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Research Article

Effects of Pitaya Peel Supplementation on Nutritional Quality, Overall Sensory Acceptance, *In Vitro* Glycemic Index, and Antioxidant Release from Fiber-Enriched Cookies

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Pitaya peel is a by-product of fruit processing. In this study, the effects of pitaya peel supplementation in the cookie recipe on the nutritional quality, in vitro glycemic index, and antioxidant release from the enriched fiber cookies were investigated. The higher the ratio of pitaya peel powder (PPP) in the recipe, the greater the dietary fiber, betacyanin and phenolic contents, and antioxidant activities of the product. Cookies supplemented with 10–25% PPP were classified as having a low glycemic index, ranging from 51.9 to 45.7 in relation to glucose reference. The release of betacyanins, phenolics, and antioxidant activities from the cookie samples was recorded at the salivary, gastric, intestinal, and colon steps during in vitro sequential digestion, and the gastric digestion showed the highest release of antioxidant content and activity. The increased PPP ratio in the cookie recipe improved the antioxidant activities of the aqueous fractions at the four digestive steps. *Statement*. This study has a preprint entitled "Effects of the ratio of pitaya peel powder on the product quality, predicted glycemic index, and antioxidant release during in vitro sequential digestion" (DOI: https://doi.org/10.21203/rs.3.rs-2322871/v1). This preprint was posted on the research square website on December 5th, 2022.

1. Introduction

Cookie is a well-known bakery product due to its low manufacturing cost, convenience of use, and long shelf-life. However, conventional cookies are high in starch, sugar, and fat but low in dietary fiber and antioxidants which make them unhealthy for daily use [1]. The addition of antioxidant dietary fiber (ADF) to cookie formulation has attracted great attention. Different ADF sources originated from plant-by-products have been added to cookie recipes as functional ingredients [2]. However, the bioavailability of bioactive compounds in ADFs which were added to the cookie recipe depends on many factors such as their chemical structure, molecular interactions with other compounds in the food matrix, as well as their release during gastrointestinal digestion [3].

Pitaya peel powder (PPP) has a high fiber content, approximately 79% dw, and the ratio of insoluble fiber to soluble fiber is about 1.32; it also contains phenolics, betacyanins, and ascorbic acid with high antioxidant activities [4]. In 2016, wheat flour was replaced by PPP at ratios of 5%, 10%, and 15% in the cookie recipe, and the obtained product had improved crude fiber and ash content [5]. However, the effects of the PPP ratio in the cookie formulation on the dietary fiber composition as well as the antioxidant activity of the product have not been reported. Recently, the influence of particle size of PPP on the proximate composition and antioxidant activities of the

cookie samples incorporated with 10% PPP was examined. The results showed that reduction in particle size of PPP decreased the dietary fiber content of the cookie but enhanced its antioxidant content and activity [6]. Nevertheless, the glycemic index as well as the release of antioxidants from the PPP-incorporated cookies during in vitro digestion remained unknown.

In this study, different ratios of PPP were used in the cookie formulation. The objective of this work was to investigate the effects of PPP supplementation on the nutritional quality of cookie samples, especially on their fiber composition and antioxidant activity. In addition, the glycemic index and antioxidant release from the PPP-added cookies were in-vitro evaluated to clarify the potential use of this ADF source in the making of healthy food products.

2. Materials and Methods

2.1. Materials. Pitaya fruits with purple-red peel and white-flesh (*Hylocereus undatus*) were harvested from a local farm in Dak Lak province (Vietnam). At the laboratory, the fruits were washed with tap water and drained; the pulp was manually separated, and the peel was then cut into 5×2 cm pieces. Each drying batch was conducted with 1.5 kg of fresh peel pieces at 60°C in a dryer (SF30, Memmert, Büchenbach, Germany) for 8 h, and the moisture content was achieved at 10 ± 1 (%). The dried peel was crushed in a cutter mill and passed through a 70-mesh sieve. From 50 kg of pitaya fruits, about 12 kg of fresh peel and 1.4 kg of pitaya peel powder were collected. The obtained pitaya peel powder was preserved in polyethylene bags at 4°C for experimentation.

Cookie ingredients include wheat flour from DP Flour Milling Co., Ltd. (Can Tho, Vietnam), fresh chicken eggs from Ba Huan Co. (Ho Chi Minh City, Vietnam), Anchor unsalted butter from Fonterra Ltd. (Auckland, New Zealand), isomalt from Vikibomi Corp. (Ho Chi Minh City, Vietnam), table salt from Salt Group JSC (Ho Chi Minh City, Vietnam), vanilla flavor from Thien Thanh Co., Ltd. (Ho Chi Minh City, Vietnam), baking powder from Church & Dwight Co. (Ewing, NJ, USA), and acesulfame potassium from Vitasweet Co., Ltd. (Changzhou, Jiangsu, China) were purchased from a local supermarket.

Termamyl® SC, Dextrozyme® DX, and Alcalase® 2.5L with alpha amylase (120 kilo Novozyme α-amylase units/g), glucoamylase (340 amyloglucosidase units/g), and protease (2.5 Anson units/g) activities, respectively, originated from Novozymes Co. (Copenhagen, Denmark) and were used for fiber quantification. Analytical chemicals include 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,4,6- tri (2-pyridyl)-s-triazine, gallic acid (GA), Folin–Ciocalteu reagent, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), pepsin from porcine gastric mucosa (≥250 units/mg), pancreatin from porcine pancreas ($4 \times$ USP specifications), and Pronase E with protease activity from *Streptomyces griseus* (≥3.5 units/mg), Viscozyme L with a cell wall degrading enzyme complex from *Aspergillus* sp. (100 fungal beta-glucanase units/g) were bought from Sigma-Aldrich Co. (St. Louis, MO, USA) and used for in vitro digestion; 3,5-dinitrosalicylic acid and D-glucose were bought from Merck Co. (Darmstadt, Germany); sodium chloride, sodium hydroxide, potassium chloride, sodium bicarbonate, hydrochloric acid, sodium phosphate monobasic dihydrate, diethyl ether, and sodium phosphate dibasic dodecahydrate were obtained from Xilong Scientific Co. (Shantou, Guangdong, China), and all were of analytical grade.

2.2. Cookie Preparation. The cookie recipe included 150 g of wheat flour and PPP, 46.61 g of isomalt, 0.18 g of acesulfame potassium, 0.66 g of table salt, 46.61 g of fresh chicken eggs (yolk and white), 74.51 g of butter, 1.61 g of baking soda powder, 0.6 g of vanilla, and 13.01 g of water. In the cookie preparation, the weight of PPP to the total weight of PPP and wheat flour was changed: 0 (control), 10, 15, 20, and 25% (w/w). The cookie-making procedure was performed as described elsewhere [6].

2.3. Chemical Analysis

2.3.1. Proximate Composition. Lipids were determined by Soxhlet extraction using diethyl ether solvent. Total protein was estimated by the Kjeldahl method, using a nitrogen-to-protein conversion factor of 6.25. Soluble dietary fiber (SDF) and insoluble dietary fiber (IDF) contents were quantified by the AOAC 993.19 and AOAC 991.42 methods, respectively [7]. Total dietary fiber (TDF) content was computed as the sum of insoluble and soluble fiber content. Total starch was analyzed using the AOAC 996.11 method [7]. Ash was evaluated by the AOAC 930.30 method [7]. Total carbohydrate was determined by subtracting the sum of lipid, protein, and ash percentages from 100%.

2.3.2. Betacyanin Content. In order to extract betacyanin, 1 g of cookie samples were put into a 50 mL beaker containing 20 mL of distilled water. The extraction was performed with an ultrasonic probe (VC750, Sonics, Newtown, CT, USA) at 150 W for 15 min. The extraction temperature was kept at roughly 30°C by placing the beaker in a bath (WCB-22, Daihan, Seoul, Korea) with cold water. The slurry was then centrifuged at 25°C and $3500 \times g$ and for 5 min (ROTOFIX 32A, Hettich, Tuttlingen, Germany). The supernatant was collected, and the procedure was carried out three times. The resulting extracts were well mixed. Betacyanin content (Bc) was measured with a spectrophotometric method described by Ee et al. [8] and calculated as follows:

$$\mathrm{Bc}\left(\frac{\mathrm{mg}}{\mathrm{kg}}\right) = \frac{(A \times M \times f \times V \times 1000)}{(\varepsilon \times L \times W)}, \quad (1)$$

where A is the absorbance (538 nm); M is the molecular weight of betacyanins ($M = 550\ \mathrm{g\ mol^{-1}}$); f is the dilution factor and V is the pigment solution volume (mL); ε is molar extinction coefficients of betacyanins ($65{,}000\ \mathrm{L\ mol^{-1}\ cm^{-1}}$); L is the path length of the cuvette (1 cm); W is the dry weight of the cookie sample used for extraction (g).

2.3.3. Total Phenolic Content. About 1 g of cookie samples were added to a 100 mL beaker with 40 mL of a 60% (v/v) methanol solution for phenolic extraction. The process of extraction was carried out using an ultrasonic probe (VC750, Sonics, Newtown, CT, USA) at 150 W for 15 min. The extraction temperature was also kept at roughly 30°C as previously mentioned. The slurry was separated by centrifugation (ROTOFIX 32A, Hettich, Tuttlingen, Germany) at 25°C and 3500 × g for 10 min, and the leftover material was extracted once more under the same circumstances. The pooled supernatants were utilized to calculate the total phenolic content. By using the Folin–Ciocalteau reagent and spectrophotometric method, the total phenolic content was determined [9].

2.3.4. Determination of Antioxidant Activity. The phenolic and betacyanin extracts were mixed and utilized to assess the antioxidant capacity, which was measured by ferric reducing antioxidant power (FRAP) [10] and DPPH radical scavenging activity assays [11]; the results were presented as μmol Trolox equivalent per 100 g dry weight of the sample.

2.4. Sensory Evaluation. After 24 h of baking, the cookie samples were evaluated by sixty untrained panelists who were casually recruited from the staff and students of Tay Nguyen University. A nine-point hedonic scale was used to evaluate the overall acceptability of Toledo et al. [12]. The panelists were asked to give score from 1 point (extremely dislike) to 9 points (extremely like).

2.5. Estimation of the In Vitro Glycemic Index. The in vitro glycemic index of cookie samples was estimated following the method of Englyst et al. [13] with some adjustments. Approximately 2 g samples of cookies incorporated with 0, 10, 15, 20, and 25% PPP and white bread as a reference food item were crushed by a crusher (Model A11, Ika, Staufen, Germany) and mixed with 30 mL distilled water; the pH value was changed to 2.5 by 0.8 mL of 1 M HCl solution and maintained for 10 min. Then, 1 mL pepsin solution (10% w/v prepared in 0.05 M HCl) was supplemented and kept in the dark at 120 rpm and 37°C for 30 min using an incubator shaker (Innova 40, New Brunswick Scientific, NJ, USA). After that, the pH value was modified to 6.9 by 1 mL 1 M $NaHCO_3$ solution before adding 5 mL pancreatin and 0.1 mL amyloglucosidase solution (2.5% w/v prepared in 0.01 M phosphate saline buffer with pH of 6.9). The mixture was filled up to 50 mL with 0.01 M PBS pH 6.9 and further incubated at 37°C and 120 rpm. Sampling was performed at 0, 15, 30, 60, 120, and 180 min of the incubation; about 1 mL of the aliquot was separated and mixed with 4 mL of 99.5% v/v ethanol for enzyme inhibition. The centrifugation was performed at 3000 × g for 15 min. The supernatants were then collected to measure reducing sugar content by the spectrophotometric method using 3,5-dinitrosalicylic acid reagent and glucose as a standard [14]. The hydrolyzed starch content was calculated by multiplying the reducing sugar content by 0.9. The starch hydrolysis kinetics were described by a nonlinear model suggested by Goñi et al. [15] as follows:

$$C = C_\infty \left(1 - e^{-kt}\right), \tag{2}$$

where C is the hydrolyzed starch content (g/L) at time t, C_∞ is the hydrolyzed starch content (g/L) after 180 min, k is the kinetic constant, and t is the reaction time (min).

The digestion curves were simulated and regressed using OriginLab OriginPro 8.5 software (OriginLab Corporation, Northampton, MA, USA) to obtain the value of k.

The area under the curve of starch hydrolysis S was computed as follows:

$$S = C_\infty\left(t_f - t_0\right) + \frac{C_\infty\left[1 - e^{-k\left(t_f - t_0\right)}\right]}{k}, \tag{3}$$

where $t_0 = 0$ and $t_f = 180$ min.

The hydrolysis index (HI) was computed while white bread was used as reference food:

$$\mathrm{HI} = \left(\frac{S_{\text{investigated sample}}}{S_{\text{reference sample}}}\right) \times 100. \tag{4}$$

The in vitro glycemic index (GI) based on the bread reference was calculated as follows:

$$\mathrm{GI} = 39.71 + 0.059 \times \mathrm{HI}. \tag{5}$$

When glucose is used as a reference food item (GI = 100), the glycemic index of white bread is 71. The obtained results in this study were therefore multiplied by 0.71 to convert them into glycemic indexes based on glucose [16].

2.6. Evaluation of Antioxidant Release Using In Vitro Sequential Digestion. Cookie samples with PPP ratios of 0 (control), 10, 15, 20, and 25% were subjected to in vitro sequential digestion following the method described by Colantuono et al. [17] with minor modifications. Briefly, four steps of digestion including salivary, gastric, intestinal, and colon steps were carried out.

In the salivary digestion step, 2.5 g of cookie samples were crushed by a crusher and added to 15 mL of distilled water, the pH value of which was adjusted to 6.5; the mixture was incubated for 2 min and centrifuged. The obtained pellet was suspended in 15 mL of distilled water for the next gastric digestion step; the suspension pH was modified to 2.2 using 6 M HCl; about 2.5 mL of pepsin solution (12.5 mg/mL dissolved in 0.1 M HCl) was added; the resulting mixture was further incubated for 1 h and centrifuged. In the intestine digestion step, 15 mL of distilled water was supplemented to the resulting pellet; the pH value was altered to 7.5 using 1 M NaOH; about 2.5 mL of pancreatin solution (10 mg/mL dissolved in 1 M NaOH) was added; the obtained mixture was then incubated for 4 h and centrifuged. In the colon digestion step, the pellet was resuspended in 15 mL of distilled water and the pH value was changed to 8.0 before adding 2.5 mL of pronase E (1 mg/mL); the mixture was incubated for 1 h and centrifuged. Finally, the pellet was added to 17.5 mL of distilled water; the pH was then adjusted

to 4.0, followed by the addition of 75 μL of Viscozyme L; the mixture was incubated for 16 h and centrifuged. For the salivary, gastric, intestinal, and colon steps of the in vitro digestion, the incubation was carried out at 37°C and 120 rpm in the incubator shaker. At each step, the supernatants (soluble fractions) were collected by centrifugation at 4°C and $4924 \times g$ for 15 min (Allegra X-15R, Beckman Coulter, IN, USA) for the measurement of the release of betacyanin and total phenolic contents as well as antioxidant activities by FRAP and DPPH assays, whereas the pellets were used for the next steps. Blanks of reagents were also made in each step of the in vitro digestion. The in vitro sequential digestion test was carried out in triplicate.

The release percentage (RP) of antioxidant activity and content from the cookie samples was calculated as follows:

$$\mathrm{RP} = \frac{(N_1 + N_2 + N_3 + N_4)}{N} \times 100\%, \quad (6)$$

where N_1, N_2, N_3, and N_4 are the antioxidant activity or the content of betacyanins or phenolics released from the investigated cookie sample at the salivary, gastric, intestinal, and colon steps, respectively; N is the initial antioxidant activity or the content of betacyanins or phenolics of the investigated cookie sample.

2.7. Statistical Analysis. Each cookie sample was conducted in triplicate; the results were shown as means ± standard deviation ($n = 3$). One-way analysis of variance and multiple range tests with a significant level of $p < 0.05$ were performed by Statgraphics Centurion XV.I software (Manugistics Inc., Rockville, MN, USA).

3. Results and Discussion

3.1. Effects of Pitaya Peel Supplementation on Nutritional Quality and Overall Acceptability of Cookies. When the ratio of PPP in the formulation increased from 0 to 25%, the total lipid content of the PPP-supplemented cookies was similar to that of the control cookies, while the protein, starch, and total carbohydrate content of the PPP-added cookies decreased by 7%, 21%, and 2%, respectively (Table 1). In contrast, the ash content of PPP-fortified cookies was 83–220% higher than that of the control cookies. Some important minerals for human nutrition such as calcium, potassium, magnesium, zinc, and iron are identified in pitaya peel [6].

Enhancement in the PPP ratio from 0% to 25% increased the total insoluble and soluble fiber contents of the cookies by 7.1, 7.4, and 6.7 times, respectively. Cookie samples supplemented with 15% PPP or higher ratio were considered as foods high in fiber since they had a total dietary fiber content of over 6% dw [18]. Furthermore, when the level of PPP increased from 10 to 25%, the IDF:SDF ratio slightly changed, ranging from 1.85 to 1.93. The IDF:SDF ratio of food is an important index for improvement in health benefits associated with dietary fiber consumption; this ratio should be varied from 1.0 to 2.3 to achieve the healthy physiological effects of both soluble and insoluble fractions [19]. Different fiber materials have various IDF:SDF ratios. When bulgur bran was added to the cookie formulation at 5–20%, the IDF and SDF of the obtained product ranged from 2.80% to 9.61% and 0.43% to 0.63%, respectively, and the IDF:SDF ratio of the cookies was therefore increased from 6.5 to 15.3 [20]. Dietary fiber materials with high insoluble fiber content are reported to be pretreated for the conversion of insoluble into soluble fiber before being supplemented to cookie formulation to improve the balance of insoluble and soluble fractions [21]. It can be concluded that PPP is a potential dietary fiber source for cookie fortification since the obtained product had a proper IDF:SDF ratio.

The control cookies did not contain betacyanins since they were not identified in wheat flour. The addition of PPP to the cookie recipe highly improved the antioxidant content and activity of the product. When the level of PPP increased from 0 to 25%, the phenolic content of the product enhanced by 204%. The DPPH radical scavenging activity and FRAP of the cookies supplemented with 25% PPP were 7.0 and 5.4 times, respectively, greater than those of the control without PPP addition.

Table 1 also shows that all PPP-added cookie samples were deemed to be acceptable due to their overall acceptance scores being greater than 5. When the ratio of PPP increased from 0 to 25%, the sensory acceptability of the cookies declined by 20.4%, which was probably due to the enhanced hardness (data not shown) and the obvious change in color (Figure 1). It can be noted that the change in the PPP ratio from 0 to 15% did not affect the sensory acceptability of the product.

3.2. Effects of Pitaya Peel Supplementation on the In Vitro Glycemic Index of Cookies. In this work, the selected nonlinear model was well fitted to the experimental data with $R^2 > 0.94$ (the data are not shown); this model was therefore used to calculate the GI of cookies samples. The GI of all cookie samples varied from 79.9 to 64.4 in relation to white bread as a reference food or from 56.7 to 45.7 in relation to glucose reference. The effects of PPP supplementation to the cookie recipe on the anticipated glycemic index of the product based on glucose reference are depicted in Figure 2.

It should be noted that the control cookie sample (GI = 56.7) was categorized as having a medium GI (GI = 55–69), while all PPP-supplemented cookie samples (GI = 45.7–51.9) were classified as having a low GI (GI < 55). The control cookies with a medium GI in this study could be attributed to the use of isomalt instead of sucrose in the product recipe. An increase in the PPP ratio in the cookie formulation from 0 to 25% reduced the GI of the product by about 19%. The reduction in GI of PPP-supplemented cookies was due to their enhanced levels of SDF which enhances the viscosity of the digesta, thus impeding amylase access to their substrates [22]. In addition, there was evidence that amylase-catalysed starch hydrolysis may be affected due to the interaction between pectin and amylase [23]. Polygalacturonic acid, a pectin component of pitaya peel [24], might interact electrostatically with α-amylase and

Table 1: Proximate composition, antioxidant activity, and overall acceptability of cookie samples supplemented with different pitaya peel levels.

Ratio of pitaya peel powder (% of the total weight of pitaya peel powder and wheat flour)	0	10	15	20	25
Lipid (% dw)	24.15 ± 0.13^{a}	24.20 ± 0.15^{a}	24.44 ± 0.35^{a}	24.26 ± 0.11^{a}	24.34 ± 0.42^{a}
Protein (% dw)	7.97 ± 0.02^{a}	7.66 ± 0.16^{b}	7.60 ± 0.04^{b}	7.40 ± 0.02^{c}	7.39 ± 0.05^{c}
Ash (% dw)	0.87 ± 0.03^{e}	1.59 ± 0.05^{d}	1.93 ± 0.04^{c}	2.47 ± 0.06^{b}	2.78 ± 0.02^{a}
Starch (% dw)	47.63 ± 1.65^{a}	42.33 ± 0.74^{b}	40.99 ± 0.92^{bc}	39.28 ± 0.04^{cd}	37.61 ± 0.79^{d}
Total fiber (% dw)	1.44 ± 0.18^{e}	4.93 ± 0.01^{d}	6.73 ± 0.06^{c}	8.83 ± 0.37^{b}	10.21 ± 0.20^{a}
Insoluble fiber (% dw)	0.91 ± 0.12^{e}	3.20 ± 0.03^{d}	4.41 ± 0.02^{c}	5.82 ± 0.28^{b}	6.71 ± 0.18^{a}
Soluble fiber (% dw)	0.52 ± 0.06^{e}	1.73 ± 0.02^{d}	2.32 ± 0.04^{c}	3.01 ± 0.10^{b}	3.50 ± 0.03^{a}
Ratio of insoluble fiber/soluble fiber	1.75 ± 0.06^{b}	1.85 ± 0.03^{ab}	1.90 ± 0.03^{a}	1.93 ± 0.05^{a}	1.92 ± 0.04^{a}
Total carbohydrate (% dw)	67.01 ± 0.10^{a}	66.57 ± 0.31^{a}	66.03 ± 0.30^{b}	65.87 ± 0.14^{bc}	65.49 ± 0.43^{c}
Betacyanins (mg/kg dw)	—	27.00 ± 1.07^{d}	31.30 ± 1.38^{c}	35.58 ± 1.14^{b}	45.43 ± 3.36^{a}
Total phenolics (mg GAE/100g dw)	113.46 ± 2.74^{e}	201.34 ± 4.03^{d}	265.81 ± 8.87^{c}	299.92 ± 11.67^{b}	345.40 ± 7.57^{a}
Antioxidant capacity by the DPPH assay (μmol TE/100 g dw)	49.80 ± 1.90^{e}	160.63 ± 5.20^{d}	251.24 ± 8.32^{c}	326.25 ± 9.59^{b}	397.35 ± 11.68^{a}
Antioxidant capacity by the FRAP assay (μmol TE/100 g dw)	77.42 ± 3.09^{e}	234.36 ± 8.01^{d}	288.21 ± 5.43^{c}	399.48 ± 7.45^{b}	491.43 ± 12.79^{a}
Overall acceptability	6.47 ± 0.5^{a}	6.33 ± 0.48^{a}	6.32 ± 0.47^{a}	5.98 ± 0.34^{b}	5.15 ± 0.36^{c}

Values with different subscripts in the same row are significantly different ($p < 0.05$); dw: dry weight; GAE: gallic acid equivalent; DPPH: 2, 2-diphenyl-1-picrylhydrazyl; TE: trolox equivalent; FRAP: ferric reducing antioxidant power.

Figure 1: Picture of cookie samples with different ratios of pitaya peel powder; C0, C10, C15, C20, and C25: cookie samples with 0, 10, 15, 20, and 25% pitaya peel powder, respectively.

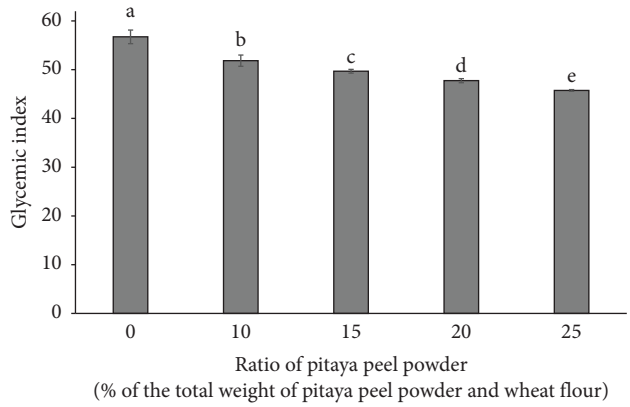

Figure 2: *In vitro* glycemic index based on glucose reference; the values with different lowercase letters (a–e) above the columns which have with the same pattern fill are significantly different ($p < 0.05$).

act as a noncompetitive inhibitor of this enzyme [25]. Moreover, cookies incorporated with a high PPP ratio contained more phenolic compounds which might reduce the glycemic index by inhibiting α-amylase and/or α-glucosidase as well as the interactions between polyphenol and starch molecules [26].

3.3. Release of Betacyanins, Phenolics, and Antioxidant Activities from Cookie Samples Incorporated with Different Ratios of Pitaya Peel Powder during the In Vitro Sequential Digestion. Table 2 shows that the betacyanins of all PPP-incorporated cookies were released during the four steps of the digestive tract. The content of betacyanins liberated at the salivary step was much higher than that at the three remaining steps, and the value accounted for 60.0–60.5% of the initial content. It can be explained by their high water solubility [27]. The higher the PPP ratio in the cookie recipe, the greater the content of betacyanin released at the salivary, gastric, and intestinal steps. It is probably due to the high initial betacyanin levels of cookies with the increased PPP ratio. Previously, betanin was not detected in colon fermentation fluid when the dosage of 23 mg/mL was used in human simulated gastrointestinal digestion [28]. In this study, the betacyanin content liberated at the colon step was statistically similar for all fiber-enriched cookies and the value was 9.3% of the original level. The presence of betacyanins in the colon can protect humans from diet-induced obesity and its related metabolic disorders thanks to the improvement of inflammatory status and modulation of the gut microbiota [29]. The release percentage of betacyanins was nearly 99%, and this value was statistically similar for all cookies fortified with various PPP ratios (Figure 3). According to Tesoriere et al. [30], when the purified betalains were subjected to in vitro digestion, their loss was observed in the intestinal step, while the loss did not occur when raw red beet or cactus red fruit with betalains were used in the in vitro model. It can be confirmed that the bioavailability of betalains depends not only on their chemical stability in the digestive tract but also on the proximate composition of the food matrix. For the first time, fiber-enriched cookies were proven to have great potential for betacyanin protection in the in vitro digestion model.

Similarly, phenolics were also released during the four steps of the digestive tract (Table 2). At each digestion step, the content of released phenolics tended to increase with the increased PPP ratio in the cookie recipe. The highest release of phenolic compounds was recorded at the gastric step, and the obtained value varied from 103.3 to 188.6 mg GAE/100 g dw sample. It was anticipated that the low pH and the presence of pepsin in the gastric step would create favorable conditions for the release of some phenolics that were covalently or noncovalently bound to the protein in the food matrix, making these bioactives more accessible [31]. In contrast, some polyphenols in pomegranate peels and pomegranate peel-enriched cookies were released most at the simulated salivary step [17]. Various results are probably due to different impacts of the food matrix. Phenolic

Table 2: Changes in phenolic and betacyanin contents and antioxidant activities of cookie samples with different ratios of pitaya peel powder during the in vitro sequential digestion.

Digestion step	Ratio of pitaya peel powder (% of the total weight of pitaya peel powder and wheat flour)	Betacyanin (mg/kg dw)	Total phenolic (mg GAE/100 g dw)	DPPH activity (μmol TE/100 g dw)	FRAP activity (μmol TE/100 g dw)
Salivary digestion	0	—	21.1 ± 1.2^{eD}	16.6 ± 2.1^{eC}	26.7 ± 0.7^{eB}
	10	16.2 ± 0.5^{dB}	69.7 ± 1.3^{dC}	60.6 ± 3.9^{dD}	119.2 ± 2.3^{dC}
	15	17.8 ± 0.6^{cB}	78.7 ± 0.2^{cC}	84.2 ± 6.6^{cD}	142.9 ± 3.6^{cC}
	20	20.9 ± 1.2^{bB}	85.1 ± 1.7^{bC}	95.9 ± 4.0^{bD}	172.6 ± 4.9^{bC}
	25	27.5 ± 0.4^{aB}	94.4 ± 2.4^{aC}	105.6 ± 2.8^{aD}	195.5 ± 8.2^{aC}
Gastric digestion	0	—	103.3 ± 4.1^{dB}	52.6 ± 6.6^{eA}	71.7 ± 1.3^{eA}
	10	3.8 ± 0.4^{dC}	154.7 ± 3.8^{cB}	121.5 ± 5.4^{dB}	160.3 ± 5.0^{dB}
	15	6.5 ± 0.9^{cC}	165.1 ± 5.7^{bB}	183.0 ± 8.2^{cB}	199.5 ± 10.4^{cB}
	20	7.9 ± 0.3^{bC}	182.8 ± 2.8^{aB}	227.8 ± 7.9^{bB}	245.2 ± 6.0^{bB}
	25	9.4 ± 0.4^{aC}	188.6 ± 3.3^{aB}	318.1 ± 11.0^{aB}	267.8 ± 5.3^{aB}
Intestinal digestion	0	—	32.4 ± 1.2^{dC}	39.7 ± 1.2^{eB}	26.8 ± 2.8^{eB}
	10	3.2 ± 0.3^{bD}	42.9 ± 2.8^{cD}	98.7 ± 4.5^{dC}	43.4 ± 1.0^{dD}
	15	3.2 ± 0.5^{bD}	45.1 ± 1.1^{bcD}	120.6 ± 5.7^{cC}	54.7 ± 1.3^{cD}
	20	3.7 ± 0.5^{abD}	47.1 ± 0.7^{abD}	131.2 ± 1.9^{bC}	60.5 ± 2.1^{bD}
	25	4.6 ± 0.7^{aD}	49.5 ± 1.2^{aD}	138.7 ± 3.4^{aC}	63.9 ± 1.2^{aD}
Colon digestion (Pronase E)	0	—	9.5 ± 0.4^{eE}	16.1 ± 1.4^{eC}	15.8 ± 1.3^{eC}
	10	2.1 ± 0.8^{aE}	13.1 ± 0.3^{dE}	32.1 ± 4.6^{dE}	35.6 ± 1.1^{dE}
	15	2.1 ± 0.5^{aE}	14.6 ± 0.2^{cE}	71.9 ± 2.4^{cDE}	44.6 ± 2.6^{cE}
	20	2.3 ± 0.2^{aE}	16.2 ± 1.3^{bE}	90.9 ± 4.7^{bD}	50.4 ± 1.6^{bD}
	25	2.9 ± 0.4^{aDE}	19.4 ± 0.7^{aE}	106.8 ± 3.8^{aD}	55.9 ± 2.0^{aD}
Colon digestion (Viscozyme L)	0	—	6.8 ± 0.3^{eE}	9.7 ± 0.7^{eD}	9.9 ± 3.0^{cD}
	10	0.4 ± 0.1^{aF}	13.8 ± 0.6^{dE}	39.5 ± 3.4^{dE}	16.4 ± 0.4^{bF}
	15	0.4 ± 0.1^{aF}	15.5 ± 0.2^{cE}	74.0 ± 5.5^{cE}	18.2 ± 0.5^{abF}
	20	0.5 ± 0.2^{aF}	18.2 ± 0.3^{bE}	88.4 ± 1.1^{bD}	18.7 ± 0.2^{abE}
	25	0.5 ± 0.2^{aE}	21.0 ± 1.2^{aE}	98.1 ± 3.0^{aD}	21.2 ± 2.5^{aE}
Total (for the four steps)	0	—	173.2 ± 6.3^{eA}	134.8 ± 9.6^{eA}	150.9 ± 5.1^{eA}
	10	25.7 ± 1.8^{dA}	294.1 ± 0.8^{dA}	352.4 ± 4.7^{dA}	375.0 ± 2.1^{dA}
	15	30.0 ± 1.4^{cA}	319.1 ± 7.0^{cA}	533.7 ± 6.9^{cA}	459.9 ± 8.3^{cA}
	20	35.2 ± 0.4^{bA}	349.5 ± 0.6^{bA}	634.3 ± 8.3^{bA}	547.3 ± 9.5^{bA}
	25	45.0 ± 0.9^{aA}	372.9 ± 4.0^{aA}	767.3 ± 7.1^{aA}	604.2 ± 14.7^{aA}

In the same column and in the same step of the *in vitro* digestion, the values with different lowercase letters (a–e) are significantly different ($p < 0.05$); in the same column and with the same ratio of pitaya peel powder, the values with different capital letters (A–C) are statistically different ($p < 0.05$); dw: dry weight; GAE: gallic acid equivalent; DPPH: 2,2-diphenyl-1-picrylhydrazyl; TE: trolox equivalent; FRAP: ferric reducing antioxidant power.

compounds were still liberated from all PPP-incorporated cookie samples throughout the large intestine step. It is possible that Viscozyme L and Pronase E were responsible for the release of nonextractable polyphenols associated with dietary fibers [31] and/or some low-molecular-weight phenolics linked to the melanoidins formed during the baking [32]. From the health point of view, the potential release of dietary phenolic compounds in the colon is useful. It is reported that phenolic compounds affect gut health since these bioactives impact the balance between major groups of gut bacteria including *Bifidobacterium* spp., *Bacteroidetes,* and *Firmicutes*, in comparison to the controls [33]. Furthermore, in the colon, dietary phenolic compounds appear to improve the formation of some useful metabolites by gut bacteria and/or restrict their production of detrimental molecules. Following the in vitro incubation of rutin, quercetin, chlorogenic acid, and caffeic acid, an increase in the overall synthesis of short-chain fatty acids was recorded; these metabolites provide energy for the growth of colonocytes and play a major role in preventing colorectal cancer [33]. Conversely, consumption of proanthocyanidin-rich extracts from grape seeds has the potential to reduce toxic products from the protein fermentation in the large intestine such as ammonia, phenol, p-cresol, and skatole [34].

The total content of phenolics released from all cookie samples ranged from 173.2 to 372.9 mg GAE/100 g dw in the in vitro digestive tract. The released percentage of phenolic compounds ranged from 108 to 153% of the initial content of the undigested cookie samples and tended to decrease as the PPP ratio in the cookie formulation increased (Figure 3). It is hypothesized that increased soluble fiber content in cookie samples might reduce the release of phenolic compounds. Food matrix with highly soluble fiber content increases the viscosity of gastrointestinal fluids, restricting the peristaltic mixing process that promotes the transport of digestive enzymes to their substrates [35]. As a result, the release of phenolics which are bound with fiber and other compounds such as carbohydrates, proteins, or lipids [31] in the food matrix is limited.

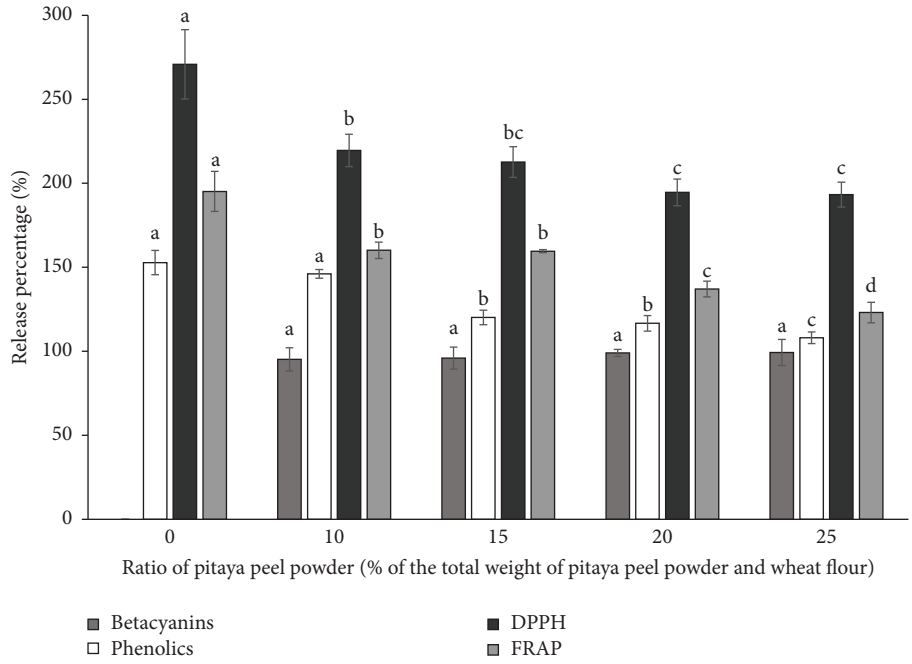

Figure 3: Antioxidant release from cookies supplemented with different ratios of pitaya peel powder in the *in vitro* digestion; the values with different lowercase letters (a–e) above the columns which have with the same pattern fill are significantly different ($p < 0.05$).

Table 2 also reveals that the antioxidant activities of the PPP-incorporated cookies were all significantly greater than those of the control cookies at all digestion steps. In the salivary step, the accessible antioxidant activities of the cookie incorporated with 25% PPP evaluated by FRAP and DPPH assays were 7.3 and 6.4 times, respectively, greater than those of the control cookies. At each digestion step, the increase in the PPP ratio in the cookie recipe significantly enhanced the antioxidant activity of the digested aqueous fractions. The highest antioxidant activities were recorded at the gastric digestion step for all cookie samples; the value varied from 71.7 to 267.8 μmol TE/100 g dw for FRAP and from 52.6 to 318.1 μmol TE/100 g dw for DPPH radical scavenging capacity. This is probably related to the highest content of phenolic compounds liberated from the cookie samples at this step; the content of released phenolics positively correlated with the FRAP and DPPH radical scavenging capacity; their correlation coefficients (r) were 0.99 ($p < 0.01$) and 0.92 ($p < 0.05$), respectively.

The total FRAP and DPPH radical scavenging capacities of all digested cookie samples were greater than those of the predigested samples. The released percentage of antioxidant activities evaluated by FRAP and DPPH assays varied from 123 to 195% and 193 to 271%, respectively. This can be explained by the release of bound phenolics from the cookie matrix during the in vitro sequential digestion. In addition, the Maillard reaction products which are produced during the cookie baking [36] as well as the cookie proteins of which might be cleaved to release peptides [37] may contribute to the in vitro antioxidant activity. Nevertheless, when the ratio of PPP increased from 0 to 25%, the released percentage of antioxidant activities in the cookie samples decreased. This result was in accordance with the recent study, in which the increase in decaffeinated coffee silverskin from 0 to 6% in the biscuit recipe resulted in a decreased release percentage of antioxidant activity from 745 to 394% [38].

4. Conclusions

Increased levels of PPP in the cookie formulation significantly enhanced betacyanin, phenolic, and dietary fiber contents as well as the antioxidant capacities of the product. The sensory acceptability of cookies added with 15% PPP and that of the control cookies were statistically similar. The supplementation of PPP reduced the predicted GI of the product. In the in vitro sequential digestion, betacyanins, phenolics, and antioxidant activities of cookie samples were released at all salivary, gastric, intestinal, and colon steps, and the highest release of antioxidants was recorded at the gastric step. The enhanced level of PPP in the cookie recipe significantly improved the antioxidant activities of the aqueous fractions at all digestive steps. PPP would be a prospective antioxidant and dietary fiber ingredient to be added to cookie products.

Data Availability

Data are available on request.

Ethical Approval

The study was covered by a general approval from the Ethics Review Board at the University of Social Sciences and Humanities, Vietnam National University – Ho Chi Minh City (VNU-HCM). Participants were instructed to read an information sheet and sign a consent form. Participants gave

voluntary consent and were assured that their responses would remain confidential. They were informed that they could withdraw at any point without any consequences. Participants received monetary compensation for their participation.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors' Contributions

Thi Hai Anh Mai was responsible for investigation, methodology, formal analysis, and writing of the original draft. Thi Thu Tra Tran was responsible for visualization, data curation, and supervision (equal). Van Viet Man Le was responsible for conceptualization, project administration, supervision (equal) and writing, reviewing, and editing.

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