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Effect of Partial Replacement of Wheat Flour with Whole Leaf and Chloroplast-Rich Fraction from Thai Jasmine Rice Grass on Nutritional and Physicochemical Properties of Cookies

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Abstract
Nowadays, the nutritional value of cookies is relatively low. To reduce wheat flour usage in the cookies, the freeze-dried whole leaf (WL) and chloroplast-rich fraction (CRF) derived from Thai jasmine rice grass were used as a food ingredient to increase the nutrients of cookies. Chloroplast is a rich source of macro and micronutrients. Thus, the use of chloroplast might improve the nutrients in food products. This research aimed to study the physical and chemical characteristics of freeze-dried WL and CRF from Thai jasmine rice grass. The nutritional and physical properties of the butter cookies substituted wheat flour with WL or CRF (0%, 2%, and 4% (w/w)) were also investigated. The result showed that CRF derived from Thai jasmine rice grass contained a significantly larger amount of macro and micronutrients (protein, lipid, ash, β-carotene, and total carotenoids) compared with WL ($P \leq 0.05$), except for carbohydrate, fiber, total chlorophyll, and total phenolic compound. Furthermore, the cookies replaced wheat flour with 4% WL from Thai jasmine rice grass, containing the highest fiber led to a decreased spread ratio and increased the hardness of cookies. Whilst

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the cookies substituted with 4% CRF (w/w) were similar in texture to the cookies containing only wheat flour (P>0.05). Moreover, the replacement of wheat flour with freeze-dried WL and CRF derived from Thai jasmine rice grass enhanced nutrients and antioxidant activity compared with cookies in the absence of WL and CRF. The butter cookies with 4% replacement of WL showed the highest level of chlorophyll and total phenolic; the cookies with 4% replacement of CRF contained the largest amount of β-carotene (provitamin A) and total carotenoids. However, both cookies containing either WL or CRF were not significantly different in antioxidant activity (P>0.05).

Introduction

Cookies have become a widely consumed snack product due to their convenience, such as easy storage and ready-to-eat, affordability, and relatively stable shelf life.[1] As a result, people of all ages prefer them. The cookies are rich in wheat flour, butter, and sugar but have low amounts of dietary fiber and bioactive compounds.[2] Nowadays, the bakery industry faces challenges from consumers' increasing desire for healthy food. They require product development with enhanced sensory, physicochemical, and nutritional qualities.[3] The addition of novel food ingredients in cookies, such as spinach powder,[4] sweet detar, and moringa leaf,[5] sweet potato flour based high protein,[6] and Pleurotus albidus mycoprotein flour,[7] have been studied to improve the physical properties and micronutrient composition of cookies.

Thai jasmine rice (*Oryza sativa* L.), also known as "Khao Dowk Mali" is the most significant aromatic rice variety cultivated in Thailand. The cultivars used to produce the jasmine rice include Kor Kho 15 (RD15) and Khao Dowk Mali 105 (KDML 105). These grains are characterised by unique appearance, aroma, and cooking quality.[8, 9] The rice seedlings are rich sources of nutrients and antioxidant activity.[10] In addition, it has been reported that Thai jasmine rice grass (KDML 105 variety) contains calcium (415 mg/100 g), sodium (29 mg/100 g), potassium (1.181 g/100 g), magnesium (194 mg/100 g), iron (8.09 mg/100 g), chlorophyll (8.69 mg/100 g), vitamin B1 (0.51 mg/100 g), vitamin B2 (0.90 mg/100 g), vitamin C (5.91 mg/100 g), and vitamin E (5.34 mg/100 g).[11] Chloroplasts are the organelles found in plants and algae that are responsible for converting light energy into chemical energy. The majority of nutrients found in green plant materials are located in the chloroplast thereby, it is possible to concentrate these by simply releasing intact chloroplast.[12] The previous research demonstrated that the chloroplast-rich fraction (CRF) derived from postharvest pea vine field residue [12-15] and spinach leaves[12, 16-18] contained a high level of macro and micronutrients. Furthermore, Gedi *et al.*,[17] illustrated the effects of partially replacing fish meal with spinach CRF on zebrafish growth. However, there has been no research on using CRF in the human diet. Thus, this study aimed to compare the physical properties and nutrient compositions of the freeze-dried whole leaf (WL) and freeze-dried chloroplast-rich fraction (CRF) derived from Thai jasmine rice grass. The effect of the partial replacement of wheat flour with WL and CRF on the nutritional and physiochemical properties of the butter cookies was also investigated in this study.

Materials and Method

Materials

Thai jasmine rice seeds (KDML 105) were washed and soaked in water for 24 h. The floating seeds were discarded. After that, the wet cheesecloth was placed over the soaked seeds to allow them to sprout for 48 h. The sprouts were then placed on the wet soil and watered twice a day. After 15 days, the rice seedlings were harvested by cutting about 0.5 inch from the soil.

Chemicals

The High-Performance Liquid Chromatography (HPLC) grade solvents (methanol, acetone, acetonitrile, chloroform, and ethyl acetate) were all acquired from Fisher Scientific (Loughborough, UK). β-Carotene (HPLC grade), gallic acid, 2,2-diphenyl-1-picrylhydrazyl (DPPH), butylated hydroxytoluene (BHT), and triethylamine (TEA) were obtained from Sigma Aldrich (Merck, Darmstadt, Germany). Other chemicals were analytical grade and bought from KemAus (New South Wales, Australia).

Freeze-Dried WL from Thai Jasmine Rice Grass

The WL of Thai jasmine rice grass were washed with tap water, and the extra water was then eliminated using a salad spinner (KitchenCraft®, Birmingham, UK). After washing, the leaves were blanched in hot water at 85°C for 3 min to deactivate endogenous enzymes. They were then promptly submersed in an ice-water bath to cool down to room temperature. The blanched WL was then frozen at -40°C for overnight prior to freeze drying (Alpha 1-4 Plus Freeze Dryer, Martin Christ GmbH, Osterode, Germany) for 3-5 days. The freeze-dried WL was blended using a blender (Philips HR2118/02, Indonesia) and followed by using an ultra-centrifugal mill (ZM 200, Retsch GmbH, Haan, Germany) to get fine powder (under 200 µm). The WL powder was kept in a vacuum-sealed foil pouch (10x15 cm, 0.13 mm, thickness, 130 µm) at 4°C for further analysis.

Isolation of the CRF

The CRF was isolated using a slightly modified Wattanakul *et al.*,[12] technique. The washed Thai jasmine rice grass was blanched in hot water at 85°C for 3 min to knock out endogenous enzymes and then rapidly placed in an ice-water bath to cool down to room temperature. The blanched Thai jasmine rice grass was juiced with a one-gear juicer (Oscar juicer, DA1200, South Korea). The homogenate was then filtered through a 154 µm stainless steel mesh sieve. The filtrated juice was centrifuged at 10,000 rpm (Digicen 21R with RT 153 Rotor, Ortoalresa, Madrid, Spain) for 10 min at 4°C. The pellet containing the CRF has remained. In order to produce another pellet, the supernatant was centrifuged again under the same conditions. The CRF was combined, weighed, and freeze-dried for 3-5 days using a freeze dryer (Alpha 1-4 Plus Freeze Dryer, Martin Christ GmbH, Osterode, Germany). After the CRF was freeze-dried, it was homogeneously ground using a pestle and mortar and sieved at 200 µm. The CRF was subsequently stored at 4°C in a vacuum-sealed foil bag for further analysis.

Butter Cookies with Freeze-Dried WL and CRF

Cookies were processed with ingredients purchased at the local market, according to the following basic formulations with modified formulation: wheat flour (100 g); butter (50 g); whole egg (36 g); sugar (20 g), baking powder (2.5 g), vanilla flavour (4.8 g).19 The freeze-dried WL or CRF powders were substituted at 0% (control), 2% WL (w/w), 4% WL (w/w), 2% CRF (w/w), and 4% CRF (w/w) levels with wheat flour. The ingredients were weighed and mixed for 5 min using a food mixer (KVL4100S Kenwood, Hampshire, UK) until a homogenous mixture was obtained. The mixture was poured into a cylindrical mould (approximately 4 cm in diameter and 0.85 cm in thickness). It was then baked for 10-15 min in an oven (Smeg, Guastala, Italy) at 180°C. After cooling, cookies were stored in an aluminium foil zip lock pouch at room temperature for further analysis.

Water Activity (a_w)

The water activity of the powder was measured at 25°C using a water activity meter (LabMaster-AW, Novasina, Lachen, Switzerland).

Determination of Color

The color measurement on the surfaces of freeze-dried WL, freeze-dried CRF, and cookies were evaluated using an UltraScan Vis spectrophotometer (UltraScan Vis, Hunterlab, Reston, USA) equipped with a three-parameter system of readings (a*, b*, L*). The parameter a* is associated with the green color dimension (negative values) and red (positive values), whereas the parameter b* is related to the colors blue (negative values) and yellow (positive values). The parameter L* represents lightness, ranging from 0 (dark sample) to 100 (light sample).

Spread Ratio

After baking and cooling at room temperature, the cookies were recorded for diameter (D) and thickness (T) using a calliper. The spread ratio (D/T) was calculated following the guidelines of Stoffel *et al*.[7]

Texture

The hardness of cookies was measured with a TA.XT Plus texture analyzer (Stable Micro Systems, Surrey, UK) equipped with Exponent software version 6.1.21.0. The test setting included a probe HDP/3PB, load cell of 5 kg, pre-test speed of 1.0 mm/s, test speed of 3.0 mm/s, post-test velocity of 10.0 mm/s, and an initial probe distance of 25 mm. The maximum force (N) required to break the cookie.

Proximate Analysis

Based on the AOAC method[20], the nutritional components of freeze-dried WL and CRF from Thai jasmine rice grass and cookies were characterized by their proximate composition. For moisture analysis, the sample (2 g) of each material was weighed in a moisture can and then placed in a hot air oven (FD 52 Binder, Binder Gmbh, Tuttlingen Germany) at 105°C and reweighed until a consistent weight was obtained. For protein analysis, the sample (1 g) was digested with celite (1 g) and concentrated sulfuric acid (25 mL) using the speed digester K-439 (Büchi Labortechnik, Flawil, Switzerland). The distillation and back titration were then performed with KjelFlex K-360 (Büchi Labortechnik, Flawil, Switzerland). The protein content was calculated using the nitrogen-to-protein conversion factor (kp) of 6.25. For fat analysis, the dried sample (1 g) was filled into a Soxhlet extraction thimble and then placed in fat extractor E-500 (Büchi Labortechnik, Flawil, Switzerland). The petroleum ether was used as the solvent for lipid extraction. Whilst the determination of ash, the sample (1 g) was added to a porcelain crucible and placed in the muffle furnace (CWF 1100, Carbolite-Gero, Derbyshire, UK) at 550°C for 3 h. Then, the crucible was left in the desiccator and reweighed until the constant weight. For crude fiber analysis, the defatted sample (1 g) and celite (1 g) were added in a new filter glass crucible and placed in a fully automated fiber analyzer (Fibertec™ 8000, Foss, Hillerød, Denmark) containing 1.25% (v/v) of sulfuric acid and 1.25% (w/v) of sodium hydroxide. Carbohydrate content was calculated as followed: equation 1.

Carbohydrate (g/100g)=100-[moisture +ash +protein+fat+fiber] ...(1)

Lipid Extraction for Analysis of Total Chlorophylls and Total Carotenoids

Lipid extraction was conducted using the method of Folch *et al*.[21] with a slight modification. A 2:1 mixture of chloroform and methanol (1.2 mL) was used to dissolve the sample, and it was vortexed for 1 min. Following the addition of sodium chloride solution (150 mM; 1 mL), the mixture was vortexed once for 1 min and centrifuged (Rotofix 32A with 1613 rotor, Andreas Hettich GmbH, Tuttlingen, Germany) at 3,000 rpm for 10 min. Following this, the mixture was separated into three phases. The lipid fraction containing the lowest phase was moved to a different tube. The remaining two phases were combined with 1.2 mL of a 2:1 Chloroform: methanol mixture, vortexed, and then centrifuged once more as previously described. After being extracted, the lipid was combined with the initial lipid layer. The pooled lipid phase was centrifuged again under the same condition to separate the lipids from any remaining chemicals. The lipid extract was then filtered through a 0.45 μm polytetrafluoroethylene (PTFE) filter membrane and subsequently dried. The dried lipid extracts were used for the analysis of total chlorophyll and total carotenoids in section 2.12.

Total Chlorophyll and Total Carotenoids Analyses

The pigment contents were determined using a UV-Vis spectrophotometer (Libra S70, Biochrom Ltd, Cambridge, UK). The lipid extracts of freeze-dried WL, freeze-dried CRF, and cookie were diluted with acetone. The sample solution was measured at three different wavelengths: 470 nm, 645 nm, and 662 nm. Total Chlorophyll and total carotenoid concentrations (μg/mL) were calculated using equation 2-5 described earlier by Lichtenthaler and Buschmann.[22]

Chlorophyll a

Chlorophyll a (μg/mL)=(11.24xA_{662})-(2.04xA_{645}) ...(2)

Chlorophyll b

Chlorophyll b (μg/mL)=(20.13 x A_{645})-(4.19 x A_{622}) ...(3)

Total Chlorophylls

Total chlorophyll (μg/mL)=Chlorophyll a+ Chlorophyll b ...(4)

Total Carotenoids

Total carotenoids (μg/mL)=((1,000 x A_{470})-(1.90 x Chlorophyll a)-(63.14 x Chlorophyll b))/214 ...(5)

β-Carotene Analysis

The amount of β-carotene was analysed using High-Performance Liquid Chromatography (HPLC) with UV detection (Agilent 1260, USA) using a modified method from Wattanakul *et al*.[14] The dried lipid extract was dissolved in acetone (containing 0.1% BHT and filtered through 0.45 μm PTFE filter membrane into amber HPLC vials. At a flow rate of 0.5 mL/min, the mobile phase was composed of acetonitrile, methanol, and ethyl acetate with 0.05% triethylamine (TEA). The solvent ratios were 95:5:0 at the beginning of the run, however, after 25 min, they changed to 60:20:20 and held in this proportion until the finish of the run. It took 15 min to re-equilibrate. Sample (10 µL) was injected via a guard column (Ascentis, Supelco, Germany) and separated on a Supelco, Ascentis C18 analytical column (3 µm, 4.6 x 150 mm). The temperature of the column was maintained at 22°C. β-Carotene was detected at a wavelength of 454 nm. A standard curve and a linear equation were used to determine the β-carotene concentration.

Extraction for Analyses of Total Phenolic and Antioxidant Activity

For the evaluation of phenolic content and antioxidant activity (DPPH), the methanolic extracts of WL, CRF, and cookie were obtained according to a slightly modified method described by Stoffel *et al*.[7] The sample (1 g) was extracted with 10 mL of an 8:2 mixture of methanol and reverse osmosis (RO) water. The solution was vortexed for 10 s, every 15 min for 1 h at room temperature. After that, the mixture was immersed in an ultrasonic bath (Elmasonic E70H, 50 Hz, Elma GmbH, Singen, Germany) for 10 min at room temperature. The solution was centrifuged at 2,000 rpm for 10 min (Rotofix 32A with 1613 rotor, Andreas Hettich GmbH, Tuttlingen, Germany). The phenolic and antioxidant activity of the supernatant was measured.

Total Phenolic Content

The total phenolic compound was evaluated using the methods described by Singleton and Rossi[23] with minor modifications. The methanolic extract (0.05 mL) was mixed with 0.25 mL of Folin-Ciocalteu reagent and vortexed. The 20% (w/v) sodium carbonate (0.25 mL) was added and adjusted the volume using RO water until 5 mL. The solution was incubated in the dark for 30 min at room temperature. The absorbance was measured at 765 nm using a UV-Vis spectrophotometer (Libra S70, Biochrom Ltd, Cambridge, UK). The total phenolic content was calculated from the standard curve of gallic acid. The result was expressed as mg of gallic acid equivalent (GAE) per gram of the sample.

DPPH Radical Scavenging Assay

The DPPH radical scavenging capacity was performed according to the method described by Yamaguchi *et al.*,[24] with some modifications. The methanolic extract (0.1 mL) was mixed with 3 mL of 0.1 mM DPPH in ethanolic solution. The mixture solution was vortexed and incubated in the dark for 30 min at room temperature. The absorbance was measured at 517 nm using a UV-Vis spectrophotometer (Libra S70, Biochrom Ltd, Cambridge, UK). The percentage of inhibition of the DPPH radical was expressed using equation 6.

$$\%\ \text{DPPH inhibition} = (A_{control} - A_{sample})/A_{contro} \times 100 \quad ...(6)$$

Where $A_{control}$ is the absorbance of the control assay at 517 nm; A_{sample} is the absorbance of the sample at 517 nm.

Statistical Analysis

All measurements were conducted in triplicate and the data were expressed as mean ± standard deviation. The experimental data exhibited normally distributed distribution and were analysed using an independent-sample t-test, or a one-way analysis of variance (ANOVA) followed by a Duncan posthoc test using IBM SPSS Statistic for Windows Version 27.0 (IBM Corp, Armonk, NY, USA), with statistically significant at $P \leq 0.05$.

Results and Discussion

Physical Properties of Freeze-Dried WL and CRF from Thai Jasmine Rice Grass

The aw has a significant effect on the shelf life of food products. It determines the amount of free water available in a food system in response to metabolic reactions.[25] In this study, the aw values of freeze-dried WL and CRF powders were 0.414 ± 0.003 and 0.456 ± 0.002, respectively (Table 1). Both freeze-dried WL and CRF powders derived from Thai jasmine rice grass could be considered microbiologically stable because the aw values of them were all less than the minimum value (0.6) required for the multiplication of microorganisms.[26] Similarly, Östbring *et al.*,[27] reported low aw (0.29-0.46) of freeze-dried spinach thylakoid. In addition, the color of freeze-dried WL and CRF derived from Thai Jasmine rice grass was assessed through three color characteristics: lightness (L*), green-red color intensity (a*), and blue-yellow color intensity (b*) as illustrated in Table 1. The CRF powder significantly decreased the parameter of L* compared with WL powder ($P \leq 0.05$). On the other hand, the a* and b* values of freeze-dried CRF powder increased compared to freeze-dried WL. This implied that CRF powder was lighter and yellower than that of WL powder. The color parameter correlated with the pigment concentration in the plants, especially for chlorophyll (green pigments) and carotenoid (yellow pigments). Our findings were in line trends with those of El-Sayed[28] and Östbring *et al.*.[27] who found that the freeze-dried spinach thylakoid powder (L* = 13.7 and b* = 16.10) had lower value of L*, but a greater value of b* than that of WL spinach powder (L* = 47.33 and b* = 15.36).

Table 1: Physical properties and nutrient compositions (on dry weight) of freeze-dried WL and freeze-dried CRF derived from Thai jasmine rice grass

Composition		Thai jasmine rice grass	
		Freeze-dried WL	Freeze-dried CRF
Water activity (aw)		0.414 ± 0.003^{b}	0.456 ± 0.002^{a}
lightness (L*)		58.57 ± 0.09^{a}	38.67 ± 0.05^{b}
redness (a*)		-3.94 ± 0.02^{b}	1.96 ± 0.03^{a}
yellowness (b*)		22.59 ± 0.02^{b}	28.09 ± 0.06^{a}
Moisture	(g/100 g, DW)	7.52 ± 0.08^{a}	8.03 ± 0.75^{a}
Protein	(g/100 g, DW)	2.44 ± 0.54^{b}	5.24 ± 0.38^{a}
Lipid	(g/100 g, DW)	4.62 ± 0.33^{b}	16.93 ± 0.14^{a}
Ash	(g/100 g, DW)	14.75 ± 0.14^{b}	22.10 ± 2.55^{a}
Crude fiber	(g/100 g, DW)	26.82 ± 0.40^{a}	12.48 ± 0.57^{b}
Carbohydrate	(g/100 g, DW)	43.85 ± 0.40^{a}	35.22 ± 0.57^{b}
Chlorophyll a	(mg/g, DW)	1.10 ± 0.12^{a}	0.93 ± 0.02^{a}
Chlorophyll b	(mg/g, DW)	0.62 ± 0.15^{a}	0.35 ± 0.01^{b}
Total chlorophyll	(mg/g, DW)	1.79 ± 0.03^{a}	1.28 ± 0.04^{b}
β-carotene	(mg/g, DW)	0.22 ± 0.02^{b}	0.42 ± 0.01^{a}
Total carotenoid	(mg/g, DW)	0.48 ± 0.01^{b}	0.67 ± 0.02^{a}
Phenolic compound	(mg GAE/g, DW)	9.94 ± 1.48^{a}	6.23 ± 0.73^{b}
DPPH inhibition	(%)	75.22 ± 1.79^{a}	72.76 ± 3.07^{a}

Data were expressed as a mean ± SD and analysed using an independent-sample t-test with a significance level at P≤0.0.5, a>b; DW: dry weight.

Proximate Composition, Micronutrients, and Antioxidant Activity of Freeze-Dried WL and CRF derived from Thai Jasmine Rice Grass

Proximate composition of freeze-dried WL and CRF derived from Thai jasmine rice grass is shown in Table 1. The CRF powder from Thai jasmine rice grass contained significantly higher levels of protein, lipid, and ash compared with WL powder (P≤0.05).

On the other hand, the carbohydrate and fiber contents decreased following the process of chloroplast recovery. This resulted from the separation of the cell wall component from the released chloroplast. Our results were in agreement with those of Gedi *et al.*,[16] who discovered that the CRF from spinach, kale, and grass showed a greater amount of nutrients, containing protein and lipid, but a lower level of carbohydrate compared with the whole leaf materials from these plants. The concentrations of total chlorophyll and carotenoid (carotene and xanthophyll) in the freeze-dried WL and CRF powders are illustrated in Table 1. Chlorophylls and carotenoids have also therapeutic properties, such as antioxidant and protection against age-related macular degeneration (AMD) disease.[29,30] According to our finding, the freeze-dried CRF had greater levels of β-carotene (provitamin A) and total carotenoid, but lower level of total chlorophyll compared to the freeze-dried WL derived from Thai jasmine rice grass. The total carotenoid of freeze-dried Thai jasmine rice grass in this study ranged from 48 to 67 mg/100 g on dry weight. Whereas the total carotenoid of the five species of rice grass, including Yarko, Yoo Noom, Look Lai, Khai Mod Rin, and Kaab Dum contained 7-9 mg/100 g on dry weight.[31] The phenolic compound is a polar compound and acts as an antioxidants by chelating the ion and scavenging the free radicals.[7] Hence, it dissolves well in polar organic solvents. The finding of Wattanakul *et al.*,[15] revealed that the value of the water solubility index (WSI) of freeze-dried WL was a higher value than freeze-dried CRF. Hence, the total phenolic content of freeze-dried WL powder was significantly higher content than that of freeze-dried CRF (P≤0.05) (see Table 1). In addition, the dried jasmine rice grass (KDML 105) at 65°C (1.51 mg GAE/g on dry weight)32 had a lower content of total phenolic than our result (9.94 mg GAE/g on dry weight). Several studies reported that the total phenolic content of the food products is related to antioxidant activity.[7, 32] The percentage of inhibition of DPPH radicals in the freeze-dried WL and CRF powders derived from Thai jasmine rice grass was 75.22 and 72.76, respectively (P>0.05); on the other hand, the inhibition of DPPH radicals in rice seedlings (KDML 105 variety) were at 63.38%.[32]

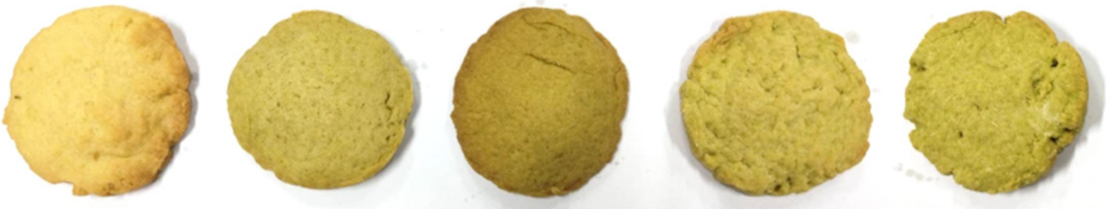

Fig. 1: Characteristics of cookies with the presence and absence of f freeze-dried WL and freeze-dried CRF derived from Thai jasmine rice grass

Table 2: Physical properties of cookies with freeze-dried WL and freeze-dried CRF derived from Thai jasmine rice grass

Cookie	L*	a*	b*	Spread ratio	Hardness (N)
Control	63.07 ± 0.88[a]	10.97 ± 0.66[a]	38.26 ± 0.40[a]	4.37 ± 0.20[a]	7.27 ± 0.64[c]
WL 2%	53.15 ± 0.25[c]	8.90 ± 1.42[b]	36.87 ± 0.20[b]	4.08 ± 0.04[b]	11.79 ± 1.50[b]
WL 4%	53.06 ± 0.24[c]	9.00 ± 1.07[b]	35.66 ± 0.11[c]	3.81 ± 0.03[c]	15.90 ± 0.95[a]
CRF 2%	56.76 ± 2.34[b]	10.27 ± 0.13[ab]	37.84 ± 0.58[a]	4.50 ± 0.15[a]	7.42 ± 1.29[c]
CRF 4%	52.86 ± 1.00[c]	11.20 ± 0.04[a]	37.01 ± 0.61[b]	4.29 ± 0.10[ab]	8.65 ± 0.44[c]

Data were expressed as a mean ± SD and analysed using ANOVA followed by a Duncan test with a significant level at P≤0.0.5, a>b; Control: cookie without WL or CRF

Physical Properties of Cookies

The partial replacement of wheat flour with different levels of freeze-dried WL and CRF from Thai jasmine rice grass in the cookies (Figure 1) affected the physical properties, especially color parameters (L*, a*, and b*), spread ratio, and hardness. The L*, a*, and b* of cookies were related to the amount of chlorophyll and carotenoid pigment in the cookie ingredients. The presence of WL in the cookies enhanced the amount of chlorophyll content of cookies (Table 1 and Table 4). Thus, it has contributed to the decreased L* and b* values compared to the control cookie (Table 3). Our result was consistent with the findings of Ahmad *et al.*[33] and Lee.[34] They discovered that L* and b* values of cookies that added green tea powder[33] or kale powder[34] were reduced compared with the cookies that added only wheat flour. In addition, the spread ratio and hardness of control cookies and cookies containing CRF did not have a significant difference (P>0.05). In contrast, the replacement of wheat flour with WL powders in cookies reduced the spread ratio and increased the hardness of cookies, especially cookies substituted with 4% WL (Table 2). The maximum of hardness of the cookies with the presence of 4% WL was 15.90 N. Perhaps this might be associated with the fiber content of cookies with freeze-dried WL derived from Thai jasmine rice grass. The spread ratio and hardness of cookies depend on the fiber content.[35] Our observation was also in line with the findings of Jeltema *et al.*,[36] who discovered that fiber content has an effect on the spread ratio and softness of the cookie. Similarly, Sharma *et al.*,[37] observed that adding *Tinospora cordifolia* leaf powder to cookies reduced the spread ratio and softness of cookies when compared to the cookies without adding the leaf powders. In addition, Lee[34] found that adding kale powder to cookies increased the hardness of cookies when compared to the cookies with the absence of kale powders.

Table 3: Proximate compositions of cookies with freeze-dried WL and freeze-dried CRF derived from Thai jasmine rice grass

Cookie	Moisture (g/100 g, DW)	Protein (g/100 g, DW)	Lipid (g/100 g, DW)	Total dietary fiber (g/100 g, DW)	Ash (g/100 g, DW)	Carbohydrate (g/100 g, DW)
Control	6.67 ± 0.21^{c}	7.79 ± 0.37^{d}	24.15 ± 0.91^{c}	0.67 ± 0.05^{c}	1.43 ± 0.06^{c}	59.29 ± 0.06^{a}
WL 2%	9.43 ± 0.34^{b}	7.82 ± 0.26^{d}	25.97 ± 0.04^{b}	1.03 ± 0.06^{b}	1.48 ± 0.01^{c}	53.96 ± 0.10^{c}
WL 4%	6.13 ± 0.07^{c}	8.95 ± 0.50^{c}	25.98 ± 0.54^{b}	1.37 ± 0.01^{a}	1.70 ± 0.09^{b}	55.28 ± 0.01^{b}
CRF 2%	11.25 ± 1.11^{a}	10.31 ± 1.00^{b}	26.79 ± 1.24^{b}	0.70 ± 0.02^{c}	1.79 ± 0.10^{b}	49.47 ± 0.01^{b}
CRF 4%	8.51 ± 0.72^{b}	12.82 ± 0.63^{a}	28.68 ± 0.92^{a}	0.76 ± 0.01^{c}	2.29 ± 0.01^{a}	47.53 ± 0.09^{e}

Data were expressed as a mean ± SD and analysed using ANOVA followed by a Duncan test with a significant level at P≤0.0.5, a>b; Control: cookie without WL or CRF; DW: dry weight

Table 4: Chlorophyll a, Chlorophyll b, total chlorophyll, β-carotene and total carotenoid contents (on dry weight) of cookies with freeze-dried WL and freeze-dried CRF derived from Thai jasmine rice grass

Cookie	Chlorophyll a (mg/g, DW)	Chlorophyll b (mg/g, DW)	Total chlorophyll (mg/g, DW)	β-carotene (mg/g, DW)	Total carotenoid (mg/g, DW)
Control	11.60 ± 0.43^{d}	4.23 ± 0.92^{d}	15.40 ± 1.31^{d}	2.24 ± 0.35^{e}	3.57 ± 0.35^{e}
WL 2%	16.81 ± 0.62^{b}	12.52 ± 1.71^{b}	29.32 ± 2.18^{b}	4.61 ± 0.24^{d}	5.69 ± 0.24^{d}
WL 4%	22.22 ± 0.63^{a}	16.48 ± 0.89^{a}	38.70 ± 0.65^{a}	6.79 ± 0.24^{c}	7.94 ± 0.24^{c}
CRF 2%	12.88 ± 0.02^{c}	8.59 ± 0.72^{c}	21.46 ± 0.70^{c}	9.90 ± 0.46^{b}	10.40 ± 0.46^{b}
CRF 4%	16.65 ± 1.18^{b}	11.04 ± 0.71^{b}	27.69 ± 0.54^{b}	11.37 ± 1.84^{a}	13.67 ± 1.84^{a}

Data were expressed as a mean ± SD and analysed using ANOVA followed by a Duncan test with a significant level at P≤0.0.5, a>b; Control: cookie without WL or CRF; DW: dry weight

Table 5: Total phenolic and antioxidant capacity (on dry weight) of cookies with freeze-dried WL and freeze-dried CRF derived from Thai jasmine rice grass

Cookie	Total phenolic (mg GAE/g, DW)	DPPH inhibition(%)
Control	0.77 ± 010^{b}	3.31 ± 0.62^{c}
WL 2%	1.27± 0.06^{a}	26.50 ± 0.52^{b}
WL 4%	1.57 ± 0.38^{a}	27.34 ± 1.40^{a}
CRF 2%	1.33± 0.06^{a}	24.42 ± 2.17^{b}
CRF 4%	1.25 ± 0.21^{a}	26.95 ± 0.83^{a}

Data were expressed as a mean ± SD and analysed using ANOVA followed by a Duncan test with a significant level at P≤0.0.5, a>b; Control: cookie without WL or CRF; DW: dry weight

Proximate Composition of Cookies

The results for the nutrient composition of cookies made with the presence of WL and CRF powder derived from Thai jasmine rice grass of varying concentrations are presented in Table 3. The cookies with the absence of WL and CRF showed the largest amount of carbohydrates. On the other hand, they were had the lowest amount of nutrients, including protein, lipid, ash, and fiber. Our findings were supported by the previous report, which found that the addition of dried Sesbania flower powder in butter cookies increased protein, lipid, ash, and crude fiber, except carbohydrates.[38] Furthermore, the cookies containing WL and CRF were significantly larger amounts of protein compared to that of the control cookies (see Table 3). It might be due to the addition of freeze-dried WL and CRF powder from Thai jasmine rice into the cookies. Both materials were high content of protein as described in Table 1. Our observation was agreed with the previous research, which revealed that the addition of basil leaves in the cookies gave a larger amount of protein than cookies prepared only with wheat flour.[39] Furthermore, it was noticed that the cookies with the presence of WL from Thai jasmine rice grass increased crude fiber compared to the other cookies. This might be due to a high crude fiber content found in WL as shown earlier in Table 1. On the other hand, the replacement of wheat flour with CRF did not significantly improve the level of crude fiber when compared to control cookies (Table 3). Perhaps, it might be the fact that the plant cell wall of Thai jasmine rice grass was removed during the CRF preparation. Moreover, the presence of CRF and WL enhanced lipid content in cookies (Table 3). It was due to the high amount of lipid in WL and CRF from Thai jasmine rice grass as shown in Table 1. However, our findings were not in line with the previous reports of Khumkhom[38], Somya *et al*.[39] and Cheng *et al*.[40] They discovered that increasing the powder of basil leaf, jering, and sesbania flower resulted in a decrease in lipid content in cookies.

Micronutrient Composition of Cookies

The incorporation of freeze-dried WL and CRF powder had an effect on the levels of chlorophyll a, chlorophyll b, total chlorophylls, β-carotene, total carotenoid, and total phenolic in cookies as shown in Table 4. The cookies that added CRF derived from Thai jasmine rice grass had a higher content of all micronutrients than the cookies containing WL, except for chlorophyll and total phenolic, which showed a reverse trend. It was due to the fact that WL included more chlorophyll and total phenolic than CRF as described earlier in section 3.2. Furthermore, the cookies with the presence of 4%CRF had the highest levels of β-carotene (provitamin A) and total carotenoids. A similar study by Dachana *et al*.,[41] discovered that cookies added with dried moringa leaves powder increased β-carotene and total carotenoid compared to the cookies without moringa powder. The cookies substituted wheat flour with WL and CRF powders derived from Thai jasmine rice grass accelerated the total phenolic content (approximately 1.65 – 2.04 times compared with the cookies without adding WL or CRF powders). The inclusion of 4% WL in cookies showed the largest amount of phenolic content compared with other cookies (Table 5). It was due to the fact that the freeze-dried WL included a cell wall. Even though chloroplast is a rich source of phenolic compounds, the cell wall also contains pectin, cellulose, hemicellulose, and phenolic compounds (i.e., lignin, hydroxycinnamic acid, ferulic acid).[42] Hence, the cookies containing WL had higher levels of total phenolic compounds than cookies containing CRF.

Antioxidant Activity of Cookies

Normally, the phenolic compound has an antioxidant activity. Therefore, the sample containing more phenolic compounds should also have more antioxidant activity.[43] Even though the cookies made with the presence of CRF had lower DPPH radical scavenging activity than the cookies made with the inclusion of WL, there were no statistically significant differences (P>0.05) (Table 5). It was possible that the antioxidant activity was influenced by the level of carotenoids rich in the CRF sample. Even though carotenoids are not phenolic compounds, it is an antioxidant properties.[44] Our results were similar trend with the studies of Lim and Lee[45] and Najjar *et al*.[46] They found that cookies formulated with date seed powder[46] and persimmon leaf powder[45] enhanced antioxidant capacity compared with the cookies without adding those materials.

Conclusion

This study demonstrated that the freeze-dried CRF derived from Thai jasmine rice grass had a greater level of macro and micronutrients compared with WL, except for carbohydrates and fiber. The partial replacement of wheat flour with freeze-dried WL and

CRF increased the nutrients in cookies, containing protein, fat, crude fiber, ash, β-carotene (provitamin A), total chlorophyll, total carotenoid, and phenolic compound compared with cookies contained only wheat flour. Regarding the color characteristics, the addition of WL made the cookies turn to green. On the other hand, the adding of CRF powders made the cookies turn to yellow. The texture of cookies containing a higher level of WL made the cookies harder compared with cookies with the absence of WL or CRF powder. The replacement of wheat flour with freeze-dried WL and CRF derived from Thai jasmine rice grass in butter cookies enhances the level of micronutrients (especially phenolic compounds, chlorophyll, and carotenoids) and antioxidant activity compared with the control cookie. The butter cookies substituted with WL 4% showed the highest concentration of chlorophyll and phenolic; the cookies substituted with CRF 4% had the highest content of β-carotene, a carotenoid. However, both of them were no significant differences in DPPH inhibition. Our findings suggested that the best formulation of cookies for physical and micronutrient properties were cookies substituted with 4% CRF. Thus, the results showed that utilizing freeze-dried WL and CRF derived from Thai jasmine rice grass can improve the nutritional and physicochemical properties of cookies. However, tests of sensory analysis and acceptability with potential consumers should be performed in the future.

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Conflict of interest statement

The authors declare that there are no conflicts of interest.

Authors' contribution

Chonthira Sarawong: Conceptualization, Methodology, Formal analysis, Writing. Krittika Norajit: Conceptualization, Methodology, Formal analysis, Writing. Rungtip Wongtom: Conceptualization, Methodology. Racharat Yampuang: Conceptualization, Methodology. Jutarat Wattanakul: Conceptualization, Methodology, Formal analysis, Validation, Supervision, Writing (review and editing).

Data Availability Statement

Not applicable.

Ethics Statement

This study was not involved an experiment on humans and animals.

Informed Consent Statement

Not applicable.

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