%, respectively, which was higher than that of WFB (12.3%). Thus, RS content dramatically increased when there was an addition of modified cassava,

sweet potato, and potato starch whose RS content in our earlier research was 40.9, 39.8, and 41.9%, respectively. These data corresponded to the work done by Ozturk *et al.* (2009) and Babu *et al.* (2015) where the addition of commercial resistant starch presented a massive increase in RS content of bread. In this study, these data also implied that RS in modified tuber starches not only persisted, but also formed more during baking period. Re-association of additional amylose and short-chain molecules of the starches which were gelatinized during baking and retrograded eventually were the major reasons for an enhancement in the amount of RS. This is because food processing, which involves heat and moisture like bread, in most cases, destroys RS type I and RS type II, but may form RS type III (Faraj *et al.*, 2004).

Table 3: Starch digestibility (RDS, SDS and RS fractions) of breads supplemented with 20% of mixture of citric acid and heat-moisture treated cassava, potato or sweet potato starches and vital gluten (9:1, w/w)^{1,2}

Fractions	WFB	20CSB	20PSB	20SPSB
RDS	62.0 ± 2.0 ^c	51.6 ± 0.3 ^a	53.1 ± 0.2 ^b	51.8 ± 0.7 ^a
SDS	25.7 ± 2.7 ^c	16.4 ± 0.5 ^b	15.6 ± 0.8 ^{ab}	14.9 ± 0.8 ^a
RS	12.3 ± 0.8 ^a	32.0 ± 0.6 ^b	31.3 ± 0.6 ^b	33.3 ± 0.4 ^c

¹RDS, rapidly digestible starch; SDS, slowly digestible starch; RS, resistant starch.

²Data followed by the same superscript letter in the same row are not significantly different ($P < 0.05$).

3.4 Sensory evaluation of starch-substituted breads

Organoleptic properties of breads enriched with modified tuber starches and vital gluten (9:1, w/w) are demonstrated in Table 4. No conspicuous disagreement was marked between WFB and breads substituted with 20% of mixture of modified cassava or potato starch and vital gluten (9:1, w/w) in term of color, appearance, texture, odor and flavor, and overall acceptability. 20SPSB had significantly lower mean sensory score as compared to that of WFB in term of all sensory attributes, while no substantial discrepancy was also confirmed between 20CSB, 20PSB, and 20SPSB. This finding was very

compatible with the works of Reed (2012) that observed bread with 20% RS from cooked rice replacement was best on overall acceptability judged by sensory panels. In addition, according to Majzoobj *et al.* (2014), supplementation of less than 30% corn resistant starch would give no significant influence on the sensory attributes of the cake, while a maximum level of corn resistant starch in cake recipe was concluded as an acceptable product amounted to 20%. Consequently, the result of the sensory evaluation pointed out that the substitution of up to 20% of mixture of citric acid and heat-moisture treated cassava starch and vital gluten gave moderately satisfactory overall acceptability (around 4.10 over 5.00).

Table 4: Mean sensory score of breads supplemented with 20% of citric acid and heat-moisture treated cassava, potato or sweet potato starches and vital gluten (9:1, w/w)

Sensory attribute	WFB	20CSB	20PSB	20SPSB
Color	4.37 ± 0.54 ^t	4.00 ± 0.74 ^{at}	4.03 ± 0.62 ^{at}	3.68 ± 0.72 ^ε
Appearance	4.33 ± 0.78 ^t	4.13 ± 0.86 ^{at}	3.93 ± 0.74 ^{at}	3.71 ± 0.71 ^ε
Texture	4.33 ± 0.62 ^t	4.03 ± 0.72 ^{at}	4.03 ± 0.70 ^{at}	3.68 ± 0.72 ^ε
Odor and flavor	4.22 ± 0.80 ^t	4.20 ± 0.55 ^{at}	4.03 ± 0.72 ^{at}	3.75 ± 0.70 ^ε
Overall acceptability	4.22 ± 0.80 ^t	4.10 ± 0.61 ^t	4.03 ± 0.62 ^{at}	3.64 ± 0.62 ^ε

Data followed by the same superscript letter in the same row are not significantly different ($P < 0.05$)

4 CONCLUSIONS

In this research, the supplementation of 20% of mixture of modified cassava, potato, or sweet potato starch and vital gluten (9:1, w/w) boosted the RS content in the bread as compared to that of WFB determined by *in vitro* digestibility method, but remarkably reduced the specific volume of bread loaves and increased the hardness and gumminess of bread crumbs. Among three kinds of breads supplemented with sweet potato, potato, or cassava starch under CAHMT and vital gluten (9:1, w/w), 20CSB exhibited intermediate RS content, and hardness and gumminess values, but highest specific volume, and score of overall acceptability. Based on these aforementioned results, the substitution 20% of mixture of citric acid and heat-moisture treated cassava starch and vital gluten (9:1, w/w) into wheat flour had satisfied bread qualities, overall acceptability as well as health benefits.

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