Utilization of Watermelon Rind Waste as a Potential Source of Dietary Fiber to Improve Health Promoting Properties and Reduce Glycemic Index for Cookie Making

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Abstract Watermelon rind powder (WRP) is a rich source of dietary fiber and bioactive compounds, hence it could be used in the development of functional foods such as cookies. Different replacement levels of wheat flour with either WRP or hi-maize starch (HMS) (10-30%) on the quality of the cookies made were studied. The dietary fiber content in the cookies increased with incorporation of increasing level of either WRP or HMS. Increasing the proportion of the WRP in the cookie making resulted in an increase in the total phenolic content, 2,2-diphenyl-1-picrylhydrazyl radical scavenging activity, and the ferric reducing antioxidant power. Using up to 20% of WRP and 30% of HMS in the cookie making promotes: increase in dietary fiber, decrease in predicted glycemic index to medium (for WRP) and low (for HMS) levels, and improving the antioxidant activity. These changes still produce an acceptable cookie.

Keywords: cookie, watermelon rind powder, glycemic index, hi-maize starch, non-enzymatic browning, antioxidant activity

Introduction

Cookie is a widely consumed popular snack item that has a long shelf life. The major ingredients usually used to make traditional cookie are wheat flour, sugar, and fat; therefore, it can be claimed that cookie is rich in carbohydrates. In addition, it lacks other essential nutritional components, such as dietary fiber, vitamins, and minerals, which are removed during the refinement of the wheat flour. There is a nutritional trend to reduce the consumption of glycemic carbohydrates in food products by increasing the level of indigestible carbohydrates. Starch is one of the most important glycemic carbohydrates in cereals, roots, and tubers that are consumed in diets worldwide. The rate of digestion and absorption of glucose in the small intestine determines the glycemic index of starchy foods. Normally, wheat flour is rapidly digested and absorbed in the duodenum and proximal regions of the small intestine. This leads to a rapid elevation of blood glucose and is usually followed by a subsequent episode of hypoglycemia. One technique that could be applied to reduce the glycemic index in bakery products is the partial substitution of wheat flour with indigestible carbohydrates (1-3).

The various fibers from plant sources have been used in the cookie making to improve the texture, color, aroma, and nutritional characteristics and reduce the energy of the final product. For examples, there are some plants, claimed to be rich-sources of fiber,

that can be utilized in cookie or biscuit making such as apple pomace (4), pomegranate peel (5), guduchi leaf (6), green tea powder (7), brown rice (8), sugarcane bagasse (9), and psyllium fiber (10). However, the quality of the finished products varied in accord with the type and content of fiber used. The watermelon (*Citrullus lanatus*) is a fruit commonly consumed in many countries. It is widely grown due to its large edible fruits that contain a hard green rind and a watery reddish or yellowish pulp. It is an important agricultural product used in the production of various food products such as fruit cocktails, juices, and nectars. The most useful part of the watermelon used in the food industry is its pulp. The rind is usually discarded, but it is edible, and sometimes used as a vegetable. Al-Sayed and Ahmed (11) studied the use of watermelon rinds as a natural fiber in the manufacture of cake. Overall, they recommended that the substitution of wheat flour with 5% of watermelon rind powder (WRP) produced acceptable cake. In addition, WRP is a good source of dietary fiber and phenolic compounds. Currently, no report related to the application of WRP in the cookie making has been found. Hence, the objective of this work was to study the physical, chemical, antioxidant, and sensory properties as well as the starch digestibility and glycemic index of cookies made. This was in relation to the influence of partial substitution of wheat flour with WRP in comparison with hi-maize starch (HMS), a commercial resistant starch.

Materials and Methods

Chemicals and Reagents All the chemicals used were of analytical grade. Gallic acid, 2,2-diphenyl-1-picrylhydrazyl (DPPH), 5-hydroxymethylfurfural (HMF), 2,4,6-tripyridyl-s-triazine (TPTZ), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (trolox), and the total dietary fiber assay kit were purchased from Sigma-Aldrich (St. Louis, MO, USA). All enzymes used, such as α -amylase (from porcine pancreas, type VI-B, A3176-500KU), amyloglucosidase (from *Aspergillus niger*, 200 unit/mL), and pepsin (from porcine gastric mucosa), were also obtained from Sigma-Aldrich.

Preparation of watermelon rind powder (WRP) Watermelon rind waste was obtained from a fresh-cut fruit producer in Bangkok province, Thailand. After collection, the watermelon rinds were chopped into small pieces and blended with blenders to obtain the watermelon rind paste. The watermelon rind paste was dried in a hot air oven (60°C) until its moisture content was less than 10%. After the drying process, the dried watermelon rind was ground with a laboratory mill, and sieved to a fine powder (100 mesh) to obtain watermelon rind powder (WRP). Hi-maize starch (HMS), a commercial resistant starch, was supplied by National Starch and Chemical (Thailand) Limited.

Physicochemical properties of wheat flour blended with either WRP or HMS Blends containing 0, 10, 20 and 30% of either WRP or HMS replacing wheat flour were performed by gradual mixing in a rotary mixer. Each blend was evaluated for the water absorption capacity (WAC), oil holding capacity (OHC) and pasting properties. Both WAC and OHC were measured as described by Sharma *et al.* (6) and Al-Sayed and Ahmed (11), respectively. Both the WAC and OHC were expressed as gram of water or oil bound per g of the sample on a dry basis (db). The pasting properties were determined using a Rapid Visco-Analyzer (Perten RVA 4500; Newport Scientific Narrabeen, New South Wales, Australia). The temperature profile used was the standard protocol of RVU Cal. The Thermocline windows software was used to process the data (Perten RVA 4500; Newport Scientific Narrabeen).

Cookie preparation The basic formula used for the cookie making was as follow: 190 g wheat flour, 170 g butter, 25 g whole egg, 15 g water, 100 g sugar, 1.8 g baking powder, 1.2 g salt, 1.7 g vanilla, and 13 g milk. Three levels of wheat flour substitution (10, 20, and 30%, g/100 g) were carried out using either WRP or HMS. A control sample was prepared by using only wheat flour (without substitution). All the ingredients were mixed and kneaded for 10 min using a laboratory type mixer. The resulting dough was shaped into a circular form. The cookies were baked in an air oven at 180°C for 20 min. The cookies were cooled down to room temperature prior to analysis.

Determination of proximate compositions The proximate

compositions of the WRP and each cookie sample, including moisture, protein, fat, and ash, were determined by the AOAC methods (12). The total dietary fiber (TF) and insoluble dietary fiber (IF) were also evaluated by using a total dietary fiber assay kit obtained from Sigma-Aldrich. The soluble dietary fiber (SF) content was calculated by subtracting the amount of the IF from the TF.

Determination of 5-hydroxymethylfurfural content The 5-hydroxymethylfurfural (HMF) content was analyzed using high performance liquid chromatography (HPLC) (13). The ground sample (1 g, db) was homogenized in water, and the HMF was extracted by the addition of 2.5 mL 40% (w/v) trichloroacetic acid. The mixture was thoroughly mixed by agitation for 5 min, the suspension was adjusted to 25 mL with distilled water, and then the resulting mixture was centrifuged at 2,800 $\times$ g for 10 min. Two milliliters of supernatant were filtered through a 0.45 mm nylon filter and injected into the HPLC system (HPLC, Agilent 1100 series; Agilent, Santa Clara, CA, USA) using a C18 LUNA column (4.6 $\times$ 250 mm, 5 mm, Phenomenex, Torrance, CA, USA) and methanol/sodium acetate (0.04 M) (20/80) as the mobile phase. The HMF was detected at 280 nm.

Determination of total phenolic compounds and antioxidant activities Both the WRP and cookie samples (1 g, db) were ground and extracted with 80% ethanol (20 mL) in a flask placed in a shaking water bath for 3 h and then centrifuged at 2,800 $\times$ g for 10 min (14). The residue was further processed for extraction twice using 20 mL of 80% ethanol. The combined supernatant was used to determine the total phenolic content (TPC) and antioxidant activities. The TPC was analyzed by reaction using a Folin-Ciocalteu reagent. The gallic acid was used as the standard and the TPC was expressed as mg gallic acid equivalent (GAE) per g sample (15). The DPPH radical scavenging activity (DPPH-RSA) was assayed using the method described by Ahmad *et al.* (7) with some modifications. The ferric reducing antioxidant powder (FRAP) was measured following the protocol developed by Benzie and Strain (16). The calibration curves of trolox were done for DPPH-RSA and FRAP.

Determination of physical properties The spread ratio of the cookies was calculated by dividing values of the diameter by the thickness values. The surface colors, L* (lightness), a* (redness), and b* (yellowness) were measured using a Hunter Color Measuring System (Colorflex; Hunter Lab. Inc., Reston, VA, USA). The hardness of the cookies was measured using a Texture Analyzer (TA-XT2i; Stable Micro Systems Ltd., Surrey, UK). Hardness (N) is defined as the maximum force on a product that displays substantial resistance to deformation. The hardness was evaluated using a cylinder probe (diameter 3 mm). Water activity was measured at room temperature using a water activity meter (LabMaster-aw; Novasina, Pfaffikon, Switzerland). The sample was inserted into a sample cup, and another water activity measurement was made immediately to restrict moisture transfer from the air to the samples.

Determination of non-enzymatic browning index The assessment of the non-enzymatic browning index of the cookie samples was carried out by the method shown in the work of Sharma and Gujral (17). Each cookie sample (500 mg, db) was mixed with 5 mL of deionized water. An aliquot (1 mL) of the mixture was added to a test tube which contained 1 mL of protease solution (the enzyme was dissolved in Tris buffer pH 7 and 50 mM of calcium chloride). The test tubes were incubated for 2 h at 45°C in a water bath. The test tubes were cooled in an ice water bath, and 1.5 mL of trichloroacetic acid was added to each tube. Then tubes were centrifuged at 2,800 $\times$ g for 10 min. The absorbance of the supernatant was measured at two wavelengths (420 and 550 nm) on a spectrophotometer. The non-enzymatic browning index (ΔA) was calculated as follows:

$$\Delta A = \text{Absorbance at 420 nm} - \text{Absorbance at 550 nm}$$

In vitro starch digestibility and predicted glycemic index (pGI)

Starch digestibility was determined using the method described by Englyst *et al.* (18) with some modifications. The ground samples (0.5 g, db) were added to 10 mL HCl-KCl buffer, pH 1.5 and agitated for 10 min. Pepsin (1 mg in 10 mL HCl-KCl buffer, pH 1.5) was added, and the sample was then incubated in a shaking water bath (40°C) for 40 min. After that, the volume of sample was diluted to 25 mL with 0.1 M phosphate buffer, pH 6.9 and then checked using a pH meter. Pancreatic amylase (5 mL, 2.6 U/5mL) was added, and the sample was then incubated in a shaking water bath (37°C). Sample aliquots (1 mL) were removed at 0, 15, 30, 60, 90, 120, 150, and 180 min, placed in boiling water for 5 min, and then cooled in ice. The aliquots were then centrifuged at 7,800 $\times$ g for 10 min, and the supernatant (1 mL) was mixed with 1 mL 0.1 M sodium acetate buffer, pH 4.5, and then checked using a pH meter. The amylo-glucosidase (30 mL) was added, and the sample was then incubated in a shaking water bath (60°C) for 45 min. Glucose release (mg/g dry sample) in each system was quantified by using a Glucose liquicolor (Human, Wiesbaden, Germany).

The hydrolysis index (HI) was calculated on the basis of the starch hydrolysis curve (0-180 min) as the percentage of total glucose released over 180 min in comparison to that released from white bread over the same period. The glycemic index (GI) was then estimated by using the following equation of Goni *et al.* (19): $GI = 39.71 + 0.549HI$.

Sensory evaluation A hedonic test was used to determine the degree of overall-liking for the cookies. Fifty untrained panelists were asked to score the samples in terms of color, appearance, texture, flavor, and overall acceptability using a nine-points hedonic scale ranging from 1- dislike extremely, 5- acceptable, and 9- like extremely.

Statistical analysis The data were subjected to statistical analysis using one-way analysis of variance (ANOVA). The means were compared using the Duncan multiple range test with a significance level of 5%.

Results and Discussion

Chemical compositions of WRP It is important to know the chemical compositions of fiber when developing formulas for new bakery products. The results showed that the WRP had lower contents of moisture (5.38 g/100 g), fat (0.25 g/100 g), and protein (7.52 g/100 g) than wheat flour, whereas the ash (8.15 g/100 g), total dietary fiber (68.43 g/100g), and total phenolic (6.21 mg GAE/g) contents were higher. The protein, ash, fat, and moisture contents of wheat flour were 11.32, 1.52, 1.31, and 9.45 g/100 g, respectively. Wheat flour generally contains low fiber content because the bran fractions were removed during the milling process, and thus the wheat flour is a poor source of fiber. A high total dietary fiber content in the WRP is an indication of its potential application for enrichment in food products to produce high fiber, value-added, healthy foods. The major type of fiber found in WRP is insoluble fiber (50.32 g/100 g), which has been associated with many health benefits including a reduced risk of cardiovascular diseases and preventing weight gain, diabetes and some types of cancers (10). Comparing the results with the previous work, a higher dietary fiber content was found in the WRP than those of cereals such as wheat bran (47 g/100 g), rice bran (40 g/100 g), oat bran (20 g/100 g), and barley bran (45 g/100 g) (20). Results similar to those obtained in this study were found in the wastes from the processing of fruits such as orange (67 g/100 g) and lemon (73 g/100 g) (21). In addition, a higher total phenolic content (TPC) was detected in the WRP than in wheat flour (0.98 mg GAE/g). Al-Sayed and Ahmed (11) reported that the major phenolic compounds detected in watermelon rinds were 4-hydroxybenzoic acid and vanillin. Therefore, WRP is a good source of insoluble dietary fiber and TPC. The enrichment of cookies with WRP could enhance the health appeal of the product.

Physicochemical properties of wheat flour blended with either WRP or HMS

Both the WAC and OHC of the blends were investigated as shown in Table 1. It was found that higher WAC was observed in all blends containing either WRP or HMS when compared with only wheat flour ($p < 0.05$). Increasing the level of either WRP or HMS in the blends progressively increased the WAC ($p < 0.05$). The increase in the WAC with the addition of fiber is due to the greater number of hydroxyl groups existing in the fiber structures which allows more water interactions through hydrogen bonding (6). Similar observations were found in the case of wheat flour blended with fiber rich powders such as mango peel (14), wheat bran, rice bran, barely bran (20), and bamboo shoots (15). OHC is the ability of materials to physically bind fat by capillary attraction. In addition, food materials with a high OHC may act as a flavor retainer, and also increases the mouth feel of foods (22). According to the results, a higher OHC was also found in all blends containing either WRP or HMS compared to that of the blend containing only wheat flour ($p < 0.05$). In addition, an increase in the OHC was observed with an increase in the WRP and HMS contents in the blends ($p < 0.05$). The surface of a fiber normally

Table 1. Properties of the blended flour and cookies prepared by substitution of wheat flour with various levels of either watermelon rind powder (WRP) or hi-maize starch (HMS) in the formulation

Properties/Treatments	Control	HMS10%	HMS20%	HMS30%	WRP10%	WRP20%	WRP30%
Physical properties of blended flour							
WAC (g/g)	3.29±0.24 ¹⁾	3.45±0.31 ^{ef}	3.58±0.26 ^e	4.02±0.42 ^d	4.91±0.37 ^c	5.84±0.51 ^b	6.94±0.54 ^a
OHC (g/g)	3.73±0.51 ^d	3.87±0.25 ^d	4.12±0.41 ^{cd}	4.57±0.36 ^c	4.49±0.40 ^c	5.21±0.34 ^b	6.34±0.44 ^a
Pasting properties of blended flour							
PT (°C)	87.95±0.07 ^c	87.95±0.08 ^c	88.80±0.15 ^b	90.10±0.57 ^a	87.98±0.04 ^c	88.05±0.37 ^c	88.78±1.10 ^b
PV (RVU)	136.21±0.88 ^a	93.88±0.41 ^c	61.54±1.00 ^e	37.75±2.12 ^f	111.08±2.24 ^b	94.25±0.47 ^c	75.21±0.06 ^d
BK (RVU)	43.75±0.66 ^a	28.33±0.62 ^c	16.25±0.73 ^e	8.83±0.58 ^f	35.75±0.61 ^b	29.58±0.56 ^c	21.83±0.46 ^d
SB (RVU)	46.21±1.36 ^a	42.46±0.77 ^b	35.42±1.30 ^d	29.04±0.88 ^e	39.58±1.06 ^c	28.63±0.88 ^e	15.38±1.59 ^f
FV (RVU)	182.42±2.24 ^a	136.33±0.35 ^c	96.96±0.29 ^e	66.79±3.01 ^g	150.67±1.18 ^b	122.88±0.41 ^d	90.58±1.53 ^f
Physical properties of cookies							
L*	63.41±1.43 ^c	69.88±0.47 ^c	70.39±2.27 ^b	76.42±1.47 ^a	58.98±3.47 ^d	49.07±2.13 ^e	47.98±1.17 ^e
a*	12.94±1.76 ^c	8.06±0.56 ^d	8.41±1.20 ^d	8.15±1.10 ^d	14.80±0.36 ^b	15.41±0.60 ^b	16.49±0.44 ^a
b*	35.36±1.66 ^a	33.09±0.79 ^b	33.24±1.20 ^b	32.99±1.41 ^b	33.71±1.07 ^b	31.68±2.09 ^c	29.01±1.30 ^d
Spread ratio	4.26±0.15 ^b	4.52±0.14 ^b	4.75±0.08 ^a	4.93±0.20 ^a	4.05±0.17 ^b	3.78±0.15 ^c	3.40±1.21 ^d
Water activity	0.42±0.01 ^d	0.46±0.02 ^c	0.47±0.01 ^c	0.50±0.01 ^b	0.45±0.01 ^c	0.49±0.01 ^b	0.54±0.01 ^a
Hardness (N)	686.56±23.45 ^a	487.33±32.54 ^d	442.45±27.33 ^e	345.36±17.45 ^f	566.24±12.65 ^b	537.16±11.47 ^c	517.48±18.98 ^c
Chemical properties of cookies							
Protein (g/100g)	6.02±0.26 ^a	5.20±0.21 ^b	4.81±0.36 ^{bc}	3.62±0.25 ^c	5.54±0.18 ^b	5.02±0.29 ^b	4.84±0.22 ^{bc}
Lipid (g/100g)	35.44±1.53 ^a	35.24±0.31 ^a	34.65±0.13 ^a	33.85±0.11 ^b	34.48±0.11 ^a	34.73±0.14 ^a	33.20±0.24 ^b
Ash (g/100g)	0.93±0.21 ^{bc}	0.76±0.18 ^c	0.63±0.12 ^d	0.58±0.11 ^d	0.58±0.13 ^d	1.14±0.18 ^b	1.63±0.06 ^a
Moisture (g/100g)	2.55±0.61 ^c	2.63±0.17 ^c	3.02±0.15 ^b	3.23±0.39 ^a	2.91±0.25 ^{bc}	3.54±0.04 ^a	3.93±0.96 ^a
TPC (mg GAE/g)	2.22±0.36 ^d	2.14±0.41 ^d	2.05±0.25 ^d	1.43±0.32 ^e	3.87±0.34 ^c	4.89±0.27 ^b	6.72±0.31 ^a
DPPH-RSA (µg TE/g)	57.40±0.48 ^d	50.15±0.52 ^e	48.32±0.67 ^f	41.74±0.44 ^g	67.87±0.61 ^c	74.13±0.55 ^b	93.65±0.72 ^a
FRAP (µg TE/g)	102.23±2.61 ^d	96.46±2.19 ^e	91.13±3.47 ^f	83.66±3.24 ^g	116.76±2.46 ^c	159.78±3.14 ^b	204.73±2.33 ^a

¹⁾Means in the same row followed by different letters differ significantly ($p<0.05$).

has a high capacity to hold oil by mechanical process, thus a high fiber content could enhance the OHC of the blends (17). The holding of the oil is partly related to chemical composition, but it is more related to the porosity of the fiber structure rather than the affinity of fiber molecules to oil. The structure of a fiber is normally more porous than that of wheat flour, and this leads to the promotion of oil entrapment (23). Similar increases in the OHC with increases in the fiber level were also observed in the substitution of wheat flour with buckwheat flour (22) and barely flour (17) in the blends. In addition, the blends containing WRP had higher WAC and OHC than those of the blends containing HMS.

The pasting properties of wheat flour blended with either WRP or HMS are also shown in Table 1. It can be seen that the pasting properties of all blends were significantly affected by level of either WRP or HMS ($p<0.05$). All of the pasting parameters except for the pasting temperature (PT) showed a decreasing trend upon increasing ratio of either WRP or HMS in the blends. The PT is related to water binding capacity of the starch granules. Incorporation of increasing levels of either WRP or HMS slightly increased in PT ($p<0.05$). This result might be explained by either WRP or HMS competed with wheat flour to bind water, leading to the limitation of free water to enhance the swelling of starch. Similar results were found in case of wheat flour blended with the powders of sugarcane bagasse (9) and pomegranate peel (5). In addition, the PT of all blends containing HMS tended to be higher than those of the blends containing WRP.

Lower WAC of the blends containing HMS was responsible for high PT of the blends. Peak viscosity (PV) is associated with the degree of granule swelling during heating. Starch with higher swelling capacity generally presents an increase in PV. It was found that the addition of either WRP or HMS caused a decrease in PV of the blends ($p<0.05$). The increment in the content of either WRP or HMS contributed to a lower value of PV ($p<0.05$). The result indicated that WRP might limit the swelling of starch granules. According to the previous works, PV tended to decrease as the level of fiber rich plants such as sugarcane bagasse powder, *Moringa* leave powder, and sweet potato fiber power increased in the flour blends as reported by Dachana *et al.* (24), Sangeetha *et al.* (9), and Mais and Brennan (25), respectively. They also revealed that fibers could reduce PV due to the reduction in starch for gelatinization and less water available for initial swelling of starch granules. In addition, Ogunsina *et al.* (26) also reported that a reduction in available water caused the limitation of starch granule swelling, thus explaining the lower PV of pastes. HMS is a natural bioactive ingredient isolated from a special corn that is high in amylose content. This starch is restricted to swell because its structure is rigid, resulting in the reduction in PV. Breakdown (BK) refers to the stability of the already broken starch granules at the cooking temperature. It was found that BK decreased with an increasing content of either WRP or HMS in the blends ($p<0.05$). This result indicated that viscosity loss due to wheat flour replacement could be compensated by the presence of either WRP or HMS. In addition,

lower BK was found in all blends containing HMS compared to those of all blends containing WRP. This result indicated that the addition of HMS to the blends could enhance the resistant of the paste to heat and shear. The rigidity of HMS granule by limiting swelling could explain why all blends containing HMS had a smaller BK than those of all blends containing WRP. Final viscosity (FV) and setback (SB) are commonly used to indicate the ability of the material to form a viscous paste or gel after cooking and cooling. FV and SB are remarkably influenced by the retrogradation of soluble amylose upon cooling. Both FV and SB decreased as a function of both WRP and HMS contents ($p < 0.05$). This result indicated that the addition of fiber could reduce the interaction of amylose recrystallization, resulting in the retardation of retrogradation. The bulky molecules of fiber might reduce the formation of hydrogen bonding among the starch molecules, particularly amylose by steric hindrance. These factors prevent the close association of chains and restrict the formation of inter-chain hydrogen bonds, thus the tendency to retrograde will be retarded. In addition, a decrease in water availability due to WRP addition could lead to restricted amylose leaching and, thus, to less cold paste viscosities (1). Similar decreases in FV and SB with increasing fiber level in the blends were also found as reported by Dachana *et al.* (24) (dried *Moringa* leave), Mais and Brennan (25) (sweet potato fiber), and Ogunsina *et al.* (26) (de-bittered *moringa* seed flour).

Physical properties of cookies

Color: The color of the cookies is one of the characteristics firstly perceived by consumers, and this affects the acceptance of the product. Color parameters such as L^* (lightness), a^* (redness), and b^* (yellowness) values among the samples differed significantly ($p < 0.05$). Substituting wheat flour with either WRP or HMS in the formulation significantly influenced the surface color of the cookies ($p < 0.05$). Compared with the control sample, all cookies containing HMS presented higher L^* value and lower a^* , b^* values (Table 1). In addition, an increase in the HMS content in the formulation caused higher increase in lightness and a decrease in the redness. However, more brown color formation was observed with an increase in the level of WRP. The color of the cookie is normally generated during baking from the Maillard reaction between reducing sugar and protein. Starch dextrinization and caramelization are induced by heating and also affect the cookie color. Chevallier *et al.* (27) suggested that the protein content was negatively correlated with the lightness of the cookie, indicating that the Maillard reaction played the major role in color formation as noted earlier. Thus, an increase in lightness by incorporating HMS could be attributed to the decrease in total protein content in the formulation of cookies. Chung *et al.* (8) also reported that an increase in white rice flour in the formulation caused an increase in the lightness of cookies. In addition, muffins produced with an increase in the level of resistant starch were whiter than the control prepared without added resistant starch (28). Similar results were also found in the work of

Laguna *et al.* (29), who found that the lightness of biscuits increased as the proportion of resistant starch used in the formulation was increased. However, as the level of substitution of the WRP increased, the lightness decreased and the redness increased (Table 1). This may be due to plant fibers containing free sugars, and this might lead to the promotion of the Maillard reaction. In addition, the difference in color can be attributed to the original pigment or initial color of the fiber. It was hypothesized that the Maillard reaction was responsible for the brown color of the WRP. Brown color of the WRP was formed during drying process via the Maillard reaction. Thus, incorporating WRP in the preparation of cookies also resulted in a reduction of the lightness of the cookies while the white color found in HMS could enhance the increase in lightness of cookies as noted above. Similar increases in the dark color of the cookies was also reported with increases in the content of plant fibers such as sugarcane bagasse (9), multigrain (30), psyllium fiber (10), bamboo shoots powder (15), green tea powder (7), and dried *Murraya koenigii* leaf powder (31).

Spread ratio: The spread ratio is another important characteristic in determining the quality of the cookies. According to the results, incorporating either WRP or HMS significantly influenced the spread ratio of the cookies ($p < 0.05$) (Table 1). As the substitution level of the HMS increased, the spread ratio of the cookies also increased ($p < 0.05$). The spread ratio is highly dependent on the viscosity of the dough (8). The substitution using HMS caused a lowering of the dough viscosity, and therefore the spread ratio increased. The lower WAC of the HMS compared with the wheat flour could have contributed to lower spread ratio. The HMS used in the present work could be seen to be resistant starch. The granules of the enzyme-resistant high amylose maize starch are not completely gelatinized even at the boiling point of water; this leads to the lowering of the WAC (29). However, the addition of WRP in the cookies significantly reduced the spread ratio, and this decreasing trend was found as the level of WRP increased ($p < 0.05$). The greater absorption capacity of the WRP during the mixing of the dough resulted in a reduction of spread ratio. This was because the water available in this system would be insufficient to dissolve sugar during the baking, thus increasing the viscosity and resulting in a lower spread ratio (21). In addition, McWatters (32) proposed that the mechanism relating to the reduction of the spread ratio was affected by the replacement of wheat flour with fiber. During mixing, a rapid partitioning of free water to hydrophilic sites took place, resulting in an increase in the dough viscosity. This led to a limitation of the cookie spread. The reduced spread ratio has also been reported of cookies made from wheat flour substituted with fiber rich sources such as buckwheat flour (22), dried guduchi leaf powder (6), brown rice (8), and palm fiber (21).

Texture: Textural property is one of the major factors contributing to the eating quality of cookies. During the baking of cookie dough, a cellular solid with a thin colored surface and a porous inner structure were formed, leading to a decrease in the bulk density of the product. This was significantly affected by incorporating the non-

Fig. 1. 5-Hydroxy-methylfurfural (HMF) content and non-enzymatic browning index of the cookies made by substitution of wheat flour with various levels of either watermelon rind powder (WRP) or hi-maize starch (HMS) in the formulation.

wheat flour ingredients in two ways: by type of ingredient and content. All the cookies that contained either WRP or HMS showed a lower hardness than did the control sample ($p < 0.05$). As the level of substitution of wheat flour with either WRP or HMS increased, the hardness tended to decrease ($p < 0.05$) (Table 1). This data indicated that the cookies tended to become softer as the level of either WRP or HMS increased. The decrease in hardness induced by incorporating either WRP or HMS in the cookie making could be mainly attributed to the changes in the gluten content. The dilution of the gluten content was due to the addition of less gluten content ingredients (HMS or WRP) in the preparation of the cookies, and may be responsible for softer cookies. The reduction of gluten in cookie dough resulted in the retardation of the forming of the gluten matrix, which contributed to the reduction in hardness (8,10,25). Similarly, the hardness of the cookies decreased by incorporating fiber rich powders such as sweet potato fiber (25), debittered *Moringa oleifera* seed flour (26), sugarcane bagasse (9), brown rice (8), and green tea (7) in the formulation. In addition, the moisture content in the final product might affect the hardness. Increase in moisture content with the proportion of either WRP or HMS levels might be another factor that was responsible for lower hardness of the cookies.

Water activity: Increasing the proportion of either WRP or HMS in the formulation significantly increased the water activity of cookies ($p < 0.05$) (Table 1). This may be because higher levels of fiber can promote the holding capacity of water. Sharma and Gujral (17) also reported that the water activity of cookies increased with the addition of fiber in the preparation.

Non-enzymatic browning index: The non-enzymatic browning index of the cookies was influenced by the incorporation of either WRP or HMS (Fig. 1). A significant increase in the non-enzymatic browning index was found as the WRP was raised to higher levels in the cookie formulation ($p < 0.05$). The increase in non-enzymatic browning index correlated well with the lightness and redness. This result could be explained by the free sugars naturally present in the plant fiber enhancing the occurrence of the Maillard reaction during baking. An

increase in browning is associated with Maillard browning occurring during the baking of cookies. It has been widely accepted that Maillard browning is influenced by many factors and these further influence the intensity of the brown pigment produced. In addition to the Maillard reaction, browning may also be due to the caramelization of sugar. However, the non-enzymatic browning index of the cookies decreased as the levels of HMS increased during the preparation ($p < 0.05$). This may be attributed to the possible dilution of the sugars and proteins as affected by the addition of the HMS.

5-Hydroxymethylfurfural (HMF) content 5-Hydroxymethylfurfural can be used to access the quality of thermally processed foods. HMF is not present in fresh, untreated foods, but it is formed as an intermediate product in the Maillard reaction. In addition, it is also formed from the degradation of sugars as a result of caramelization. Although the toxicological relevance of HMF is not clear (*in vitro* studies on genotoxicity and mutagenicity have given controversial results), its accumulation in the cookies during baking is considered undesirable (13). The results indicated that the HMF content of all cookies made from HMS were found to be lower than in the control sample and the HMF content decreased when the level of HMS increased ($p < 0.05$) (Fig. 1). This indicates that the addition of HMS in the cookie making could retard the occurrence of non-enzymatic browning, thus leading to a reduction of the formation of HMF. On the other hand, the HMF content increased with an increase in the level of WRP ($p < 0.05$). This result may be because the WRP might contain higher free sugar and protein contents than the HMS. Therefore, the increase in the level of WRP in the cookie making could enhance the formation of HMF via the Maillard reaction. However, the HMF content detected in the present study was still lower than those in the previous report (13). In addition, the result of the HMF content was in agreement with non-enzymatic browning index.

Proximate compositions Table 1 also showed the proximate

Fig. 2. Soluble dietary fiber (SF), insoluble dietary fiber (IF) and total dietary fiber (TF) contents of the cookies made by substitution of wheat flour with various levels of either watermelon rind powder (WRP) or hi-maize starch (HMS) in the formulation.

composition of the cookies as affected by substitution of wheat flour with either WRP or HMS in the formulation ($p < 0.05$). The moisture content of cookies increased when either WRP or HMS was increased in the formulation, and the cookies with both WRP and HMS added tended to contain higher moisture content than the control sample. This could be attributed to the system having a higher capacity for moisture retention, and this increased in line with the proportion of either WRP or HMS. It has also been reported that biscuits fortified with resistant starch extracted from banana and high amylose maize starch (29) showed increments in moisture and water activity. Regarding the effect of the addition of WRP, dietary fiber can normally retain significant amounts of water in food products when it is added as a functional ingredient. Thus, the increased amount of fiber in the cookies as affected by the addition of WRP was responsible for the increased moisture retention in the cookies. Rajiv and Soumya (30) and Drisya *et al.* (31) reported that the moisture content of biscuits increased with an increase in the level of fiber rich powders from multigrains and *Murraya koenigii* leaf, respectively. In addition, the moisture content of cookies containing HMS was lower than in those containing WRP. As noted previously, WRP consists of soluble and insoluble fibers. Normally, soluble fibers can absorb water easily, resulting in the promotion of moisture retention capacity in the samples containing WRP. Regarding the protein content, a small decrease in the protein content was observed as the substitution level of either WRP or HMS increased. This pattern is due to the dilution effect produced by the addition of either WRP or HMS which contains lower protein content in comparison to that of wheat flour. The lipid content of the cookies with 10-20% of either WRP or HMS added did not significantly change compared to the control sample ($p \geq 0.05$). At the highest levels of either WRP or HMS, the fat content slightly decreased. The decrease in ash content was observed with increasing level of HMS in the formulation, while the ash content of all cookies supplemented with WRP increased with an increase in the level of WRP ($p < 0.05$). The increase in ash content may be due to a higher mineral content in the WRP. An increase in

total dietary fiber content was detected in the cookies with partial substitution of either WRP or HMS ($p < 0.05$) (Fig. 2). The amount of TF increased when the content of either WRP or HMS increased in the formulation ($p < 0.05$). The results reveal that both the WRP and HMS are important sources of dietary fiber, especially IF. Hence, supplementing cookies with either WRP or HMS, each of which is rich in dietary fiber, could enhance the cookies as health products as noted earlier. According to Thai legislation, the minimum values necessary to identify a product as a source of dietary fiber are 2.5 g of fiber per 100 g of product. Therefore, the cookies produced by replacing wheat flour with either WRP or HMS in the range of 10-30% could be claimed to be sources of dietary fiber.

TPC and antioxidant activity Increasing the level of the HMS in the cookie making tended to decrease the TPC of the cookies ($p < 0.05$). This may be attributed to the lower phenolic content contained in HMS in comparison to that in wheat flour. However, the TPC apparently increased as the proportion of WRP in the formulation increased ($p < 0.05$). This result may be because wheat flour has less phenolic content as compared to WRP as noted earlier. Similar trends were found in biscuits enriched with the powders of bamboo shoots (15) and mango peel (14). There are some reports that indicate an improvement in the antioxidant potential of the cookies incorporated with natural plant fibers. Therefore, their radical scavenging activity was assessed using DPPH-RSA while the FRAP method was used to determine the ferric reducing power of the investigated samples. All cookies containing HMS had lower DPPH-RSA and FRAP than did those of the control sample ($p < 0.05$) (Table 1). However, slight decreases in the DPPH-RSA and FRAP were found with an increase in the HMS level in the formulation ($p < 0.05$). This reduction may be attributed to the lower TPC of the samples. It is well known that the potential health benefits of bioactive components, especially phenolic compounds, may be attributed to their antioxidant activities. Normally a high antioxidant capacity can be correlated with the presence of a high phenolic content in the samples. On the other

hand, increasing the level of WRP in the formulation progressively increased the DPPH-RSA and FRAP ($p < 0.05$). This is attributed to higher TPC of the WRP as compared to wheat flour. In addition, the increase in antioxidant activity may be attributed to the formation of a dark color pigment (brown color) during the baking process. This is due to a Maillard reaction, and these pigments have been reported to have antioxidant activity (33).

In vitro starch digestibility and PGI Glucose release during *in vitro* digestion of cookies was shown in Fig. 3, and corresponding hydrolysis parameters were also shown in Fig. 4. Glucose release was measured as the reducing sugars degraded from starch by digestive enzymes. Overall, all the cookie samples showed a rapid increase in starch hydrolysis in the first 30 min of the test and thereafter tended to decrease. Incorporating either WRP or HMS in the cookie making led to a progressive decrease in the hydrolysis index (HI) ($p < 0.05$). The replacement of digestible carbohydrate (wheat flour) with indigestible carbohydrates, such as WRP and HMS, could be responsible for the decrease in HI and glucose release during the test. During cooking, starch granules are disrupted in a process commonly known as gelatinization. This markedly increases the susceptibility of starch to amylolytic degradation due to the swelling of the starch and the loss of a crystalline structure. The increase in the resistant starch content through enrichment with HMS could be attributed to the intact structure of the cellular wall, entrapping starch granules and hindering their complete gelatinization. In addition, part of the resistant starch formed in the cookie could occur due to retrogradation of some amylose chains following starch gelatinization, resulting in resistance to hydrolysis caused by the amylolytic enzyme (1,34). This phenomenon is responsible for the reduction in HI as the proportion of HMS increased in preparing the cookies. Regarding the effect of fiber on starch digestibility, Soong *et al.* (34) and Regand *et al.* (35) proposed that the viscosity of fiber plays a key role in reducing starch digestibility. In addition, fiber can compete with wheat flour to bind with water, resulting in the limiting the free water to promote starch

Fig. 3. *In vitro* starch digestibility of the cookies made by substitution of wheat flour with various levels of either watermelon rind powder (WRP) or hi-maize starch (HMS) in the formulation. WB=white bread

gelatinization. This phenomenon may lead to the retardation of enzyme accessibility for hydrolyzing starch molecules. However, the reduction in starch digestibility, as influenced by fiber, still remains unclear. Studying the activity of fiber in reducing starch digestibility is necessary and important for developing an understanding of the mechanisms of the action of fibers in reducing glycemic responses in real food systems.

Classification through pGI is useful to predict the likely *in vivo* glycemic response of food. The results showed that the pGI decreased significantly as the proportion of either WRP or HMS increased in the formulation ($p < 0.05$) (Fig. 4). In addition, a lower pGI was observed in all the samples containing HMS compared to those of the samples containing WRP ($p < 0.05$) (Fig. 4). The HMS possesses reduced enzyme availability which derives from the double helical form with its more compact structure of amylose upon recrystallization. This

Fig. 4. Hydrolysis index (HI) and predicted glycemic index (pGI) of the cookies made by substitution of wheat flour with various levels of either watermelon rind powder (WRP) or hi-maize starch (HMS) in the formulation.

Fig. 5. Sensory evaluation of the cookies made by substitution of wheat flour with various levels of either watermelon rind powder (WRP) or hi-maize starch (HMS) in the formulation.

leads to a lower pGI by impeding the enzyme accessibility as noted earlier. Researchers classify high, medium, and low glycemic index foods as good, better, and the best choices for nutrition. With regards to this approach, foods are classified with respect to glucose as reference as: low glycemic index foods ($GI < 55$); medium glycemic index foods ($56 < GI < 69$); and high glycemic index foods ($GI > 70$). According to this classification, the cookies containing 10-20% HMS and 20-30% WRP fall into the medium GI category while the cookies containing 30% HMS can be classified as having a low GI. According to the results, it can be concluded that incorporating both WRP and HMS could reduce the predictive glycaemic response of the cookies, thus reducing the calorific content. This in turn may have positive effects in terms of weight management and the potential glycaemic impact of readily digestible starchy foods.

Sensory evaluation The sensory profiles of the cookie samples were evaluated in terms of color, appearance, texture, flavor, and overall acceptability as shown in Fig. 5. It can be seen that incorporating either HMS or WRP in the formulation influenced the sensory characteristics of the cookies ($p < 0.05$). No differences in color and appearance scores were found between the control sample and the sample containing 10-30% HMS ($p \geq 0.05$). This result may be because the application of HMS increased the lightness of the cookies, leading to their being acceptable to consumers. In addition, no differences in the scores for color and appearance were observed between the control sample and the sample containing 10% WRP ($p \geq 0.05$). However, further increasing the level of WRP from 20 to 30% decreased the scores for color and appearance ($p < 0.05$). It could be suggested that an increase in the level of WRP caused an increase in the dark color of crumbs. This result was in accord with decreases in the lightness and redness values. Similar decreases in color score were also reported in the cookies fortified with the powders of mango peel and bamboo shoots (14-15). This was because of the increment in the dark color of the cookies. There were no significant differences in the texture scores of the control

sample and all samples containing either 10-20% HMS or 10% WRP ($p \geq 0.05$). However, further increases in either WRP or HMS levels caused a slight decrease in the texture score ($p < 0.05$). This may possibly be due to the increase in the level of either HMS or WRP caused a decrease in the hardness, leading to the texture of the cookies becoming softer. However, the texture score was still above the predetermined acceptable level (5: not liked or disliked) in the nine-point hedonic scale and were slightly lower than the control sample. Cookie samples made from substituting wheat flour with 10-30% HMS showed no significant differences in flavor score compared to the control sample ($p \geq 0.05$). This result indicated that the application of HMS in the making of cookies did not affect the flavor of cookies. On the other hand, the flavor score tended to decrease as the proportion of WRP in the formulation increased ($p < 0.05$). This may be because of the slight bitterness and sourness that is presented in the WRP due to a high polyphenol content. Similar observations were also found in the biscuits enriched with a high content of mango peel (14), bamboo shoot powder (15), and *Murraya koenigii* leaf (31). They also reported that the high phenolic content and intensity of the volatile flavors found in both fiber powders were responsible for the decrease in flavor score. No significant difference was found in the overall acceptability score between the control sample and all samples containing HMS ($p \geq 0.05$). However, a slight decrease in the overall acceptability score was found with an increase in the level of WRP ($p < 0.05$). This result may be explained by the increase in the level of WRP causing a decrease in color, appearance, texture, and flavor scores. However, the cookies containing 20% WRP remained acceptable, receiving a score of approximately 6.72 by the panel. With respect to the color, appearance, texture, flavor, and overall acceptability scores, the cookies of an acceptable quality can be prepared by using up to 30% of HMS and 20% WRP. Thus WRP, as a by-product from a producer of fresh-cut fruit, could be utilized for the production of the cookies with improved functional, health promoting, and nutraceutical properties. In addition, the use of WRP may be useful in the

development of new products for sectors of the population with reduced caloric and glycemic requirement.

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