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PRE-TREATMENT OF VEGETABLES AND FORMULA DEVELOPMENT OF CALCIUM-FORTIFIED VEGETABLE CRISPY WAFFLES WITH RICEBERRY USING A MIXTURE DESIGN

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ABSTRACT

Crispy waffles are popular snacks typically containing wheat flour, sugar, and oil. Excessive consumption may be unhealthy. This study aimed to produce healthier crispy waffles using vegetables, specifically Chinese kale (CK), False Pak Choi (FPC), and Thai basil (TB), as sources of calcium. These vegetables underwent three pre-treatments: blanching, steaming, and stir-frying. The study then focused on the formulation of calcium-fortified crispy vegetable waffles with riceberry (CVWR) using a mixture design experiment. Calcium content in TB, FPC, and CK was found to be 313.96, 309.88, and 246.15 mg/100g, respectively. Pre-treatment results revealed that steaming resulted in the least reduction of calcium content, while stir-frying caused the least reduction of total chlorophyll and total polyphenol contents. The proportions of the crispy waffles components were 0-30%CK, 0-30% FPC, 0-30%TB, and 70% riceberry. The physical quality of CVWR, considering crispness and calcium content, was determined using a linear regression equation. The analysis showed that CVWR with TB as the primary component had the highest calcium content and crispness, followed by FPC and CK, respectively. In terms of sensory characteristics, FPC had the highest fracture coefficient, followed by TB and CK, respectively. The optimal formulation, determined by overlapping diagrams, consisted of 14.88% FPC, 13.11% TB, 2.01% CK, and 70% riceberry. The resulting CVWR contained 286.19 mg/100g calcium and was a source of total dietary fiber and anthocyanin. Consequently, CVWR offers a healthier, high-nutritional-value snack option suitable for health-conscious consumers.

Keywords: mixture design, calcium, riceberry, vegetable crisps, Chinese kale, False pak choi, Thai basil

INTRODUCTION

Crispy waffles are currently among the most popular dessert products due to their convenience and ease of consumption. Crispy waffles typically contain wheat flour, eggs, butter, sugar, milk, emulsifiers, preservatives, and flavors. They are cooked using a waffle maker, resulting in a thin, round sheet with a distinctive pattern (**Asavarujanon *et al.*, 2022**). Overconsumption of crispy waffles can contribute to obesity and diabetes. Consequently, there is growing concern about inadequate nutrition and the burden of childhood malnutrition in many countries. The role of food processing in developing new, healthy foods or freshly prepared foods that are tasty and acceptable to consumers with high nutritional value, and low calorie, salt, and fat content is crucial, particularly in the vast snack market. The COVID-19 pandemic has led to lifestyle changes and increased interest in using fruits and vegetables as substitutes for chips and other snacks. This trend is expected to continue in the coming years. Consumers are seeking convenient, ready-to-eat snacks that focus on health, providing significant growth opportunities in the fruit and vegetable market. Both young people and health-conscious adults are willing to pay more for nutritious snacks. The Marketeer Online Thailand Snack Market Sales Report 2021 shows an 8.4% decline from 2019, as consumers look for snacks rich in vitamins and minerals. The demand for snacks containing nutrients such as calcium, fiber, and antioxidants is expected to increase. **Phoowachinnapong *et al.* (2017)** reported that Thai adults of both genders consumed an average of 361 mg of calcium daily, accounting for 30% of their daily calcium intake. Meanwhile, students at the Faculty of Public Health, Khon Kaen University, had an average calcium intake of 223 mg per day (27.9% of DRI), which is considerably low compared to the Thai Recommended Daily Intake (Thai RDI) 2020. The recommended daily allowance (RDA) for calcium is 800-1000 mg per day.
Calcium is an essential mineral for bone formation and maintenance. If the body does not receive enough calcium, it draws calcium from the bones. When calcium is regularly extracted, bones can become fragile, leading to osteoporosis symptoms **(Rangsipaht *et al.*, 2010).**
The sources of calcium include milk, yogurt, cheese, and fish with bones. However, some people are allergic to milk while some people are vegetarians.
In this study, researchers are interested in using plant-based calcium sources to create crispy waffles from vegetables and riceberry. The calcium content are high in three vegetables, Thai basil, Chinese kale, and False pak choi **(Narasri, 2021).** The researchers also used riceberry as a raw material for vegetable crisps production because it is a dark purple grain (*Oryza Sativa*) derived from a cross between the Hom Nin variety, a local non-glutinous purple rice, and Khoa Dawk Mali 105, known for its high antioxidant properties **(Poosri *et al.*, 2019). Settapramote *et al.* (2018)** studied riceberry from different locations in Thailand and found a quantitative composition of total polyphenol content ranging between 179.16-327.61 mgGAE/100 g. The percentages of elimination capacity were 39.25 to 71.54 for DPPH, 27.29 to 43.03 for ABTS, and 24.69–272.76 mg/100 g for anthocyanins. Nowadays, consumers are increasingly interested in consuming riceberry due to the essential substances mentioned above, which can help reduce inflammation caused by cancer, diabetes, and cardiovascular disease. Riceberry also contributes to improving the regenerative changes of the pancreas, kidneys, heart, and liver, and reducing high blood pressure **(Sirichokworrakit *et al.*, 2015)**. Numerous studies have explored the use of vegetables or riceberry in snacks, such as dried ivy gourd sheets **(Saencom *et al.*, 2011)**, black glutinous rice snacks **(Anukulwattana & Sukkasem, 2018)**, high-fiber puffed snacks made from sinlek rice flour, black sorghum flour, and inulin **(Kunna *et al.*, 2020)**, okra and water spinach sheets **(Saengthongpinit *et al.*, 2015)**, crispy fried lotus leaves **(Tanganurat *et al.*, 2018)**, wapee fruit leather **(Roskhrua & Kitchaicharoen, 2017)**, mixed mulberry bars with longans **(Thamee *et al.*, 2018)**, and riceberry snacks **(Kantrong *et al.*, 2018)**.
This study on crispy waffles from vegetables with riceberry used vegetables as main ingredients. It is necessary to pre-treat vegetables before food production to preserve their physicochemical value and achieve a soft texture for easier processing. Various pre-treatment technologies exist, including water blanching, steam blanching, chemical treatments, ultrasound, and high pressure. Water blanching and steam blanching methods are commonly used commercially due to their ease of use. **Sun *et al.* (2020)** investigated the effects of citric acid pre-

treatment, steam blanching at 100°C for 1–2 minutes, and water blanching at 95°C for 1–2 minutes on the vitamin C content of fresh potatoes. They found that the three pre-treatments had no effect on the drying rate of potatoes. **Liaotrakoon and Liaotrakoon (2020)** studied the pre-treatments of some indigenous vegetables before drying, focusing on the physical-chemical properties. They compared water blanching, immersion in potassium metabisulfite (KMS) solution, citric acid, and sodium hydroxide (NaOH) treatments to fresh vegetables. They found that water blanching resulted in the shortest drying time compared to chemical soaking (KMS, NaOH, and citric acid) but caused a greater color change in vegetables than in chemical soaking.
The objectives of this study were to investigate: 1) the pre-treatment (water blanching, steam blanching, and stir-frying with soybean oil) on the color and chemical composition of vegetables, and 2) the formulation ratios of three types of vegetables, namely Chinese kale, False pak choi, and Thai basil, for the production of calcium-fortified crispy vegetable waffles with riceberry. The study also aimed to assess the effects of these formulations on the physical, chemical, and sensory properties of the products using a mixture design.

MATERIAL AND METHODS

Raw materials and chemicals

Three types of vegetables were used: Chinese kale (CK; *Brassica alboglabra* L.H. Bailey), False pak choi (FPC; *Brassica rapa* L), and Thai basil (TB; *Ocimum basilicum* Linn). These were purchased from local sources in Suphanburi Province. Riceberry *(Oryza sativa* L.*)* was obtained from Central Food Retail Co., Ltd. Thailand. All other reagents and solvents used were of analytical grade.

Preparation of vegetables by pre-treatments

Vegetable processing is crucial for producing crispy waffles for consumption and as a food ingredient. It is necessary to preserve their pigments and chemical composition during vegetable processing. Additionally, the production of crispy waffles requires cooked vegetables with a soft texture for easy processing. The leaves of the three types of vegetables were washed and reduced in size. The different pre-treatments of vegetables involved three methods:
1. Steaming: A steamer basket was placed over boiling water. 50 g of vegetables were placed in the steamer basket. The lid was closed, and the vegetables were steamed at 100°C for 3 minutes.
2. Blanching with 1% NaCl: 500 ml of water was brought to a boil, and 5 g of NaCl (1% NaCl) was added. 50 g of vegetables were placed in the boiling water, the lid was closed, and the vegetables were boiled at 100°C for 3 minutes. Sodium chloride helps stabilize the chlorophyll and green color of vegetables, reduces the chlorophyll degradation rate, and prevents the change in the structure of chlorophyll into pheophytin (dark greenish-brown) **(Ezekiel *et al*., 2011).**
3. Stir-frying with soybean oil: 50 g of vegetables and 10 g of soybean oil were placed in a hot pan. The vegetables were stir-fried at 150°C for 3 minutes.
The treatments were designed experimentally using a Completely Randomized Design (CRD). All treatments were used for the analysis of calcium content, chlorophyll-A and chlorophyll-B, total polyphenol content, and color (L^*, a^*, b^*).

Physicochemical analysis of pre-treatments of vegetables

Calcium content: The calcium content was determined using atomic absorption spectrometry according to the **AOAC 2019 (985.35)** method.

Chlorophyll-A and Chlorophyll-B

Chlorophyll-A and chlorophyll-B were determined following the method described by **Rajput and Patil (2017).** Approximately 1 g of vegetable was mixed with 80% acetone, homogenized, and centrifuged at 5,000 rpm for 5 minutes. The supernatant was adjusted to 100 ml. After that, samples were measured with a UV-Vis spectrophotometer at 645, and 663 nm. Chlorophyll-A and chlorophyll-B were calculated using the following equations:
Chlorophyll-A mg/gsample=$[((12.7(A_{663})\text{-}2.69\ (A_{645})xV)/1000))]xW$
Chlorophyll-B mg/gsample=$[((22.9(A_{645})\text{-}4.68(A_{663})xV)/1000))]xW$
Where, A= Absorbance value; V=Final volume of chlorophyll extract from the sample in 80% acetone; W=Weight of the sample

Total polyphenol content

The total polyphenol content was determined using the Folin Ciocalteu colorimetric method **(Lu *et al*., 2007).** 1-2 g of the sample was extracted with 20 mL methanol and then centrifuged at 6,000 rpm for 30 minutes. Polyphenol analysis was performed by mixing 0.4 ml of sample extract, 2 ml of 10% Folin-Ciocalteu reagent, and 1.6 ml of 5% Na_2CO_3. The samples were incubated in the dark for 30 minutes at room temperature. After that, samples were measured with a UV-Vis spectrophotometer (model 1601; Shimadzu Corp; Kyoto, Japan) at 765 nm. The results of the total polyphenol content (TPC) were expressed as mg of gallic acid equivalent per 100g dry basis.

Color measurement

The color (L^*=black to white; a^* = green to red, and b^*= blue to yellow) of sample were measured using a Hunter colorimeter (Minolta Camera Co., Osaka, Japan).

Preparation of crispy vegetable waffles with riceberry (CVWR)

The CVWR was prepared using mixture design experiments for the three components. The proportions of the three components were 0-30% TB, 0-30% CK, and 0-30% FPC (WW), equivalent to a total of 30%, while riceberry was fixed at 70%. All materials were weighed and mixed at varying ratios as follows: T1 (5:20:5:70), T2 (0:30:0), T3 (10:10:10:70), T4 (20:5:5:70), T5 (0:15:15:70), T6 (5:5:20:70), T7 (0:0:30:70), T8 (15:0:15:70), T9 (15:15:0:70), and T10 (30:0:0:70). All treatments were used for the analysis of calcium content, moisture, Aw, texture (hardness and crispness), color, and sensory evaluation. The ingredients used to produce CVWR included riceberry, TB, CK, FPC, and riceberry. The flow diagram for the production was modified from **Asavarujanon, Ratanasumawong and Rumpagaporn (2022)** and presented in Figure 1.

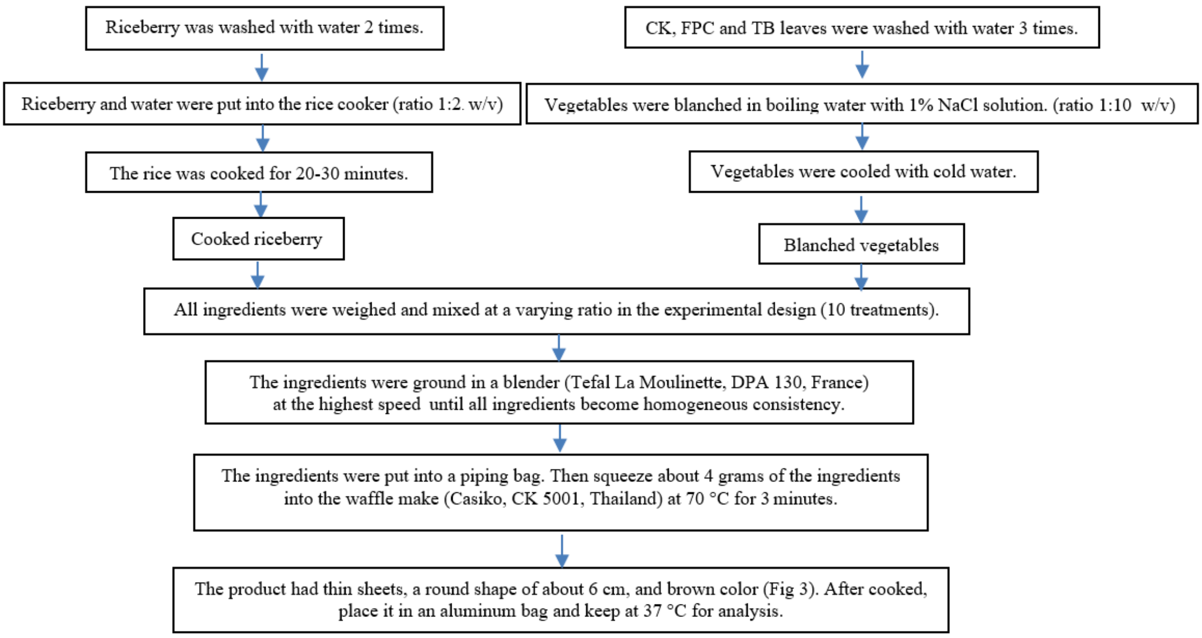

Figure 1 Process flow diagram for production of CVWR

Nutrition composition

Chemical composition was determined using the method of **AOAC (2019)**; total fat (922.06), protein (991.20), total carbohydrate (by calculation), total dietary fiber (by calculation), insoluble dietary fiber (991.42), soluble dietary fiber (993.19), sodium (985.35), vitamin B1(942.23), vitamin B2 (970.65), calcium (985.35), iron (985.27), ash (930.30), moisture (927.04) and vitamin A according to the method modified from **Kangsdalampai and Sungpuang (1984).**

Total polyphenol content (Similar to analysis of pre-treatments of vegetables)

DPPH free radical scavenging assay

The DPPH radical scavenging activity was determined using a modified method from **Katsuke *et al.* (2007).** The assay was performed by mixing 2 ml of sample extract and 2 ml of DPPH reagent (200μM). The solution mixtures were kept at room temperature for 30 minutes. After that, samples were measured with a UV-Vis spectrophotometer at 515 nm. The DPPH radical scavenging activity was expressed as mM of Trolox equivalent per100 g (mM Trolox/100g).

Antioxidant activity by FRAR assay

The FRAR assay was determined according to the method modified from **Benzie and Strain (1996)**. The stock solutions for analysis were prepared by mixing 300 mM acetate buffer pH 3.6, 20 mM $FeCl_3 6H_2O$, and 10 mM TPTZ in 10 mM HCL (10:1:1). The FRAP assay analysis was performed by mixing 50 ml of sample extracted and 50 ml of FRAP solution. The solution mixtures were kept at room temperature for 4 minutes. After that, samples were measured with a UV-Vis spectrophotometer at 593 nm. The FRAP value was expressed as μM of Trolox equivalent per 100g. (μM Trolox/100g).

Total anthocyanin

Total anthocyanin was determined according to the method modified from **Zhang *et al.* (2004)**. The stock solutions for analysis were performed by mixing water, methanol, and concentrated HCl (33:50:17). The anthocyanin analysis was performed by mixing 2.0 g samples and 10 ml of the stock solutions. The mixtures were sonicated for 20 min, and extracts were then placed in a boiling water bath for 1 h. Cooled extracts were centrifuged at 2000 rpm for 30 min. The sample extracts were collected for analysis. Total anthocyanin was analyzed using the calculated dilution factor 2 pH differential (pH 1 and 4.5). The first pH with 0.5 ml sample extracted and 4.5 ml pH 1.0 buffer (0.025 M KCl) and the other pH with 0.5 ml sample extracted and 4.5 ml pH 4.5 buffer (0.4 M CHOONa) Acetate). The mixtures were measured within 20 min for 2 pH solutions by using a spectrophotometer at 520 nm and 700 nm. The anthocyanin was expressed as cyanidin-3-glucoside equivalents were calculated using the following equation: ((AxMWxDFX1000)/eL).

Where: $A=(A_{520\ nm}-A_{700\ nm})\ pH_{1.0}-(A_{520\ nm}-A_{700\ nm})\ pH_{45,}$

Where: MW=449.2 g moL^{-1} for cyanidin-3-glucoside, DF=dilution factor, L=path-length (cm),

e=26,900 molar extinction coefficient, in L/mol cm for cyanidin-3-glucoside

10^3 =Factor for conversion from g to mg.

Color measurement (Similar to analysis of pre-treatments of vegetables

Textural analysis

Textural analysis The CVWR texture properties were measured using the Manual of TAXT plus Texture Analyzer (Stable Micro System Ltd., U.K.). The settings for hardness were as follows: probe: HDP/3PB, pre-test speed: 1.0 mm/s, test speed: 1.0 mm/s, post-test speed: 10.0 mm/s, distance: 3 mm, trigger type: 5 g, and data acquisition rate: 500 PPS. For crispness, the settings were: probe: P/O 255, pre-test speed: 1.0 mm/s, test speed: 1.0 mm/s, post-test speed: 10.0 mm/s, distance: 3 mm, trigger type: 5 g, and data acquisition rate: 500 PPS (method modified from **Saencom *et al.* (2011).** The force of each sample was recorded as data.

Sensory evaluation

The CVWR was packaged in plastic bags and served in 5 treatments each time, for a total of 10 treatments, to the panelists. The samples were evaluated by 50 panelists comprising Food Technology and Culinary Technology and service students at Suan Dusit University. The panelists evaluated the CVWR using a 9-point hedonic scale (1=disliked extremely, 9=liked extremely). The attributes evaluated were color, odor, taste, fracture, and overall liking. the approved questionnaire was examined for accuracy and appropriateness by the Ethical Review Subcommittee for Human Research at the Research and Development Institute of Suan Dusit University, COA.NO: SDU-RDI-HS 2022-002 (Subprotocol No: HS003/2022).

Microbiological properties

The microbial study of the sample was conducted according to the standard methods of **BAM online (2022)**. Total plate count (Chapter 3), Yeast and Mold (Chapter 18), *Escherichia coli* (Chapter 4), *Salmonellae* spp. (ISO 6579-2002), *Staphylococcus aureus* (Chapter 12), *Bacillus cereus* (Chapter 14), and *Clostridium perfringens* (Chapter 16) were assessed.

Statistical analysis

Statistical analysis was performed using SPSS Statistic Version 20.0 (SPSS Inc., Chicago, IL, USA). To compare the results Duncan's multiple range test one-way ANOVA analysis was used. The statistically significant level for all tests at $p<0.05$. The data of crispy waffles from vegetables with riceberry were analyzed by physicochemical properties and sensory analysis using the Response Surface Methodology (RSM) by linear regression model ($Y=\beta A+\beta B+\beta C$). The data from the linear regression model was then used to determine the optimal suitable mixture from the overlay plot by employing Design Expert (Version 13.0.9.0), Design Type: I-optimal, Design Model.

RESULTS AND DISCUSSION

Physicochemical of pre-treatments of vegetables

The effect of pre-treatments on three types of vegetables (Chinese kale, False pak choi, and Thai basil) was studied prior to producing the crispy waffles from vegetables with riceberry. Three pre-treatments, including water blanching, steam blanching, and stir-frying with soybean oil, were compared to fresh vegetables. The results for calcium content, chlorophyll-A and chlorophyll-B, total polyphenol content, and color (*L**, *a**, *b**) are shown in Table 1.

Table 1 Different pre-treatments on physicochemical of Chinese kale, False pak choi, and Thai basil

Treatments	Calcium (mg/100g)	Total Chlorophyll (mg/100g)	Chlorophyll A (mg/100g)	Chlorophyll B (mg/100g)	Total polyphenol content (mg eqGA/100g)	Color		
						*L**	-*a**	*b**
Chinese kale (CK)								
Fresh	246.15±0.99 ^{a}	84.81±0.52^{a}	53.47±0.32^{a}	31.34±0.21^{a}	176.82±3.03^{a}	36.45±0.23 ^{a}	-2.63±0.17^{d}	24.17±0.06^{b}
Blanching	207.02±1.40^{c}	64.49±0.50^{d}	43.91±0.15^{d}	20.59±0.35^{c}	158.70±1.69 ^{c}	33.51±0.17^{b}	-3.83±0.11^{c}	29.78±0.18^{a}
Steaming	230.56±0.84^{b}	68.05±0.41^{c}	46.87±0.30^{c}	21.18±0.11^{c}	164.98±1.23 ^{b}	30.28±0.09^{c}	-4.31±0.05^{b}	29.54±0.24^{a}
Stir-frying	194.63±1.12^{d}	76.45±0.48^{b}	51.20±0.22^{b}	25.25±0.25^{b}	166.31±1.13 ^{b}	25.86±0.03^{d}	-5.52±0.04^{a}	19.66±0.23^{c}
False pak choi (FPC)								
Fresh	309.88±1.60^{a}	85.25±0.57^{a}	54.87±0.20^{a}	30.38±0.37^{a}	204.85±2.66^{a}	40.07±0.08 ^{a}	-2.38±0.09^{d}	24.40±0.15^{b}
Blanching	281.99±1.39^{c}	63.70±0.48^{d}	45.26±0.22^{d}	18.44±0.26^{c}	177.32±3.26^{b}	35.87±0.16^{b}	-3.12±0.08^{c}	28.96±0.07^{a}
Steaming	292.08±1.31^{b}	69.82±0.53^{c}	48.03±0.30^{c}	21.78±0.23^{b}	179.74±3.48^{b}	34.57±0.08^{c}	-3.47±0.06^{b}	28.72±0.09^{a}
Stir-frying	227.56±1.44^{c}	81.68±0.59^{b}	52.06±0.35^{b}	29.62±0.23^{a}	203.82±2.57^{a}	26.77±0.17^{d}	-4.36±0.13^{a}	20.45±0.11^{c}
Thai basil (TB)								
Fresh	313.95±1.26^{a}	110.98±0.47^{a}	71.86±0.34^{a}	39.16±0.13^{a}	710.68±1.06 ^{a}	32.69±0.09 ^{a}	-2.59±0.14^{c}	24.58±0.06^{c}
Blanching	288.66±1.32^{c}	84.34±0.48^{d}	58.59±0.32^{b}	25.76±0.17^{d}	698.13±2.40 ^{c}	29.27±0.14^{b}	-3.18±0.06^{b}	29.52±0.06^{a}
Steaming	306.19±0.88^{b}	94.38±0.53^{c}	59.09±0.23^{b}	35.28±0.30^{c}	703.16±1.49 ^{b}	26.58±0.15^{c}	-3.37±0.07^{b}	27.44±0.18^{b}
Stir-frying	224.89±0.89^{d}	109.49±0.36^{b}	71.29±0.24^{a}	38.20±0.12^{b}	706.24±1.15 ^{ab}	23.39±0.10^{d}	-4.47±0.13^{a}	18.54±0.29^{d}

Legend: Difference letters in the same column indicate that the values are significant difference ($p<0.05$), one-way ANOVA.

Calcium content

Calcium plays an essential role in building bones and protecting against osteomalacia. Sources of calcium include milk, cheese, other dairy foods, and green leafy vegetables. Therefore, this research selected green leafy vegetables that are sources of calcium, namely Thai basil, Chinese kale, and False pak choi. Analysis of calcium content found that the highest concentration of calcium was in TB (313.95 mg/100 g), followed by FPC (309.88 mg/100 g) and CK (246.15 mg/100 g) respectively **(**Table 1). Among the three pre-heating methods, blanching reduced the calcium content the most in vegetables compared to steaming, as some of the calcium dissolves in water. The stir-frying method also reduced the calcium content in vegetables due to direct heat causing calcium decomposition.

Chlorophyll-A and Chlorophyll-B

Chlorophyll is the primary pigment, consisting of chlorophyll-A and chlorophyll-B, and is an essential parameter for evaluating vegetables. The three pre-treatment methods for all three vegetables showed that chlorophyll-A and chlorophyll-B contents were reduced in all pre-treatments compared to fresh vegetables, with significant differences (p<0.05) (Table 1). This reduction is due to losses during processing, such as dissolving in boiling water or heat from the stove. Another reason is that chlorophyll-A and chlorophyll-B can degrade during thermal processing to form pheophytins and pyropheophytins. Pheophytins are formed from the displacement of the central magnesium atom from the chlorophyll porphyrin ring with hydrogen **(Belitz *et al*., 2009).** Chlorophyll-A, with its dark green color due to a methyl group (-CH_3) side chain at position 3, is highly soluble in oil. Comparing the three pre-heating methods using water and oil, it was found that using oil in stir-frying vegetables resulted in higher chlorophyll-A content than both of the other methods, as chlorophyll-A is released from the cell wall of chloroplasts by oil to bind more prominently to the vegetable surface. In contrast, chlorophyll-B, with its yellowish-green color, is more soluble in water due to its aldehyde group (-CHO) making it more polar and soluble. Blanching vegetables causes the boiled water to have a light green color that dissolves, resulting in blanched vegetables having a light green color from the dissolved chlorophyll. As shown in Table 1, blanching with 1% NaCl reduced chlorophyll-B content more than both other methods.

Total polyphenol content

The reduction in total polyphenol content may be attributed to the leaching of total polyphenol content from the vegetable tissues into water. Consequently, the total polyphenol content was highest in stir-fried treated vegetables, followed by steaming and blanching, respectively. The total polyphenol content of vegetables decreased after blanching and steaming due to the heat received from both methods, which can destroy the structure of the total polyphenol content. The loss of total polyphenol content in blanching with NaCl was greater than that in steam blanching, likely due to the loss of total polyphenol content in the boiling water. In contrast, the stir-frying method resulted in an increase in total polyphenol content compared to fresh vegetables, as soybean oil was used in stir-frying.

Color measurement

The effect of pre-treatment on vegetable color is indicated in Table 1. All three pre-treatments resulted in decreased $L*$ (lightness) values and increased $b*$ (green) values compared to fresh vegetables. Stir-frying had the lowest $L*$ (lightness) value (darker color), followed by steaming and blanching with 1% NaCl, respectively. Stir-frying also resulted in the highest -$a*$ (green) value, while having the lowest $b*$ (yellow) value. The color values correspond to the chlorophyll content (Table 1). Vegetables appear greener after pre-treatment because fresh vegetables have air between the cell walls of chloroplasts, which can obscure the color of the vegetables. When vegetables are heated or exposed to hot water, the air in the vegetable cells is released, making the vegetables appear more prominently green. On the other hand, blanching with 1% NaCl stabilizes the chlorophyll and green color of vegetables, but some green color dissolves in the water, causing the greenness of the vegetable to decrease and the brightness to increase.

The vegetables were pre-treated prior to producing the crispy waffles using three methods: blanching, steaming, and stir-frying with soybean oil. All the physicochemical data showed that steaming had the least reduction in calcium content, followed by blanching and stir-frying, respectively. Stir-frying had the least reduction in total chlorophyll and total polyphenol contents, followed by steaming and blanching, respectively. However, stir-frying using vegetable oils results in a greasy odor and may be unhealthy for consumers. Comparing steaming and blanching for equal treatment time (3 minutes), blanching resulted in softer and more tender vegetable texture than steaming, as water as a heating medium weakens cell membranes, making vegetables easier to blend. Additionally, **Chinsan and Chinsan (2018)** studied blanching vegetables using 1% NaCl to inhibit the peroxidase enzyme, as NaCl has oxidizing properties and inhibits browning reactions. Blanching can also destroy and reduce microorganisms contaminating the surface of vegetables in boiling water. For these reasons, blanching was determined to be the most suitable pre-treatment for the production of crispy waffles from vegetables with riceberry.

Physicochemical properties of CVWR

This study investigated the effects of color, moisture, water activity, calcium, hardness, and crispness on CVWR (Table 2) using an extreme vertices design of experimental mixtures for the three components (CK, FPC, and TB,), which together made up 30% of the mixture, with riceberry at a fixed rate of 70% for the testing.

Table 2 Physicochemical properties of CVWR

Treatments	Color			Moisture (%)	Water activity	Calcium (mg/100g)	Hardness (g force)	Crispness (g force)
	L*	***a****	***b****					
T1	51.79±0.20^{a}	4.61±0.10^{a}	12.23±0.03^{a}	3.63±0.08^{b}	0.267±0.00^{a}	292.21±1.73^{ab}	213.08±2.67^{f}	108.72±0.23^{d}
T2	50.29±0.15^{c}	4.56±0.08^{ab}	12.14±0.05^{ab}	3.86±0.07^{a}	0.255±0.00^{b}	242.19±1.97^{e}	284.52±2.17^{c}	144.11±1.34^{c}
T3	51.69±0.30^{a}	4.45±0.08^{bc}	11.16±0.36^{e}	2.86±0.09^{de}	0.223±0.00^{e}	289.37±3.24^{ab}	235.10±2.45^{c}	153.66±1.29^{b}
T4	50.52±0.32^{bc}	4.36±0.07^{c}	10.73±0.26^{f}	2.75±0.04^{ef}	0.218±0.00^{ef}	283.86±1.81^{b}	258.07±3.83^{d}	154.22±5.23^{b}
T5	50.62±0.37^{bc}	4.38±0.07^{c}	11.92±0.05^{bc}	3.21±0.08^{c}	0.236±0.00^{d}	255.13±1.26^{d}	327.71±1.39^{a}	224.15±4.04^{a}
T6	50.41±0.34^{bc}	4.57±0.08^{ab}	11.81±0.09^{c}	2.72±0.02^{ef}	0.245±0.00^{c}	260.90±3.73^{d}	207.94±0.27^{f}	142.68±0.78^{c}
T7	50.63±0.54^{bc}	4.65±0.17^{a}	10.92±0.40^{ef}	3.21±0.09^{c}	0.248±0.00^{c}	264.34±8.55^{cd}	207.51±1.73^{f}	150.95±0.24^{b}
T8	50.85±0.38^{b}	4.53±0.03^{ab}	11.81±0.09c	2.52±0.08^{g}	0.216±0.00^{f}	296.71±5.87^{a}	293.22±5.67^{b}	157.18±1.97^{b}
T9	50.80±0.14^{b}	4.46±0.46^{bc}	10.49±0.03^{g}	2.89±0.10^{d}	0.223±0.00e	263.57±4.58^{bc}	320.80±1.29^{a}	157.79±2.50^{b}
T10	50.42±0.24^{bc}	4.62±0.06^{a}	11.90±0.07^{bc}	2.68±0.07^{e}	0.233±0.00^{d}	271.91±0.01^{c}	283.73±5.30^{c}	222.48±5.85^{a}

Legend: Difference letters in the same column indicate that the values are significant difference (p<0.05), one-way ANOVA.
Materials were Thai basil: Chinese kale: False pak choi and riceberry and mixed at a varying ratio as follows: T1 (5:20:5:70), T2 (0:30:0:70), T3 (10:10:10;70), T4 (20:5:5:70), T5 (0:15:15:70), T6 (5:5:20:70), T7 (0:0:30:70), T8 (15:0:15:70), T9 (15:15:0:70), and T10 (30:0:0:70)

Color

Table 2 shows the color measurements for the 10 CVWR formulas, which included values for $L*$ (black to white), $a*$ (red), and $b*$ (yellow) within the ranges of 50.29-50.85, 4.36-4.65, and 10.73-12.23, respectively. Each formula consisted of a mixture of 70% riceberry and 30% green leafy vegetables blended from TB, CK, and FPC. When measuring the color value of crispy waffles made from 100% riceberry, the $L*$ value was darker at 40.23, with $a*$ at 6.61 and $b*$ at 13.45. This was due to the brownish and dark purple color of riceberry, which made the CVWR products darker, as well as more red and yellow when mixed with vegetables. Another factor was the 70% riceberry content in the CVWR. This finding aligns with previous research on black glutinous rice snacks, which reported an $L*$ value of 40.36 **(Anukulwattana & Sukkasem, 2018),** and puffed snacks made from black sorghum flour **(Saengthongpinit *et al*., 2020). Pumilia *et al.* (2004)** suggested that high heat can convert chlorophyll to pheophytin, turning the original green vegetables brownish-green or brown. This finding is consistent with **Saengthongpinit *et al*. (2015)**, who baked okra and water spinach at three temperature levels (60, 70, and 80°C) and found that the $a*$ and $b*$ color values increased with higher temperatures and longer baking times. Moreover, **Tanganurat *et al.* (2015)** studied lotus leaves and found that the frying oil temperature transformed the chlorophyll into brown, resulting in crispy lotus leaf chips with increased $a*$ and $b*$ color values.

Moisture content and water activity

The moisture content and water activity of the 10 CVWR treatments (Table 2) were analyzed, revealing a moisture content range of 2.52-3.86%. Meanwhile, water activity values ranged from 0.216 to 0.267. These values were lower than 10% for moisture content and less than 0.6 for water activity, effectively inhibiting microbial growth according to **Larrauri (1999).**

Calcium

The 10 CVWR treatments contained varying calcium levels, depending on the composition of CK, FPC, and TB, which had calcium contents of 246.15-313.96 mg/100g. Riceberry did not contain calcium, so the mixture of the three green vegetables increased the nutritional value by contributing calcium. The analyzed results showed that the calcium content of the 10 CVWR treatments ranged between 242.19-296.71 mg/100g. The highest calcium content was found in T8 (15% TB and 15% FPC) at 296.71 mg/100g, which was not statistically different from T1 (5% TB, 20% CK, and 5% FPC) and T3 (10% TB, 10% CK, and 10% FPC) with calcium contents of 292.21 and 289.37 mg/100g, respectively. The calcium content of CVWR in T8 aligns with Table 1, indicating the highest calcium content in TB, followed by FPC and CK, making it suitable for manufacturing crispy waffles. The Thai Recommended Daily Intakes (Thai RDI) 2020, issued by the Bureau of Nutrition, Department of Health, and Ministry of Public Health, recommend a daily allowance (RDA) of 800-1000 mg of calcium.

Texture

The texture analysis results for the 10 CVWR formulas showed hardness and crispness values, as presented in Table 2. Hardness is a texture property, and its value was determined through compression and penetration testing with a texture analyzer, measuring the maximum compressive force (N) required to overcome the samples' drag force. The findings revealed hardness values ranging from 207.94 to 327.71 g force and crispness values ranging from 108.72 to 224.15 g force. T5, comprising 15% CK and 15% FPC, exhibited the highest product hardness at 327.71 g force. T9 (15% TB and 15% CK) followed with a hardness value of 320.80 g force. No significant differences (p>0.05) were observed compared to T8 (15% TB and 15% FPC), which displayed a significant difference (p<0.05). When comparing the hardness value to that of crispy waffles made from 100% riceberry, it was found that the hardness value was higher (572 g force) than that of CVWR (207.94-327.71 g force). The product hardness was influenced by the varying fiber contents of the three vegetables; insoluble fiber also contributed to the products' hard texture. Fiber contents in CK, FPC, and TB were 2.91, 2.69, and 1.88%, respectively, corresponding to T5 (15% CK and 15% FPC).

Crispness is a texture property measured by a crispness texture analyzer. Breaking the samples with different characteristics produced sounds associated with crispness. Crispness is related to humidity; low humidity results in higher crispness, while high humidity can cause a product to lose its crispiness due to toughness. Desired crispiness depends on consumer acceptance. Experiments on the crispness of CVWR revealed that T5 (15% CK and 15% FPC) and T10 (30% TB) had the highest values at 224.15 and 222.48 g force, respectively, with no significant difference (p>0.05). However, both treatments were significantly different (p<0.05) compared to T3, T4, T7, T8, and T9. When comparing the crispness value to that of crispy waffles made from 100% riceberry, it was found that the crispness value was higher (254 g force) than that of CVWR (108.72-224.15 g force). This was a result of riceberry's 15.3% amylose content, low swelling power, and high gelatinization temperature **(Thiranusornkij, 2019).** A strong dough was formed from the closely arrangement of amylose chains. When the riceberry dough was heated by the waffle maker, it cooked the riceberry and reduced the moisture content, resulting in a crispy and hard texture for the product.

Sensory Evaluation p>

The sensory evaluation results of CVWR, assessing color, odor, taste, fracture, and overall preference, are presented in Table 3. No statistically significant differences were found in color and fracture across all treatments (p≥0.05). However, T6 (5% TB, 5% CK, and 20% FPC) demonstrated the highest odor, taste, and overall preference values at 7.12, 6.88, and 7.18, respectively. This outcome was due to consumer feedback indicating that Thai basil has a spicy flavor, while Chinese kale has a slightly bitter taste. T6 had the highest ratio of FPC (20%), with TB and CK having equal treatment (5%). Consequently, consumers found this treatment more acceptable than the other treatments.

Table 3 Sensory evaluation of CVWR

Treatments	Color ^{ns}	Odor	Taste	Fracture ^{ns}	Overall liking
T1	6.44±0.84	6.68±0.89^{bc}	6.70±1.17^{ab}	7.12±1.17	6.84±0.98^{ab}
T2	6.58±0.86	6.66±0.77^{bc}	6.02±1.38^{de}	7.02±1.38	6.66±0.87^{bc}
T3	6.44±0.84	6.32±0.79^{cd}	5.92±1.00^{e}	6.98±1.00	6.34±0.80^{c}
T4	6.38±1.10	5.98±1.30^{d}	6.12±1.10^{de}	7.06±1.10	6.32±1.02^{c}
T5	6.70±1.27	6.58±0.99^{bc}	6.64±1.09^{abc}	7.40±1.09	6.88±0.80^{ab}
T6	6.74±1.12	7.12±1.02^{a}	6.88±1.00^{a}	7.34±1.00	7.18±1.08^{a}
T7	6.54±1.30	6.78±1.06^{ab}	6.76±1.05^{a}	7.10±1.05	6.88±1.15^{ab}
T8	6.58±1.14	6.04±1.01^{d}	6.38±0.95^{bcd}	7.28±0.95	6.52±0.86^{bc}
T9	6.68±1.10	6.30±0.93^{cd}	6.30±1.27^{cd}	6.98±1.27	6.52±0.91^{bc}
T10	6.72±1.16	5.96±1.01^{d}	6.34±0.98^{cd}	7.06±0.98	6.48±0.91^{c}

Legend: Difference letters in the same column indicate that the values are significant difference (p < 0.05), one-way ANAOVA.
ns : not significant
Materials were Thai basil: Chinese kale: False pak choi and riceberry and mixed at a varying ratio as follows: T1 (5:20:5:70), T2(0:30:0:70), T3 (10:10:10;70), T4 (20:5:5:70), T5 (0:15:15:70), T6 (5:5:20:70), T7 (0:0:30:70), T8 (15:0:15:70), T9 (15:15:0:70), and T10 (30:0:0:70)

Mixture Optimization

Researchers statistically analyzed the physicochemical and sensory evaluation results from the 10 treatments of vegetable crisps containing different ratios of TB, CK, FPC, and riceberry using the Extreme Vertices Design of mixture experiments for the three components. The proportions of the three components were 0-30% TB, 0-30% CK, and 0-30% FPC (WW), equivalent to a total of 30%, with riceberry fixed at 70%. Regression equations were used to create a mathematical model to determine the relationships between the proportions of the vegetables affecting quality in various aspects. The R^2 ranged between 64.44-88.79 (Table 4). Aiming for the highest calcium, crispness, and sensory characteristics, the software analyzed a set of TB, CK, and FPC (A, B, and C) proportions. The optimized mixture would provide the ideal balance of these properties to produce the best quality crispy waffles. The results of the optimization can be used to guide the production of vegetable crisps with the desired characteristics and consumer acceptance.

Table 4 Equation regression forecast the optimal formula CVWR mixed TB, CK, FPC, and riceberry for attribute physical properties and sensory evaluation for their effect

Dependent variable	Predictive Model	R^2	Probability
Crispness	$Y=224.23A+140.81B+150.95C-167.25AB-121.65AC+314.34BC+4504.72A^2BC-5938.83AB^2C$	88.79	0.0412
Moisture	$Y = 2.45A+3.29B+2.82C$	73.82	0.0005
Water activity	$Y = 0.23A+0.26B+0.26C-0.27AB(A-B)$	68.25	0.1357
Calcium	$Y = 272.77A+243.05B+264.34C+112.62AC$	69.22	0.1495
Fracture	$Y=7.04A+7.04B+7.10C+0.83AC+1.31BC-34.50 A^2BC$	87.98	0.0299
Overall liking	$Y=6.50A+6.68B+6.99C$	64.44	0.0740

Legend: A = Thai basil, B = Chinese kale, C= False pak choi

The linear regression equation for the physical quality of CVWR was analyzed in terms of crispness and calcium content. The findings revealed that the calcium content of CVWR containing Thai basil (TB) as the main ingredient had the highest coefficient, followed by False Pak Choi (FPC) and Chinese kale (CK), respectively. This is in line with the observation that TB had the highest calcium content. In terms of crispness, the CVWR containing TB as the primary ingredient had the highest coefficient, followed by FPC and CK, respectively. This suggests that the amounts of TB and FPC significantly influenced the crispness of the vegetable crisps made with riceberry. Regarding sensory characteristic fractures, the crispness of CVWR with FPC as the main ingredient had the highest coefficient, followed by TB and CK, respectively. This further indicates that the quantities of TB and FPC greatly impacted the crispness of the vegetable crisps made with riceberry. Finally, the moisture and water activity of CVWR containing CK as the primary ingredient had the highest coefficient, followed by FPC and TB, respectively.

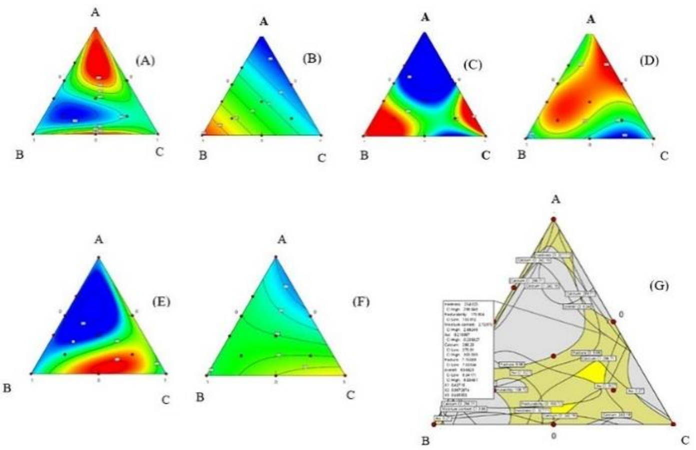

Figure 2 Contour plots for the effect of different combinations of TB(A), CK(B), and FPC(C) on (A) crispness, (B) moisture, (C) water activity (D) calcium (E) fracture (F) overall liking, and (G) overlay plot

Figure 2 illustrates the overlapping areas suitable for TB, CK, and FPC, which were combined with riceberry to create the CVWR. The optimal percentages of selected ingredients were as follows: 13.11% TB, 2.01% CK, 14.88% FPC, and 70% riceberry. It had thin sheets, round shape about 6 cm, and brown color (Fig 3). The nutritional value, chemical value, and microbiological qualities of vegetable crisps and riceberry were then analyzed using this formula (Table 5). The nutritional value of the CVWR had a moisture content of 4.82% and water activity at 0.33, which met the requirements of the Thai Community Product Standard 523/2563, where water activity must be below 0.6. This finding is consistent with **Larrauri (1999)**, who stated that product moisture content below 10% and water activity below 0.6 inhibit the growth of microorganisms.

The calcium content of the CVWR, at 286.19 mg/100g, was higher than that of crispy cricket products (149 mg/100g) **(Promkhan *et al*., 2020)** but lower than vegetable sheets made from okra and water spinach (353.70-332.12 mg per 100g), which primarily contain vegetables **(Saengthongpinit *et al*., 2015).** The Thai Recommended Daily Intake (Thai RDI) 2020 specifies a recommended calcium intake of 800 mg/day for Thais aged 6 years and older (refer to Account No. 3, Order 20). According to nutritional claim guidelines for nutrition labels in the notification of the Ministry of Public Health (No. 182, 1998), Account No. 4, Page 162, nutrients claiming calcium content must contain at least 10% of the calcium in the Thai RDI or more. A 35g serving of CVWR (one box) contains 10% of the Thai RDI for calcium, enabling it to be claimed as a source of calcium.

Regarding the nutritional value of fiber, the CVWR contained 7.07% fiber, which consisted of 5.68% insoluble dietary fiber and 1.38% soluble dietary fiber. This surpasses the fiber content found in similar snack products, such as riceberry snacks (0.2%) **(Kantrong *et al*., 2018),** black glutinous rice snacks (0.21%) **(Anukulwattana & Sukkasem, 2018)**, mulberry-longan mixed bars (0.42%) (Thamee et al., 2017), and puffed snacks made from sinlek rice flour, black sorghum flour, and inulin (3.32–4.95%) **(Kunna *et al*., 2020).** According to the Thai RDI, a product must be rich in dietary fiber, with fiber content of at least 6 g per 100 g of food (refer to Account No.3, Page 173). Therefore, 100 g of CVWR contains 7.07 g of fiber.

As previously mentioned, a product with high nutritional value in terms of vitamins and minerals must contain no less than 10% of the Thai Recommended Daily Intakes (Thai RDI*), including 10% of Vitamin A and 10% of iron (Table 5). Consequently, CVWR is rich in Vitamin A, iron, and B1. In addition, the CVWR contains 22.43 mg gallic acid/100g of total polyphenol compounds, 269.54 mmol TE/100g of DPPH free radical scavenging activity, 2887.26 µmol TE/100g of FRAP free radical scavenging activity, and an anthocyanin content of 6.50 mg cyanidin-3-glucoside/100g. All these compounds play significant roles in defending the body against free radicals, reducing inflammation and cholesterol, and inhibiting viruses.

Moreover, this study has demonstrated that incorporating vegetables and riceberry in crispy waffles enhances their nutritional value in terms of high calcium, total dietary fiber, total polyphenol content, antioxidant activity (DPPH), antioxidant activity (FRAP), and anthocyanin. On the other hand, this product has the advantage of providing low energy from fat, total fat, and total carbohydrates. In contrast, the general crispy waffle formulas contain four main ingredients: wheat flour, eggs, butter, sugar, and milk. **Asavarujanon, Ratanasumawong and Rumpagaporn (2022)** found that these general crispy waffles had high energy (478.42 kcal/100g), total fat (18.20 g/100g), and total carbohydrates (68.43 g/100g) while being low in total dietary fiber (3.23 g/100g) and total polyphenol content (1.12 mg gallic/g).

Table 5 Nutrient content of the CVWR and nutrition information for serving 35 g/box

Properties	Values	Nutrition Information (Serving size: 1 box (35g)	
			Percent Thai RDI*
Energy (Kcal)	390.9±0.54	140 Kcal	-
Energy from fat (Kcal/100g)	34.83±0.76	10 Kcal	-
Total fat (g/100g)	3.87±0.08	1.5 g	2%
Saturated fat (g/100g)	0.00	0 g	0%
Cholesterol (mg/100g)	0.00	0 mg	0%
Protein (g/100g)	12.29±0.01	4 g	
Total carbohydrate (g/100g)	76.74±0.06	27 g	9%
Total dietary fiber (g/100g)	7.07±0.04	2 g	8%
Insoluble dietary fiber (g/100g)	5.68±0.04	-	-
Soluble dietary fiber (g/100g)	1.38±0.01	-	-
Sugar (g/100g)	0.00	0 g	-
Sodium (mg/100g)	37.11±0.75	15 mg	1%
Vitamin A (µg/100g)	287.25±4.21	-	10%
Vitamin B1 (mg/100g)	0.31±0.01	-	8%
Vitamin B2 (mg/100g	0.07±0.01	-	0%
Calcium (mg/100g)	286.19±2.01	-	10%
Iron (Fe) (mg/100g)	4.21±0.02	-	10%
Ash (g/100g)	2.29±0.01	-	-
Moisture (g/100g)	4.82±0.02	-	-
Total polyphenol content (mg gallic/100g)	822.42±8.82	-	-
DPPH Assay (mM TE/100g)	269.54±1.93	-	-
FRAP Assay (µM TE/100g)	2887.26±21.41	-	-
Anthocyanin (mg cyaniding-3-glucoside/100g)	6.50±0.10	-	-

Legend: *Percentage of Thai Recommended Daily intakes for the population over 6 years of age are based on 2,000 kcal diet

Figure 3 Crispy vegetable waffles with riceberry (14.88% FPC, 13.11% TB, 2.01% CK, and 70% riceberry)

Table 6 Sensory evaluation and consumer acceptance of crispy vegetable waffles with riceberry

Sensory evaluation	Result
Color	7.26±0.70
Odor	7.04±0.69
Taste	6.90±0.87
Fracture	7.22±0.68
Overall liking	7.33±0.67

Consumer sensory testing is essential for evaluating new food products to guide product development and product improvement. Sensory evaluation in consumer acceptance crispy vegetable waffles with riceberry were evaluated by 100 panelists. They evaluated the crispy waffles using a 9 point-hedonic scale (1=disliked extremely, 9=liked extremely). The attributes evaluated were color, odor, taste, fracture, and overall liking values at 7.26, 7.04, 6.90, 7.22 and 7.33, respectively (Table 6). The results showed that consumers accepted the product moderately.

The microbial qualities of the product, revealing that the aerobic plate count was $7.4x10^2$ cfu/g, Yeast and Mold were <10 cfu/g, *Escherichia coli* <3 cfu/g, no growth of *Salmonellae* spp, *Staphylococcus aureus* <10 cfu/g, *Bacillus cereus* <100 cfu/g, and *Clostridium perfringens* <100 cfu/g. Consequently, this product meets the standard criteria for microbial qualities in accordance with the Thai Community Product Standard, as referenced by the Sticky Rice Cracker Products (Khaitane) code number 36/2562.

CONCLUSION

The three types of vegetables; Thai basil, Chinese kale, and False Pak Choi were pre-treated prior to producing the crispy waffles by three methods i.e., blanching, steaming, and stir-frying with soybean oil. The reasons blanching was a suitable pre-treatment for the production of crispy waffles from vegetables with riceberry were 1) blanching vegetables by using NaCl at 1% inhibited peroxidase enzyme, which NaCl has properties as an oxidizing agent and inhibits the brown reaction. Blanching can destroy and reduce the microorganisms contaminating the surface of vegetables in boiling water 2) blanching results in softer and more tender the texture of vegetables due to water as a medium for heating to weaken cell membranes, resulting in easy blending of vegetables 3) stir-frying using vegetable oils result in a greasy odor, and is unhealthy for consumers, if used to make products, it causes rancidity. The simplex centroid mixture design was used for the optimum formulation of vegetable crisps with riceberry. Regression models were established for apparent hardness, fracturability, moisture, water activity, calcium, fracture in sensory characteristics, and overall liking in sensory attributes. At the same time, triangular contour diagrams indicated the effects of the variables on response. The combination of 14.88 % False Pak Choi, 13.11% Thai basil, 2.01% Chinese kale, and 70% cooked riceberry is proposed as the optimized formulation that satisfies all expectations. In addition, the present study also showed high calcium content at 123.05 mg/100g, total dietary fiber of 7.07 g/100g, total polyphenol content of 822.43 mg gallic/ 100g, antioxidant activity (DPPH) of 269.54 mM TE/100g, and anthocyanin of 6.50 mg cyaniding-3-glucoside/100g.The microbial qualities of the product met the standards of Thai Community Products code number 36/2562. Therefore, crispy vegetable waffles with riceberry are suitable for entrepreneurs in the food processing industry, particularly the snack food industry, targeting consumers who are health-conscious snack lovers looking to increase the intake of calcium fiber, and anthocyanin. Inaddition, they are gluten-free, low sodium, low fat, low energy, contain no sugar, and no cholesterol. Future works should study the shelf life of the product.

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