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EFFECT OF PARTIAL SUBSTITUTION OF SPRAY DRIED RIPE HOM THONG BANANA POWDER WITH HOM THONG BANANA FLOUR ON *IN VITRO* STARCH DIGESTIBILITY AND ANTIOXIDANT PROPERTIES OF HOM THONG BANANA TABLETS AND THEIR CHEMOMETRICS

Kitisart Kraboun[1], Mayura Thongchuang[2], Aumnart Chinpongpanich[3], Kamonwan Rojsuntornkitti[4], and Teeraporn Kongbangkerd[4]*

Address(es): Kitisart Kraboun,
[1]*Division of Food Safety Management and Technology, Department of Science, Faculty of Science and Technology, Rajamangala University of Technology Krungthep, Bangkok 10120, Thailand.*
[2]*Department of Chemistry, Faculty of Science, Ramkhamhaeng University, Bangkok 10240, Thailand.*
[3]*Faculty of Science and Technology, Rajamangala University of Technology Phra Nakhon, Bangkok 10800, Thailand.*
[4]*Department of Agro-Industry, Faculty of Agriculture Natural Resources and Environment, Naresuan University, Phisanulok 65000, Thailand.*

*Corresponding author: Kitisart.k@mail.rmutk.ac.th

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ABSTRACT

Hom Thong banana flour (HTBF) contains the large amounts of the phenolic compounds and resistant starch (RS). The application of HTBF in food products improves their antioxidant activities and glycemic index (GI). The objective of research was to estimate the different ratios of spray dried ripe Hom Thong banana powder (SDRHTBP) to HTBF, 95:5 and 90:10 on the color, hardness, resistant starch (RS), non-resistant starch (Non-RS), and total starch (TS) contents and *in vitro* starch hydrolysis index (HI), estimated glycemic index determination (EGI), total phenolic content (TPC), Trolox equivalent antioxidant capacity (TEAC), and chelating ability on Fe^{2+} of Hom Thong banana tablets (HTBT). When increased substitution ratios of HTBF, the L*, a*, and b* values and the hardness value, HI, and EGI of HTBT decreased; whereas, the TPC, chelating ability on Fe^{2+} assay, TEAC, and RS content increased. Obviously, the higher substitution ratios of HTBF were related to those parameters. Using PCA, the correlation among these parameters clearly revealed which TPC and RS contents were good indicators for Non-RS content, HI, EGI, chelating ability on Fe^{2+} assay, TEAC, L*, a*, b*, and hardness. Eventually, the HTBF substitution can be an alternative way to help reducing a sugar level used in the confectionery production and to get a novel confectionary with health benefits.

Keywords: TEAC, resistant starch, starch digestibility, Hom Thong banana flour, glycemic index

INTRODUCTION

The industry of tableted confections is developing and more interesting; therefore, there are many companies establishing to get into this business. For confectionery industries, many kinds of candy tablets developed, such as milk, peanut, and milk chocolate candy tablets, which normally contain a high sugar level. Sugar is a main ingredient and used more than 30 kg for the preparation of milk candy tablets **(Othman *et al.*, 2019)**. Although the high sugar-containing candy tablets are popular with great economic significance, an excessive consumption of sugar increases by 38% of the risk from the cardiovascular disease, high blood pressure, heart attack, and stroke, etc. **(Guimarães *et al.*, 2014)**. Moreover, milk as an ingredient in the dairy products contains lactose, which causes the risk phenomenon of lactose intolerance since primary or secondary lactase deficiency leads to the symptoms, i.e. abdominal pain, distension, borborygmi, flatus, and diarrhea, etc. **(Deng *et al.*, 2015)**. To avoid the lactose intolerance phenomenon, the substitution of milk with other ingredients to develop the healthy candy tablets is required. Many researches of the lactose-free candy tablets, such as pumpkin candy tablets **(Kamgoed and Kongbangkerd, 2007)**, corn milk tablets **(Srikusalanukul *et al.*, 2007**), and cashew nut milk tablets **(Panyayong and Ubolsook, 2017)** have been studied.

In addition, the important factors affecting the qualities and shelf life of tableted confections such as carbohydrate content, water content, water activity, and glass transition are considered **(Othman *et al.*, 2019)**. For example, the higher amount of icing sugar increased the hardness and Aw values of pumpkin candy tablets **(Kamgoed and Kongbangkerd, 2007)**. **Srikusalanukul *et al*. (2007)** established that the higher substitutions of sweet corn milk powder with pumpkin powder (containing > 12% carbohydrate) enhanced the hardness of corn milk tablets. **Panyayong and Ubolsook (2017)** explained that the moisture in products depended on the ingredient level (i.e. carbohydrate level) and affects the physical characteristics, e.g. vapor pressure (Aw).

In 2021, Hom Thong bananas were exported from Thailand and the export value of them was equivalent to 42,822,262 bath. However, a large number of bananas under the export standard were observed, estimated a low price of only 10 bath.kg^{-1} **(Sanyong*et al.*, 2020)**. They contain the compounds with antioxidant action presented in both pulp and peel parts, such as ascorbic acid, tocopherol, carotenoids, and flavonoids as well as some antioxidant enzymes associated with the phenolic compounds to enhance the antioxidant capacities **(Leong and Shui, 2002)**. **Rebello *et al*. (2014)** suggested that green banana flour contained the highest level of total phenolic substances, corresponding to high antioxidative activities. Therefore it is recommended to apply banana flour to be an ingredient of lots of bioactive compounds.

During unripe stage, Hom Thong banana flour (HTBF) comprises the large amount of resistant starch (RS) (between 40.9 and 58.5%) compared with jackfruit seed (27%) and job's tear flours (3%) **(Leong and Shui, 2002)** as well as also rich in minerals and vitamins available in pulp **(Someya *et al.*, 2002)**. The RS refers to an important component of dietary fiber (DF), which could not be hydrolyzed by the enzymes and was remained within the human colon for the fermentation by lactic acid bacteria (LAB) **(Amaral *et al.*, 2016)**. It used in many kinds of foods is an alternative way through to substitute high glycemic index foods due to increasing DF contents and tastes compared with the conventional insoluble fiber. In addition, the foods comprising RS had a decrease in calorie and glucose levels as compared with rice (a high glycemic index) **(Shukri *et al.*, 2017)**. For example, the application of RS in breads, mayonnaise, pates, pasta, cakes, muffins and biscuits was estimated **(Arp *et al.*, 2018)**. This showed that the RS improved the dough qualities, enhanced total dietary fiber (TDF) content and decreased estimated glycemic index (EGI) **(Ozturk *et al.*, 2009)**.

The HTBF indicates the abundant sources of RS and bioactive compounds for an ingredient in order to develop a new health product. Therefore, the application of HTBF could lead to the novel tableted confection with desirable estimated glycemic index (EGI) and *in vitro* antioxidant properties as well as the high level of recovered RS. This study aimed the incorporation of different levels of HTBF into spray dried ripe Hom Thong banana powder (SDRHTBP) on the RS content and *in vitro* starch digestion curve, and antioxidant and estimated glycemic properties of Hom Thong banana tablets (HTBT).

MATERIAL AND METHODS

Materials

Spray dried ripe Hom Thong banana (*Musa* sp., AAA group, cultivar Hom Thong) powder (SDRHTBP) was purchased from Bio Consumer Co., Ltd., Bangkok, Thailand and Hom Thong banana flour (HTBF) was obtained from Wai Whan community enterprise, Phetchaburi, Thailand.

Hom Thong banana tablets (HTBT)

Table 1 displays the ingredients of HTBT, which included the different ratios of SDRHTBP to HTBF (95:5 and 90:10), and other ingredients for each treatment, such as corn flour (8 g), maltodextrin (28 g), sugar (20 g), and magnesium stearate (2 g). In a mixing chamber, all the materials were thoroughly mixed before being filtered through a 600-mesh screen. The mixture was then compacted into a tablet using a single punch tablet machine (Ko Erwek, Germany) with the pressure force between 1500 and 1600 kg. Each tablet was fixed at 500 mg for the product consistency. To preserve the HTBT samples from moisture, oxygen, and light, they were kept in PET/Al/LLDPE (10x15 cm) bags. All treatments were performed in triplicate and the tablets were kept at 35ºC for further analysis.

Table 1 Ratios of SDRHTBP to HTBF (i.e. 95:5 and 90:10) and other ingredients for formulations of HTBT

Formulations	SDRHTBP (g)	HTBF (g)	Corn flour (g)	Maltodextrin (g)	Sugar (g)	Magnesium stearate (g)
SDRHTBP	42	-	8	28	20	2
SDRHTBP:HTBF (95:5)	39.9	2.1	8	28	20	2
SDRHTBP:HTBF (90:10)	37.8	4.2	8	28	20	2

SDRHTBP, Spray dried ripe Hom Thong banana powder; HTBF, Hom Thong banana flour

Color measurement

The L*, a* and b* values of HTBT were determined via a color meter (WS Series; FRU, China) behind calibrating with a referent plate (X = 98.66, Y = 85.19, and Z = 90.17), which indicated light, red, and yellow tones, respectively.

Hardness

The hardness of HTBT was analyzed by means and expressed as force (kp) by using the hardness tester (GS-719N, TECLOCK, Japan).

Resistant (RS), digestible (Non-RS), and total starch (TS) contents determination

The levels of RS, Non-RS, and TS starches were assessed using the Megazyme Resistant Starch Assay Kit (Megazyme Ltd., Wicklow, Ireland), with slight modifications, according to **Englyst *et al.* (1992)**.

In vitro starch hydrolysis index (HI) and estimated glycemic index determination (EGI)

The HI and EGI were explained by **Goñi *et al.* (1997)**. 80 mg of HTBT was transferred into a 15-mL PE centrifuge tube, also added to 12 mL hydrochloric acid-potassium chloride buffer (pH 1.2) and 0.5 mL of pepsin solution (0.5 g pepsin in 15 mL hydrochloric acid-potassium chloride buffer (pH 1.2)), ultimately incubated at 45°C for 1.30 h. The 15 mL aliquot was mixed with 5 mL of Tris-maleate buffer (pH 7.0) and 6 mL of α-amylase solution (50 mg α-amylase.mL^{-1} Tris-maleate buffer (pH 7.0)). Then, it was shaken at 170 rpm at 40°C, which 1 mL was obtained from the mixture in 30 min intervals for 3 h. The 1 mL aliquot was transferred into a flask and incubated at 120°C in order to inhibiting the enzymes, then left it cool down prior to the analysis. 400 µL of 0.4 M sodium acetate buffer (pH 5) and 40 µL of AMG (350 U.mL^{-1}) were transferred into the aliquot to obtain glucose, which was warmed at 65°C for 35 min. Then it was added to 3.5 mL GOPOD reagent and kept at 65°C for 30 min. The mixture was estimated at 510 nm versus the blank using GENESYS 20 spectrophotometer. The amount of starch occurred in each mixture was as follows:

$$\% \text{ Starch} = \Delta A \times F \times \frac{(100)}{(0.1)} \times \frac{(1)}{(1000)} \times \frac{(100)}{(W)} \times \frac{(162)}{(180)}$$

where, ΔA = different optical density means of the sample and the blank
F = 100 µg of glucose divided by the optical density of GOPOD
W = weight of sample

The rate of starch digestion was calculated as % starch hydrolyzed which was calculated following the equation below, obtained from % starch in 30 min intervals for 3 h divided by % total starch and then multiplied by 100.

$$\% \text{ Starch hydrolyzed} = \frac{(\% \text{ starch at certain time point})}{\text{total starch (\%)}} \times 100$$

Each point for % starch hydrolysis was plotted versus relative to each time point (min) beginning at 0 min. The area under curve (AUC) was measured by using the trapezoid method. The hydrolysis index (HI) was the AUC of sample divided by the AUC of glucose (as the reference (HI = 100)). HI was expressed in % as shown below:

$$\text{HI (\%)} = \frac{\text{AUC sample}}{\text{AUC of glucose}} \times 100$$

The estimated GI (EGI) of HTBT was measured according to the equation following **Goñi *et al.* (1997)**, HI was the hydrolysis index:

$$\text{EGI} = 39.71 + (0.549 \times \text{HI})$$

Extraction of total phenolic compound

Total phenolic compound was extracted following the method of **Goñi *et al.* (1997)**. 1 g of HTBT was transferred into 30 mL of methyl alcohol, incubated for 2 h at 30°C, and centrifuged at 15,000 *g* for 15 min. The supernatant was kept at -18°C prior to the analysis.

Determination of total phenolic content

The total phenolic content was explained by **Goñi *et al.* (1997)** with some modifications. 150 µL aliquot of the extract was mixed with 9 mL of deionized water, added to 1 mL of Folin-Ciocalteu reagent, and vortexed for 5 min. Then, 1.5 mL of 6% sodium carbonate was transferred to the aliquot and mixed by a vortex. The optical density was analyzed behind 1.5 h at 725 nm using a spectrophotometer (GENESYS 20 spectrophotometer). The phenolic content was presented as mg of gallic acid equivalent (GAE) per gram of sample (mg GAE.g^{-1}). The gallic acid concentrations were prepared as follows: 0, 0.05, 0.50, 0.75, and 1.0 mg.mL^{-1}.

Sample extract preparation for antioxidant activity determination

The sample extraction was explained by **Kraboun (2019)**. Briefly, a 15-g sample was extracted with 150 mL of methyl alcohol and then shaken with a shaker at 200 rpm for 12 h. The mixture was filtered using Whatman grade no. 42. The precipitate was again extracted using 50 mL of methyl alcohol, following the method explained above. The solvent in pooled extracts was removed out until not available using an evaporator at 50°C. The extract powder was estimated for the antioxiative properties.

Chelating ability on Fe^{2+} assay

The chelating ability on Fe^{2+} was estimated following the method of **Kraboun (2019)**, with a slight modification. 300 µL of 2 mM $FeSO_4.H_2O$ was put into 1-10 mg.mL^{-1} of 600 µL extract prior to mixing 500 µL of 4 mM ferrozine. Then the incubation at 25ºC for 15 min, 10 mL of ethyl alcohol was mixed with the reacted sample which was read at 562 nm versus the reagent blank using GENESYS 20 spectrophotometer. IC_{50} (mg extract.mL^{-1}) is the potential amount indicating to chelate 50% of Fe^{2+}.

Trolox equivalent antioxidant capacity (TEAC)

For ABTS assay, the TEAC of the extract eliminating $ABTS^{\cdot+}$ was analyzed following the method of **Kraboun (2019)**. The $ABTS^{\cdot+}$ was through from the reaction of both 2.5 mL of 7.5 mM ABTS solution and 50 µL of 3.26 mM $K_2S_2O_8$ solution, kept in the darkness at 25ºC for 20 h. Then, the $ABTS^{\cdot+}$ solution was mixed with ethyl alcohol to obtain an absorbance of 0.600 $\pm$ 0.005 at 734 nm. The free radical solution was put into 500 µL of each aliquot (1-10 mg.mL^{-1}), incubated at room temperature for 10 min. The optical density of the extract was against the calibration curve of Trolox (0.05-0.5 mM). The TEAC was expressed as $TEAC_{50}$ value referred to 50% inhibition of $ABTS^{\cdot+}$ ($TEAC_{50}$).

Statistical analysis

Data were processed by SPSS 17.0 (SPSS Inc., USA) and analysed by an analysis of variance which means were separated by Duncan's new multiple range test ($p \leq 0.05$). The results were expressed as mean ± SD. Multivariate analysis was composed of principal component analysis (PCA) to study correlation among HTBT formulations and the dependent variables, such as resistant starch (RS), non-resistant starch (Non-RS), and total starch (TS) contents and *in vitro* starch hydrolysis index (HI), estimated glycemic index (EGI), antioxidant activities, phenolic content, color, and hardness.

RESULTS AND DISCUSSION

Appearance and color values of HTBT

The appearance and L*, a*, b* values of all formulations of Hom Thong banana tablets (HTBT) are presented in Table 2. The spray dried ripe Hom Thong banana powder (SDRHTBP) increasingly substituted by Hom Thong banana flour (HTBF) decreased the L*, a* and b* values of HTBT ($p \leq 0.05$). It is possible that different color appearances between SDRHTBP and HTBF were observed since the color of HTBF was darker than that of SDRHTBP, affecting lower brightness of HTBT (Table 2). **Saifullah *et al.*** (**2009**) explained that the L* value in banana flour noodles was lower than the control (noodles without banana flour). To obtain banana flour, the unripe banana pulp is cut and sliced prior to taking banana flour production, causing physical tissue damages and then becoming browning from the enzymatic browning reaction **(Leong and Shui, 2002)**. Thus, the color of banana flour is relatively browning, which impacts on the color in the products when it used as an ingredient.

Hardness of HTBT

The hardness of Hom Thong banana tablets (HTBT), varied in different ratios of spray dried ripe Hom Thong banana powder (SDRHTBP) to Hom Thong banana flour (HTBF), 95:5 and 90:10 is presented in Figure 1. The hardness of HTBT decreased with increasingly substituted by HTBF ($p \leq 0.05$). The highest hardness value of 15.54 kp was obtained from the HTBT without HTBF substitution. The important factor leading to an increase in hardness of tablet is moisture **(Ohta *et al.*, 2003)**. This indicated that the hardness change in HTBT was observed with alterations in moisture content. Hence, change in the hardness of HTBT may be a cause of moisture sorption when alterations in RH and water activity (Aw) **(Amidon *et al.*, 1995)** during the process of tablet compaction. More moisture sorption of SDRHTBP is due to a large amount of sugar leading to a cause of more polarity because its particles are bound to stronger and a greater number of hydrogen bonds of water **(Tano *et al.*, 2008)**. The Aw of SDRHTBP was 0.54 (data now shown), indicating a low Aw related to its amorphous structure, thus moisture would diffuse into the structure at a low water activity to transformation to the stronger crystalline structure **(Saifullah *et al.*, 2009)**. However, the HTBF might contain a lot of non-polar molecules, i.e. starch granules, proteins, and lipids **(Tano *et al.*, 2008)** so that SDRHTBP increasingly replaced by HTBF protected the moisture adsorption of HTBT.

Table 2 L*, a*, and b* and appearance of HTBT containing various ratios of SDRHTBP to HTBF (i.e. 95:5 and 90:10)

Formulations	L*	a*	b*	Appearances
SDRHTBP	90.75±1.74[a]	5.49±0.05[a]	6.50±1.11[a]	
SDRHTBP:HTBF (95:5)	85.37±1.47[b]	3.85±0.02[a]	5.29±1.29[b]	
SDRHTBP:HTBF (90:10)	75.14±2.75[c]	2.97±0.01[c]	4.62±0.08[b]	

Various letters within a column are significant ($p \leq 0.05$).

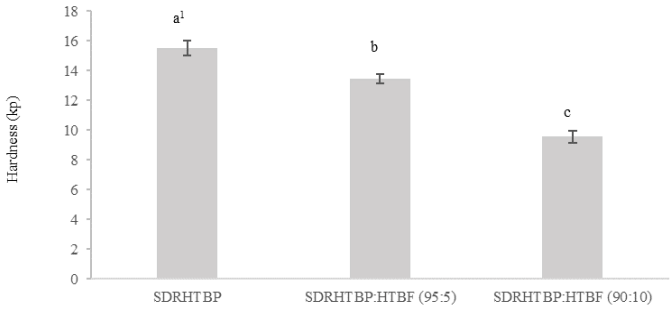


Figure 1 Hardness of HTBT containing various ratios of SDRHTBP to HTBF (i.e. 95:5and 90:10). [1]Various letters are significant ($p \leq 0.05$).

Resistant, digestible, and total starch contents and *in vitro* starch hydrolysis index and estimated glycemic index of HTBT

The data for resistant starch (RS), non-resistant starch (Non-RS), and total starch (TS) contents and *in vitro* starch hydrolysis index (HI) and estimated glycemic index (EGI) of Hom Thong banana tablets (HTBT), varied in different ratios of spray dried ripe Hom Thong banana powder (SDRHTBP) to Hom Thong banana flour (HTBF), 95:5 and 90:10 are shown in Table 3. The TS contents of HTBT of all formulations were slightly different ($p \geq 0.05$). The RS content of HTBT with 10% HTBF substitution (12.98%) was higher than that with 5% HTBF substitution (6.28%). The lowest RS level (0.22%) was available in the tablets without HTBF substitution. This indicated that the lowest level of RS led to the higher starch metabolism, showing that no addition of HTBF was relative to low RS that ultimately increases HI and EGI **(Tiboonbun *et al.*, 2011)**. **Detchewa *et al.* (2021)** suggested that the amount of unripe banana flour added in gluten-free cookies was related to RS content. The substitution of rice flour with 50-100% unripe banana flour in the formulations of gluten-free cookies increased the RS levels, ranged from 2.70 to 8.50%, respectively. **Moongngarm *et al.* (2014)** further suggested that the HTBF was rich in RS content (48.88%), and can be used as an important source for enhancing the amount of RS in food products. **Ratnasari (2018)** also noted that the substitution of wheat flour with 75% unripe banana flour in cookies increased by 14.60% of RS content. Furthermore, **Tiboonbun *et al.* (2011)** stated that the contents of banana flour between 0 and 75% replaced in rice noodles enhanced the RS contents, between 7.49 and 13.15%, respectively.

The non-RS levels of HTBT of all formulations were higher than 68%. The increased levels of HTBF replacement slightly decreased Non-RS content in HTBT ($p \leq 0.05$). The lowest Non-RS content was obtained from the HTBT with 10% HTBF substitution by 68.35%. The highest Non-RS content occurred in the

tablets without HTBF substitution was observed relative to the lowest RS content (Table 3).

The HI of HTBT of all formulations (ranged from 50.25 to 98.21%) was related to EGI (ranged from 67.29 to 93.62). The HI and EGI of HTBT trended to decrease when the amount of HTBF increased. The highest HI and EGI were noted for the tablets without HTBF substitution; whereas, the lowest was recorded for HTBT with 10% HTBF substitution. Obviously, the HI values of HTBT containing SDRHTBP replaced by 5-10% HTBF substitution were lower than 71% and corresponding EGI values were lower than 79. Thus, the HTBT containing SDRHTBP replaced by with 5-10% HTBF substitution was a medium-GI food **(Haini *et al.*, 2021)**. **Saifullah *et al.* (2009)** explained that the HI and GI of banana flour noodles produced from 10% substitution of wheat flour with banana flour were 23.3% and 53.0, respectively. The unripe banana flour could be attributed to the presence in the high contents of RS and DF **(Juarez-Garcia *et al.*, 2006)**, which are the indigestible polymer lowering the carbohydrate hydrolysis in the human digestive system simulation, leading to a decrease in metabolism **(Granfeldt *et al.*, 1992)**. Moreover, the bread supplemented with banana flour revealed to a low HI involved with lots of RS and DF contents **(Juarez-Garcia *et al.*, 2006)**.

Table 3 Resistant starch (RS), non-resistant starch (Non-RS), and total starch (TS) contents and *in vitro* starch hydrolysis index (HI) and estimated glycemic index (EGI) of HTBT containing various ratios of SDRHTBP to HTBF (i.e. 95:5 and 90:10).

Formulations	RS (%)	Non-RS (%)	TS (%)^{ns}	HI (%)	EGI
SDRHTBP	0.22±0.01^{c}	79.28±2.42^{a}	79.5±3.25	98.21±1.12^{a}	93.62±2.52^{a}
SDRHTBP:HTBF (95:5)	6.28±0.02^{b}	72.57±1.25^{b}	78.85±5.45	70.28±2.35^{b}	78.29±3.40^{b}
SDRHTBP:HTBF (90:10)	12.98±0.14^{a}	68.35±3.25^{c}	81.33±2.95	50.25±1.42^{c}	67.29±4.25^{c}

Various letters within a column are significant ($p \leq 0.05$). ^{ns}not significant ($p>0.05$).

***In vitro* starch digestion curves of HTBT**

The starch digestion curves within the enzymatic digestion 180 min of Hom Thong banana tablets (HTBT), varied in various ratios of spray dried ripe Hom Thong banana powder (SDRHTBP) to Hom Thong banana flour **(**HTBF**)**, 95:5 and 90:10 every 30 min are represented in Figure 2. At all time points, the starch digestion of HTBT of all formulations was lower than 10% as compared with the reference food, i.e. glucose. In the first 30 min, the starch digestion curves of HTBT with 5% HTBF and without HTBF substitutions were very close, with approximately 70% starch hydrolysis; whereas, beyond 60 min, the starch digestion curves of HTBT with 5% HTBF and without HTBF substitutions were slightly different for starch hydrolysis patterns. However, the curve of starch hydrolysis in HTBT with 10% HTBF substitution had the lowest starch hydrolysis at all time points. The levels of starch hydrolysis after 3 h of HTBT were highest for SDRHTBP, then 5% HTBF substitution, and lastly, 10% HTBF substitution. This is a cause of a higher source of RS content (48.88%) occurred in HTBF **(Moongngarm *et al.*, 2014)**, hence affecting stability on the enzymatic hydrolysis available in HTBT. Therefore, the enzymatic hydrolysis of HTBT with higher HTBF substitutions was lower since just a partial amount, around a quarter, of unripe Hom Thong banana flour could be digested by *in vitro* simulation of digestive enzymes **(Jaiturong *et al.*, 2020)**. Consequently, the starch of Hom Thong banana may reach the colon and have the possibility to improve LAB activity in the human colon due to its strong resistance to amylolytic and proteolytic enzymes **(Juarez-Garcia *et al.*, 2006)**. Besides, the carbohydrate digestibility also depends on the morphology, granule size, porosity, structure, and concentration **(Juarez-Garcia *et al.*, 2006)**. Normally, the B-type starch content has been presented in the HTBF by approximately 18%, thus its structure may be resistant to the enzyme hydrolysis **(Granfeldt *et al.*, 1992)**, which were higher than that found in other sources, approximately 17% for potato **(Zhang *et al.*, 2017)**, 2% for turmeric (*Curcuma longa*), 14% for canna (*Canna edulis*), 16% for canna (*Canna indica*), and 25% for tiger lily (*Lilium lancifolium*) **(Huang *et al.*, 2015)**.

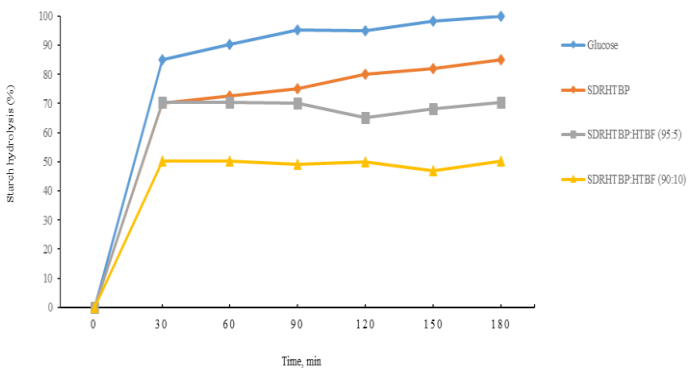


Figure 2 *In vitro* starch digestion curves of HTBT containing different ratios of SDRHTBP to HTBF (i.e. 95:5 and 90:10).

Total phenolic content, chelating ability on Fe^{2+} assay and TEAC of HTBT

The total phenolic content (TPC), Trolox equivalent antioxidant capacity (TEAC), and chelating ability on Fe^{2+} of Hom Thong banana tablets (HTBT) containing the various ratios of spray dried ripe Hom Thong banana powder (SDRHTBP) to Hom Thong banana flour (HTBF) (i.e. 95:5 and 90:10) are presented in Table 4. The HTBT samples with increased HTBF substitutions trended to an increase in TPC. The TPC in HTBT with 5-10% HTBF substitution ranged from 60.52 to 135.24 mg GAE.g^{-1} with significantly different ($p\leq$ 0.05). Whereas, the TPC of HTBT without HTBF substitution was not found. **Fatemeh *et al.* (2012)** noted that the unripe Hom Thong banana pulp had the highest content of TPC, between 69.52 and 577.52 mg GAE.100.g^{-1} dry matter. Generally, the TPC in unripe banana pulp for other cultivars, such as 50-52 mg GAE.100g^{-1} for Kluay Khai banana **(Alothman *et al.*, 2009)**, 91.3 mg GAE.100g^{-1} for Namwa banana has been reported **(Choo and Azis, 2010)**. Besides, tannins and flavonoids were water-soluble phenol derivatives found abundantly in the unripe banana, which were the distinguished derivatives of phenolic compounds **(Choo and Azis, 2010)**. **Koodkaew *et al.* (2021)** reported that the contents of tannins and flavonoids of Hom Thong banana (3.49 mg.g^{-1} and 0.35 mg QE.g^{-1}, respectively) were higher than those of other varieties, 0.001 to 0.42 mg.g^{-1} and 0.001 to 0.01 mg QE.g^{-1}, respectively **(Bashmil *et al.*, 2021)**.

The TEAC assay determines the total antioxidative capacity through a SET or HAT mechanism **(Tohma and Gulçin, 2010)**. For chelating ability on ferrous ion, in available chelating substances, the reaction between ferrozine and ferrous ion is interrupted, affecting a decreased absorbance of the estimated sample **(Dinis *et al.*, 1994)**. The $TEAC_{50}$ and IC_{50} of chelating ability on Fe^{2+} of HTBT trended to decrease because of the increased HTBF substitutions. The HTBT with 10 % HTBF substitution had the lowest values of $TEAC_{50}$ and IC_{50} of chelating ability on Fe^{2+}(0.25 mM Trolox.mL^{-1} and 0.42mg extract.mL^{-1}, respectively), followed by 5 % HTBF substitution (2.35 mM Trolox.mL^{-1} and 0.85mg extract.mL^{-1}, respectively), and lastly, without HTBF substitution (25.25 mM Trolox.mL^{-1} and 12.52 mg extract.mL^{-1}, respectively). In this experiment, the patterns of TEAC and chelating ability on Fe^{2+} values were related to TPC (Table 4). Moreover, **Bashmil *et al.* (2021)** reported that the ABTS and Fe^{2+} chelation activity (FICA) values of unripe Cavendish banana (belonged to AAA group) (2.20 mg AAE.g^{-1} and 0.05 mg EDTA.g^{-1}, respectively) were higher than those of other unripe bananas (1.91-1.98 mg AAE.g^{-1} and 0.03-0.06 mg EDTA.g^{-1}, respectively). **Hagerman *et al.* (1998)** confirmed that the tannins and flavonoids (high molecular weight of phenolic compounds) were main components found in HTBF. The flavonoids can bind to iron against iron's accessibility to oxygen molecules as well as reducing Fe^{3+} ions become inert Fe^{2+} polyphenol complexes **(Hagerman *et al.*, 1998)**. Therefore, the tannins and flavonoids would be found in HTBF being the endogenous chelating compounds **(Hagerman *et al.*, 1998)**. **Alothman *et al.* (2009)** also noted that the antioxidative properties in fruits could depend on location, strain, and physiological maturity. Moreover, it is noted that the antioxidative activity involves with the molecular weight, the number of aromatic ring, and the number and position of OH group bound to the aromatic rings within phenolic structures **(Alothman *et al.*, 2009)**.

Table 4 Total phenolic content (TPC), Trolox equivalent antioxidant capacity (TEAC), and chelating ability on Fe^{2+} of HTBT containing various ratios of SDRHTBP to HTBF (i.e. 95:5 and 90:10).

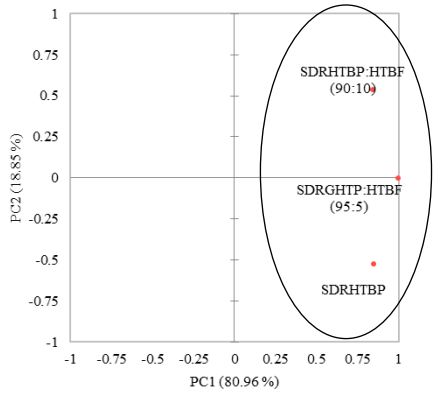
Formulations	TPC (mg GAE.g^{-1})	$TEAC_{50}$ (mM Trolox.mL^{-1})	IC_{50} chelating ability on Fe^{2+} (mg extract.mL^{-1})
SDRHTBP	ND	25.25±3.25^{a}	12.52±0.12^{a}
SDRHTBP:HTBF (95:5)	60.52±0.25^{b}	2.35±0.14^{b}	0.85±0.04^{b}
SDRHTBP:HTBF (90:10)	135.24±3.24^{a}	0.25±0.02^{c}	0.42±0.01^{c}

ND: not detected. Various letters within a column are significant ($p \leq 0.05$).

Principal component analysis (**PCA**)

PCA of various HTBF substitution formulations of Hom Thong banana tablets (HTBT) is represented in Figure 3A. Two PCs could be accounted for 99.81% of total variance. It confirmed that all formulations such as SDRHTBP, SDRHTBP:HTBF (95:5), and SDRHTBP:HTBF (90:10) were similarly grouped. The PCA of dependent variables based on RS, Non-RS and TS contents and HI, EGI, chelating ability on Fe^{2+} assay, TEAC, phenolic content, color and hardness is represented in Figure 3B. The eigenvalues of two principal components could explain 100 % of total variance. The PC1 accounted for 91.38% of total variance, which contained RS and Non-RS contents and HI, EGI, chelating ability on Fe^{2+} assay, TEAC, phenolic content, L*, a*, b*, and hardness. The result demonstrated that Non-RS content and HI, EGI, chelating ability on Fe^{2+} assay and TEAC, L*, a*, b* and hardness were positively correlated; whereas, they were negatively correlated with TPC and RS content. Whereas, the TS was observed on the PC2, which could be explained 8.62% of total variance. It showed that the RS showed a high correlation with chelating ability on Fe^{2+} assay and TEAC, with the high correlation coefficient between -0.867 and -0.888 (data not shown). Therefore, the SDRHTBP substituted by higher percentages of HTBF used for produce HTBT could directly confirm presence in higher RS contents corresponding stronger antioxidant activities.

A



B

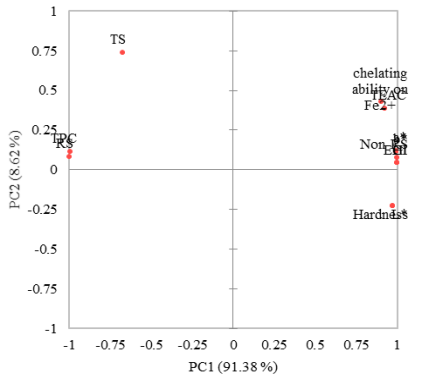


Figure 3 Principal component analysis (PCA) describing correlation among different HTBF substitution formulations of HTBT (A) and dependent variables (B) on PC1 and PC2 based on resistant starch (RS), non-resistant starch (Non-RS), and total starch (TS) contents and *in vitro* starch hydrolysis index (HI), estimated glycemic index (EGI), antioxidant activities, phenolic content, color, and hardness.

CONCLUSION

The research informed the effectiveness of HTBT obtained from the replacement of SDRHTBP by HTBF at various ratios of SDRHTBP to HTBF, 95:5 and 90:10. It has demonstrated that the SDRHTBP increasingly substituted by HTBF decreased the L*, a* and b* values and the hardness value of HTBT as well as the lower enzymatic hydrolysis after 3 h hydrolysis by the enzymes. Varietal differences in terms of RS level, HI, EGI, and starch hydrolysis curve have been clearly presented by HTBF and correlated significantly with TPC, TEAC and chelating ability on Fe^{2+}. The PCA result clearly confirmed that all formulations were in the same group. Therefore, the *in vitro* digestibility and antioxidant properties and RS level described here may also be a database for the banana farmers who are requiring and applying for other value-added products with a high nutritional value.

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