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Effect of the partial replacement of wheat flour with a whole leave and a chloroplast-rich fraction derived from holy basil leaves on the nutrient composition, physical properties, and antioxidant activity of butter cookies

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Abstract

Cookies are one of the most popular food products in the world. The demand for healthy and quality food products has encouraged interest in innovative food ingredients. With wheat flour as the main ingredient in cookies, they generally contain a low number of phytochemical compounds. To reduce wheat flour usage in cookies, innovative ingredients such as chloroplast-rich fraction (CRF) derived from holy basil leaves can be used as an alternative to increase the nutritional value. This research aimed to compare the nutritional components of the whole leaf (WL) and CRF from holy basil leaves. The variations of holy basil WL and CRF were 0% (control), 3% WL, 6% WL, 3% CRF and 6% CRF. The water activity, colour, spread ratio, texture, chlorophyll, carotenoid, phenolic compound, and antioxidant activity were evaluated in these butter cookies. The results showed that CRF derived from holy basil leaves had a higher amount of macro and micronutrients than WL, except for fibre and carbohydrates. The cookies substituted with 6% CRF had the darkest green colour. The cookies substituted with 6% WL containing higher fibre led to a decreased spread ratio and increased hardness of cookies. In addition, the replacement of wheat flour with holy basil WL and CRF can enhance nutritional and antioxidant values in bakery products. The butter cookies substituted with 6% WL showed the highest concentration of phenolic; the cookies substituted with 6% CRF had the highest contents of chlorophyll, carotenoid, and antioxidant activity.

1. Introduction

Cookie is a variety of bakery products consisting of wheat flour, butter and sugar. It contains an abundant source of calories, carbohydrates, protein, lipids, fibre, minerals, and vitamins. It is also a ready-to-eat, convenient and low-cost product (Olaoye *et al*., 2007). Nowadays, the growing demand for healthy and high quality food by consumers is a challenge for the bakery and confectionery industry. They require the development of products with improved physical-chemical, sensory, and nutritional properties (Mariotti *et al*., 2014). The addition of novel food ingredients in cookies, such as underutilised jering (*Pithecellobium jiringa* Jack.) legume flour (Cheng and Bhat, 2016), spinach powder (Galla *et al*., 2017), and *Pleurotus albidus* mycoprotein flour (Stoffel *et al*., 2021), have been studied to improve the physical and chemical properties of cookies.

Holy basil (*Ocimum sanctum*) is an annual herbaceous plant in Asia, Africa, and Central and South America (Singh and Chaudhuri, 2018). Its slightly hairy, pale green leaves are commonly used in Southeast Asia cuisine, particularly Thai stir-fries as a flavouring enhancer. Holy basil leaves have a spicy and citrusy flavour with a camphoraceous aroma (Wangcharoen and Morasuk, 2007) as well as a high nutritional content

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(Wangcharoen and Morasuk, 2007; Vidhani *et al*., 2016; Singh and Chaudhuri, 2018). Asians have used it for asthma, headaches, cramps, diarrhoea, and gastrointestinal disorders in traditional medicine (Wangcharoen and Morasuk, 2007; Cohen, 2014; Singh and Chaudhuri, 2018). Holy basil, on the other hand, has been shown to have therapeutic application in modern medicine, including the central nervous system, cardiovascular system, gastrointestinal systems, immune system, antimicrobial activity, and antioxidant activity (Wangcharoen and Morasuk, 2007; Vidhani *et al*., 2016; Singh and Chaudhuri, 2018). Toxicity studies have revealed that holy basil is a non-toxic plant that is suitable for human consumption (Gautam and Goel, 2014).

Chloroplasts are the organelles in plants and other photosynthetic organisms, that convert light energy into chemical energy. The majority of nutrients found in green plant materials are located in the chloroplast (Wattanakul *et al*., 2021), thus it is possible to concentrate these by simply releasing intact chloroplasts from their cellular confinement, centrifuging this suspension, then dehydrating the pellet to produce a chloroplast-rich fraction (CRF). The previous works demonstrated the CRF from postharvest pea vine field residue (Torcello-Gomez *et al*., 2019; Wattanakul *et al*., 2019; Wattanakul *et al*., 2021; Wattanakul *et al*., 2022) and spinach leaves (Gedi *et al*., 2017; Gedi *et al*., 2019; Wattanakul *et al*., 2019) have the nutritional credentials to be used as a food supplement or food ingredient. Despite the fact that Gedi *et al*. (2019) investigated the effects of partially replacing fish meal with spinach CRF on zebrafish growth, there has been no research on using CRF in the human diet. Wattanakul *et al*. (2021) discovered that heat treatment of biomass is an effective way of preserving the nutrients in the dry powder of pea vine haulm and blanching in hot water at 85℃ for 3 mins can inactivate the endogenous enzymes that might cause a deterioration in the quality of chloroplast-rich fraction from spinach leaves (Wattanakul *et al*., 2019).

Therefore, the materials used in this experiment were blanched before the processing of the powders. The study aimed to compare the nutritional components of freeze-dried whole leaf (WL) and freeze-dried chloroplast-rich fraction (CRF) derived from holy basil leaves and evaluated the effect of the partial replacement of wheat flour with either WL or CRF on the nutrient composition, physical properties, and antioxidant activity of the butter cookies. The water activity, colour, spread ratio, texture, nutrient composition, chlorophyll, carotenoid, phenolic compound, and DPPH inhibition were evaluated in butter cookies with WL and CRF substitution.

2. Materials and methods

2.1 Materials

Holy basil leaves were bought from a local market in Bangkok, Thailand.

2.2 Freeze-dried whole leaf from holy basil leaves

The whole holy basil leaves (200 g) were washed with tap water, and then the excess water was removed using a salad spinner (KitchenCraft®, UK). The washed leaves were blanched in hot water at 85℃ for 3 mins to inactivate endogenous enzymes and then immediately immersed in an ice-water bath to rapidly cool to room temperature. The blanched whole leaves were then frozen at -40℃ overnight prior to freeze drying (Christ Alpha 1-4 Plus freeze dryer, Germany) for 3-5 days. The freeze-dried WL was then blended using a blender (Philips HR2118/02, Indonesia) and then ground to homogeneous powder using a pestle and mortar under dim light. The WL powder was stored in a vacuum-sealed foil pouch (10×15 cm, 0.13 mm, thickness, 130 μm) at 4℃ for further analysis.

2.3 Isolation of the chloroplast-rich fraction

The chloroplast-rich fraction (CRF) was isolated according to a slightly modified method from Wattanakul *et al*. (2019). The washed basil leaves (2 kg) were blanched in hot water at 85℃ for 3 mins to inactivate endogenous enzymes and then immediately immersed in an ice-water bath to rapidly cool to room temperature. Blanched basil leaves were homogenised in a blender (Philips HR2118/02, Indonesia) for 30 s with 0.3 M sucrose solution 1:6 (w/v). The homogenate was then filtered through a 154 mm stainless steel mesh sieve. The filtrated juice was centrifuged at 10,304 RCF or 10,000 rpm (Ortoalresa Digicen 21R with RT 153 rotor, Spain) for 10 mins at 4℃. The pellet containing the CRF was retained while the supernatant was centrifuged again under the same conditions to obtain a further pellet. The CRF was pooled, weighed and frozen at -40℃ before being freeze-dried (Christ Alpha 1-4 Plus freeze dryer, Germany) for 3-5 days. The freeze-dried CRF was then ground to homogeneous powder using a pestle and mortar and stored in a vacuum-sealed foil pouch at 4℃ for further analysis.

2.4 Butter cookies with freeze-dried whole leaf and chloroplast-rich fraction

Cookies were processed with ingredients purchased at the local market, according to the following basic formulations: wheat flour (200 g); butter (100 g); whole egg (50 g); sugar (100 g), and baking powder (5 g) (Thanonkaew *et al*., 2008). The freeze-dried WL or CRF powders were substituted at 0% (control), 3% WL, 6%

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WL%, 3% CRF and 6% CRF levels in wheat flour. The ingredients were weighed and mixed until a homogenous mixture for 5 mins was obtained. The mixture was placed in a cylindrical format (approximately 4 cm in diameter and 0.85 cm in thickness) and baked in an oven (Smeg, Italy) at 180°C for 10-15 mins. After cooling, cookies were stored in aluminium foil zip lock pouch at room temperature for further analysis.

2.5 Proximate composition

Nutritional components of WL, CRF from holy basil leaves, and cookies were characterised by proximal composition according to the method of Association of the Official Analytical Collaboration (AOAC) International (2000). For moisture analysis, the sample (2 g) of each of the materials was weighed in a moisture can and then placed in a hot air oven (Binder, US) at 105°C and reweighed until the consistent weight. 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 (Buchi, Switzerland). The distillation and back titration were then performed with KjelFlex K-360 (Buchi, Switzerland). The protein content was calculated using the general factor (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 (Buchi, Switzerland). The petroleum ether was used as the solvent for lipid extraction. Whilst the determination of ash, the dried sample (1 g) was added to a porcelain crucible and placed in the muffle furnace (Carbolite, CWF 1100, UK) at 550°C for 3 hrs. Then, the crucible was left at room temperature in the desiccator and reweighed until the constant weight. For crude fibre analysis, the de-fat sample (1 g) and celite (1 g) were added to a glass crucible and placed in a fully automated fibre analyser (Fibertec™ 8000, Foss, Denmark). Carbohydrate content and the energy value were calculated using Equation 1 and Equation 2, respectively.

$$\text{Carbohydrate (g/100 g)} = 100 - [\text{moisture (g/100 g)} + \text{ash (g/100 g)} + \text{protein (g/100 g)} + \text{fat (g/100 g)} + \text{fibre (g/100 g)}] \quad (1)$$

$$\text{Energy (kcal/100 g)} = (4 \times \text{protein (g/100 g)}) + (4 \times \text{carbohydrate (g/100 g)}) + (9 \times \text{fat (g/100 g)}) \quad (2)$$

2.6 Lipid extraction for analysis of total chlorophylls and total carotenoids

Lipid extraction was performed using the method of Folch *et al*. (1957) with modification. The sample was dissolved in a 2:1 mixture of chloroform and methanol (1.2 mL) and mixed for 1 min. Sodium chloride solution (150 mM; 1 mL) was then added to the mixture, which was vortexed again (1 min) and centrifuged (Hettich Rotofix 32A with 1613 rotor, Germany) at 3,000 rpm for 10 mins after which it separated into 3 phases. The lowest phase, which contained the lipid fraction, was transferred into another tube. A 2:1 Chloroform: methanol mixture (1.2 mL) was added to the remaining two phases and then vortexed and centrifuged again as above. The lipid was removed and pooled with the original lipid layer. The combined lipid phase was then centrifuged under the same condition to separate the lipids from any residual compounds. The lipid extract was filtered through a 0.45 µm PTFE filter membrane and then dried under a flow of nitrogen. The dried lipid extracts were used for the analysis of total chlorophyll and total carotenoids in section 2.7.

2.7 Total chlorophyll and total carotenoids analyses

The pigment contents were analysed using an UV-Vis spectrophotometer (Libra S70, Biochrom, UK). The lipid extract was diluted with acetone in a ratio of 1:300 for WL and CRF powders and 1:2 for cookies. The sample solution was measured at three different wavelengths: 662 nm, 645 nm, and 470 nm. Total Chlorophyll and total carotenoid concentrations (µg/mL) were calculated using Equations 3-6 by Lichtenthaler and Buschmann (2001).

$$\text{Chlorophyll a (µg/mL)} = (11.24 \times A_{662}) - (2.04 \times A_{645}) \quad (3)$$

$$\text{Chlorophyll b (µg/mL)} = (20.13 \times A_{645}) - (4.19 \times A_{662}) \quad (4)$$

$$\text{Total chlorophylls (µg/mL)} = \text{chlorophyll a} + \text{chlorophyll b} \quad (5)$$

$$\text{Total carotenoids (µg/mL)} = ((1{,}000 \times A_{470}) - (1.90 \times \text{chlorophyll a}) - (63.14 \times \text{Chlorophyll b}))/214 \quad (6)$$

2.8 Water activity (a_w)

The water activity of the powder was determined at 25°C using a water activity meter (AquaLab Series 3, Decagon Devices Inc., USA).

2.9 Determination of instrumental colour

The colour evaluation on the surfaces of freeze-dried WL, freeze-dried CRF, and cookies was carried out using UltraScan Vis spectrophotometer (Ultrascan® Vis, Hunterlab, USA) equipped with a three-parameter system of readings (L*, a*, b*). Where the parameter L* corresponds to the lightness, ranging from 0 (dark sample) to 100 (light sample), the parameter a* is related to the green colour dimension (negative values) and red (positive values), b* is related to the colours blue (negative values) and yellow (positive values).

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2.10 Spread ratio

The cookies were measured for diameter (D) and thickness (T), after baking and cooling at room temperature, with calliper and the spread ratio (D/T) was calculated according to American Association of Cereal Chemists (AACC) (2000).

2.11 Texture

The cookies were evaluated for hardness using a TA.XT Plus texture analyser (Stable Micro Systems, UK) equipped with Exponent software version 6.1.21.0. The maximum force (N) was measured to break the cookie, under the following test conditions: 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.

2.12 Cookie extract

For the evaluation of phenolic content and antioxidant activity (DPPH), the methanolic extract of cookies was obtained according to a slightly modified method described by Stoffel *et al*. (2021). Each cookie (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 mins for 1 hr at room temperature. After, the mixture was placed in an ultrasonic bath (Elmasonic E70H, 50 Hz, Germany) for 10 mins at room temperature. The solution was centrifuged at 2,000 rpm for 10 mins (Hettich Rotofix 32A with 1613 rotor, Germany). The supernatant was used for the determination of phenolic and antioxidant activity.

2.13 Phenolic content

The total phenolic compound was determined using the methods described by Galla *et al*. (2017) and Najjar *et al*. (2022) with some modifications. The sample solution (60 mL) was mixed with 2.5 mL of Folin-Ciocalteu reagent and vortexed. The 7.5% (w/v) sodium carbonate (2 mL) was added and incubated at 50°C for 15 mins. The absorbance was measured at 760 nm. The concentrations of phenolic were calculated from the standard curve of gallic acid. The result was expressed as mg of gallic acid equivalent (GAE) per gram of the sample.

2.14 DPPH radical scavenging assay

The DPPH radical scavenging capacity was performed according to the method described by Geremu *et al*. (2016) with some modifications. The methanolic extract of cookie (3 mL) was mixed with 1 mL of 0.6 mM DPPH methanolic solution. The mixture solution was incubated at room temperature in the dark for 30 mins. The absorbance was recorded at 517 nm. The percentage of inhibition of the DPPH radical was expressed using Equation 7.

$$\% \text{ DPPH inhibition} = ((A_{control} - A_{sample})/(A_{control})) \times 100 \quad (7)$$

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

2.15 Statistical analysis

All experiments were performed in triplicate. The statistical analysis was carried out using IBM SPSS statistic 27 using post hoc analysis of variance (ANOVA) and according to the Duncan test with statistically significant at $p<0.05$ or an independent-sample t-test with statistically significant at $p<0.05$. The data were expressed as mean ± standard deviation (SD). Differences in means at $p<0.05$ were considered significant.

3. Results and discussion

3.1 Yield, water activity, moisture content and colour of freeze-dried whole leaf and chloroplast-rich fraction from holy basil leaves

The yield of freeze-dried WL was 150.86 g/kg of fresh WL of holy basil leaves. The yield of freeze-dried CRF was 26.93 g/kg of fresh WL of holy basil leaves. The water activity (a_w) of freeze-dried powder is an essential factor since it impacts the powder's shelf life. It determines the amount of free water available in a food system in response to metabolic activities (Prabhakar and Mallika, 2014). In this present study, the water activity (a_w) values of freeze-dried WL and CRF powders were 0.32±0.03 and 0.26±0.06, respectively (Table 1). Both freeze-dried WL and CRF derived from holy basil powders could be considered microbiologically stable because the a_w values were all less than the minimum value (0.6) required for the multiplication of microorganisms (Gurtler *et al*., 2014). Similarly, Östbring *et al*. (2020) obtained low water activity (0.29-0.46) after freeze-drying of spinach thylakoid.

Moisture content represents the water composition in the food system. For prolonged shelf life and good quality, a dried product's moisture level should be less than 4%. The freeze-drying techniques result in a moisture content of less than 2% (Prabhakar and Mallika, 2014). The moisture content in freeze-dried apples and strawberries was reported at 0.5% and 2%, respectively (Nowak and Jakubczyk, 2020). In this work, the moisture contents of freeze-dried WL and CRF powders reported 1.39% and 1.31%, respectively (Table 1). On the other hand, Östbring *et al*. (2020) determined

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Table 1. Physical properties and nutritional constituents (on dry weight) of freeze-dried whole leaf and freeze-dried chloroplast-rich fraction from holy basil leaves.

Composition		Holy basil leaves	
		Freeze-dried WL	Freeze-dried CRF
Water activity (a_w)		0.32±0.03[a]	0.26±0.06[b]
L*		58.19±0.24[a]	56.04±0.39[b]
a*		-7.40±0.48[a]	-11.08±0.39[b]
b*		21.44±0.75[b]	25.09±0.09[a]
Moisture	(g/100 g, DW)	1.39±0.08[a]	1.31±0.06[a]
Protein	(g/100 g, DW)	18.51±0.36[b]	29.80±0.25[a]
Lipid	(g/100 g, DW)	3.46±0.19[b]	9.91±0.82[a]
Ash	(g/100 g, DW)	37.52±0.05[b]	39.28±0.53[a]
Crude fibre	(g/100 g, DW)	26.13±0.21[a]	8.40±0.10[b]
Carbohydrate	(g/100 g, DW)	13.39±0.16[a]	11.34±0.03[b]
Chlorophyll a	(mg/g, DW)	18.97±0.59[b]	49.48±5.45[a]
Chlorophyll b	(mg/g, DW)	9.20±0.59[b]	24.43±4.36[a]
Total chlorophyll	(mg/g, DW)	28.17±1.19[b]	73.91±9.78[a]
Total carotenoid	(mg/g, DW)	6.02±0.61[b]	15.19±1.16[a]
Phenolic compound	(mg GAE/g, DW)	20.07±0.29[a]	15.57±0.35[b]
DPPH inhibition	(%)	73.09±0.48[b]	75.44±0.08[a]

Values are presented as mean±SD from three separated samples. Values with different superscripts within the same row are statistically different (p<0.05) analysed using independent t-test. DW: dry weight, WL: whole leaf, CRF: chloroplast-rich fraction.

the moisture content of freeze-dried spinach thylakoid (2.3%) was higher moisture content than that of our samples.

The colour of freeze-dried WL and CRF from holy basil leaves was evaluated through three colour parameters: luminosity (L*), green-red colour intensity (a*), and blue-yellow colour intensity (b*) as shown in Table 1. The holy basil CRF powder significantly decreased the parameter of L* and a* compared to holy basil WL powder (p<0.05). On the other hand, the b* value of CRF powder increased compared to WL powder. This indicates that freeze-dried CRF powder was darker and greener than that of freeze-dried WL. It was due to the fact that the CRF itself came in a more concentrated form, thus it has more pigments, especially for chlorophyll (green pigments) and carotenoid (yellow pigments) than WL (Table 1). Our findings were in line with those of El-Sayed (2020) and Östbring *et al*. (2020), who found that the freeze-dried spinach thylakoid powder (L* = 13.7, a* = -7.22, b* = 16.10) had lower values of L* and a* values as well as a greater value of b* than whole leaf spinach powder (L* = 47.33, a* = -3.14, b* = 15.36).

3.2 Nutritional components of freeze-dried WL and CRF from holy basil leaves

Protein, lipid, ash, crude fibre, and carbohydrate from freeze-dried CRF and its parent WL from holy basil leaves were measured and presented in Table 1. Previous research (Alikwe *et al*., 2013; Vidhani *et al*., 2016; Mustafa and El-Kamali, 2020) showed holy basil protein content between 16.35 and 20.64, g/100 g, DW, showing closer agreement with this study (18.51 g/100 g, DW). The lipid content in the green material of this study was 3.46 and 9.91 g/100 g, DW of freeze-dried WL and CRF powders, respectively. Around 10% of the dry weight of green leaves of higher plants is represented by lipids, which are concentrated in the membrane organelles, particularly in the plastids, such as chloroplasts where they provide a source of macro and micronutrients (Walker and Barber, 1976). Our findings were in agreement with those of Vidhani *et al*. (2016) and Alikwe *et al*. (2013), who found the fat content of *Ocimum sanctum* ranged from 3.6% to 4.33%. Moreover, the carbohydrate content was reduced after the chloroplast recovery process (Table 1). This was due to the separation of cell wall material from liberated chloroplast. Our result was supported by the result of Gedi *et al*. (2017), who found that the freeze-dried WL showed higher content of carbohydrate than the freeze-dried CRF in spinach, kale, and grass. In addition, the carbohydrate value (13.39%) for WL of basil leaves in Table 1 was slightly lower than that reported by Vidhani *et al*. (2016) (39.58%). On the other hand, the freeze-dried CRF was a higher amount of ash than that of freeze-dried WL. It might be due to that chloroplast is a rich source of minerals (Gedi *et al*., 2017). Both WL (37.52%) and CRF (39.28%) from freeze-dried holy basil leaves exhibited a high percentage of ash,

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indicating that both samples contain a rich source of minerals. Furthermore, the level of ash from holy basil WL had a higher concentration than the result of Alikwe *et al*. (2013), who observed an ash value of 11.38% in the holy basil. The crude fibre content of holy basil WL (26.13%) was quite higher than that discovered by Mustafa and El-Kamali (2020) (10.74%) and Alikwe *et al*. (2013) (17.54%).

Moreover, the results showed that CRF powder contained more lipid, protein, and ash than WL powder. The carbohydrate and fibre contents, on the other hand, were reduced after the chloroplast recovery process (Table 1). This was due to the separation of the cell wall. The findings in this study were in line with those of Gedi *et al*. (2017). They discovered that CRF from spinach, kale, and grass contained more protein and lipids than whole-leaf material with lower carbohydrates. However, the CRF from nettles did not follow the trend of the other CRF. It had a low protein, but high carbohydrate content.

Total chlorophyll and carotenoid (carotene and xanthophyll) concentrations of freeze-dried WL and CRF are shown in Table 1. The results obtained in this study showed that total chlorophyll and total carotenoid concentrations in freeze-dried CRF were greater amount than freeze-dried WL from holy basil leaves (Table 1). This was attributed to the fact that the CRF sample contains concentrated chloroplast, which is a rich source of chlorophylls and carotenoids. Chlorophyll and carotenoid have also therapeutic properties, such as antioxidant and protection against age-related macular degeneration (Ferruzzi and Blakeslee, 2007; Rasmussen and Johnson, 2013). The chlorophyll concentration is an indirect indicator of the chloroplast concentration. The nutrient concentration could be determined as a function of chlorophyll content, which represents the concentration of candidate nutrients relative to the actual chloroplast material instead of the total dry matter (Gedi *et al*., 2017). Furthermore, our findings were consistent with Gedi *et al*. (2017)'s findings. They reported that CRF from four different plants, including spinach, kale, nettles, and grass had higher levels of chlorophylls and carotenoids than whole leaf materials from these plants.

3.3 Water activity, moisture content and colour of cookies

Water activity (a_w) and moisture content values of cookies with holy basil WL and CRF ranged from 0.18-0.22 and 1.86-2.44 g/100 g, DW, respectively. This trend can be acceptable as the moisture levels in baked cookies are usually less than 5% (Cauvain and Young, 2008). Low moisture levels can make cookies more stable, extending their shelf-life. Generally, the cookies with the presence of WL and CRF presented lower water activity (Table 2) and moisture content (Table 3). Similar to the findings of Sowmya *et al*. (2022), they reported that the cookies containing basil, ajwain, and orange peel had lower water activity and moisture content than the cookie without added herbs. Perhaps, the addition of herbs decreased water activity and moisture content because of low water absorption capacity (Sowmya *et al*., 2022). However, it has been reported that the moisture content increased when increased the amount of kale powder (Lee, 2015) and underutilised jering seed (Cheng and Bhat, 2016) in cookies.

The replacement of wheat flour with freeze-dried WL and CRF from holy basil leaves into the cookies resulted in significant differences (p<0.05) in the colour parameter L*, a* and b*. Cookies without the addition of WL and CRF (control) showed the highest value L*, a*, and b* at 74.24, 11.14. and 42.64, respectively. Cookies produced with only wheat flour were lighter, redder, and more yellow than those with WL and CRF at any level. The presence of WL and CRF in cookies decreased the values of L*, a* and b*. The greater the amount of holy basil WL or CRF powders added to the cookies, the greener colour of the cookies appeared as shown in Figure 1 and Table 2. The lightness (L*) and green-red colour intensity (b*) of cookies were related to the amount of chlorophyll pigment in the ingredients. Since the addition of WL and CRF increased the amount of chlorophyll content of cookies (Table 1 and Table 4), this has contributed to the decreased L* and b* values compared to the control cookie (Table 3). Our findings were similar to those of Ahmad *et al*. (2015) and Lee (2015). They discovered that L* and b* values of cookies added green tea powder (Ahmad *et al*., 2015) or kale powder (Lee, 2015) dropped when compared to the cookies added only wheat flour.

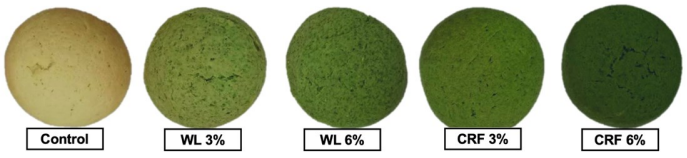

Figure 1. Colour characteristics of different cookies.

3.4 Spread ratio and texture analysis of cookies

The spread ratio was determined in the cookies produced as shown in Table 2. The control sample and 3% CRF obtained the highest value (p>0.05) of the spread ratio (3.34). On the other hand, the use of WL resulted in a decrease in the spread ratio value and an increase in hardness, especially since cookies added 6% WL (Table 2). The instrumental texture of the cookie showed values of hardness ranging from 12.29 to 14.67 N (Table 2). Cookies with the addition of 3% WL, 3% CRF and 6% CRF did not have significant differences in

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Table 2. Physical properties of cookies freeze-dried whole leaf and freeze-dried chloroplast-rich fraction from holy basil leaves.

Cookie	Water activity (a_w)	L*	a*	b*	Spread ratio	Hardness (N)
Control	0.22±0.008^{a}	74.24±1.56^{a}	11.14±1.76^{a}	42.46±1.29^{a}	3.34±0.04^{a}	12.29±0.12^{b}
3% WL	0.22±0.002^{a}	49.98±1.80^{b}	-4.31±0.16^{b}	27.38±1.38^{b}	2.85±0.05^{c}	12.84±0.25^{ab}
6% WL	0.21±0.001^{a}	45.77±0.57^{c}	-4.51±0.67^{b}	27.48±0.67^{b}	2.64±0.02^{d}	14.67±1.85^{a}
3% CRF	0.19±0.012^{b}	44.23±1.08^{c}	-7.38±0.15^{c}	29.22±0.50^{b}	3.34±0.02^{a}	12.59±1.44^{b}
6% CRF	0.18±0.003^{b}	41.04±0.45^{d}	-7.77±0.27^{c}	24.34±1.25^{c}	3.25±0.03^{b}	12.85±1.36^{ab}

Values are presented as mean±SD from three separated samples. Values with different superscripts within the same column are statistically different (p<0.05) analysed using post-hoc analysis of variance (ANOVA) with Duncan test. Control: cookie without WL or CRF, WL: whole leaf, CRF: chloroplast-rich fraction.

hardness when compared with the control cookie (p>0.05). The cookies with the presence of 6% WL had the maximum hardness (14.67 N) compared to all the cookies tested. Perhaps, this might be associated with the fibre content of cookies added to holy basil WL. Regarding spread ratio, Tangkanakul *et al*. (1995) reported that an increase in fibre content can lead to a decrease in spread ratio but increase the texture of hardness. The observation in this study was also in agreement with Jeltema *et al*. (1983), who discovered that fibre content has an effect on the spread ratio and softness of cookies. Similarly, Sharma *et al*. (2013) observed that adding *Tinospora cordifolia* leaf powder to cookies reduced the spread ratio and softness of cookies when compared to the cookies without any addition. In addition, Lee (2015) found that adding kale powder to cookies increased the hardness of cookies when compared to the cookies without the absence of kale powders.

3.5 Nutritional components of cookies

The results for the nutrient composition of cookies made with the presence of WL and CRF powder derived from holy basil leaves of varying concentrations were presented in Table 3 and the energy values of cookies ranged from 483 to 492 kcal/100 g of cookies. It was observed that the proximate values for protein, fat, crude fibre, and ash were low in cookies without the addition of WL and CRF, which served as the control. The proximate values increased with increasing the amount of WL and CRF except for carbohydrate content which showed a reverse trend. The control cookies contain 100% of wheat flour, which is rich in carbohydrates. Whilst the substitution of wheat flour with 3% or 6% green powders contains only 94-97% of wheat flour. In addition, the WL and CRF from basil leaves had 11-13% of carbohydrate content (Table 1). Thus, this is a reason why carbohydrate contents in cookies containing WL or CRF have lower content than control cookies. Similarly, Khumkhom (2018) found that increasing the amount of dried Sesbania flower powder in butter cookies enhanced protein, lipid, ash, crude fibre, except carbohydrate. The protein contents of the supplemented cookies were significantly higher compared to that of the control cookies (6.78% for 3% WL compared to control, 9.37% for 6% WL compared to control, 9.21% for 3% CRF compared to control, and 13.25% for 6% CRF compared to control). This might be the result of the incorporation of holy basil WL and CRF powders which have a higher protein content as indicated earlier in Table 2. Similar findings were previously published (Sowmya *et al*., 2022), in which the protein content of cookies incorporated with basil leaves was found to be higher than that of the control cookies prepared using wheat flour. As for the crude fibre content, it was noticed that the addition of 6% WL, resulted in an increase in crude fibre content up to 46% compared to control. This might be due to the higher crude fibre level in WL as shown earlier in Table 1. However, the addition of CRF at 3% and 6% did not significantly improve the level of crude fibre when compared to control cookies (Table 3). Perhaps, it might be the fact that the plant cell wall of basil leaves was removed during the CRF preparation. With regard to the fat content of the cookies, it increased significantly from 19.31 g/100 g, DW in control cookies to 21.83 g/100 g, DW in cookies contained 6% WL and 21.82 g/100 g, DW in cookies contained 6% CRF. It was attributed to the greater amount of fat in WL and CRF from holy basil leaves as described in Table 1. In contrast to these findings, increasing the amount of basil leaf powder and jering powder resulted in a decrease in fat content in cookies (Cheng and Bhat, 2016; Khumkhom, 2018; Sowmya *et al*., 2022). Even though the CRF powder gave a lipid content more than WL, the cookies substituted with 6% WL and 6% CRF were not significant differences of lipid. This might occur from the error of cookie sampling for lipid extraction.

3.6 Phytochemical compounds and antioxidant activity of cookies

The phytochemical analysis of cookies was made with the addition of WL and CRF powders derived from holy basil leaves of varying concentrations (0%, 3% WL, 6% WL, 3% CRF and 6% CRF). The results showed that the incorporation of freeze-dried holy basil WL and CRF has an encouraging effect on chlorophyll a, chlorophyll

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Table 3. Chemical properties (on dry weight) of cookies with freeze-dried whole leaf and freeze-dried chloroplast-rich fraction from holy basil leaves.

Cookie	Moisture (g/100 g, DW)	Protein (g/100 g, DW)	Lipid (g/100 g, DW)	Total dietary (g/100 g, DW)	Ash (g/100 g, DW)	Carbohydrate (g/100 g, DW)	Energy (kcal/100 g, DW)
Control	2.44±0.12^{a}	6.19±0.19^{d}	19.31±0.01^{b}	0.46±0.01^{c}	0.63±0.25^{c}	71.01±0.03^{a}	482.86±1.08^{c}
3% WL	2.23±0.14^{ab}	6.61±0.18^{c}	21.30±0.35^{a}	0.58±0.03^{b}	1.20±0.03^{b}	68.49±0.69^{b}	491.79±1.10^{ab}
6% WL	2.06±0.07^{bc}	6.77±0.14^{b}	21.83±0.09^{a}	0.67±0.04^{a}	1.53±0.03^{a}	67.51±0.12^{b}	493.25±0.40^{a}
3% CRF	2.00±0.14^{bc}	6.76±0.26^{b}	19.69±0.27^{b}	0.50±0.01^{c}	1.14±0.12^{b}	70.17±0.77^{a}	484.91±0.59^{c}
6% CRF	1.86±0.06^{c}	7.01±0.05^{a}	21.82±0.60^{a}	0.54±0.08^{c}	1.30±0.01^{ab}	67.20±0.63^{b}	488.82±2.38^{b}

Values are presented as mean±SD from three separated samples. Values with different superscripts within the same column are statistically different (p<0.05) analysed using post-hoc analysis of variance (ANOVA) with Duncan test. Control: cookie without WL or CRF, WL: whole leaf, CRF: chloroplast-rich fraction, DW: dry weight.

b, total chlorophylls, total carotenoids, and phenolic compounds (Table 4). The cookies that added holy basil CRF had a higher content of all bioactive compounds than the cookies containing holy basil WL, except for phenolic content which showed a reverse trend. It was due to the fact that CRF contained more chlorophyll and carotenoid pigments than WL as described earlier in section 3.2. In addition, the cookies with the presence of 6% CRF had the highest levels of chlorophyll a, chlorophyll b, total chlorophyll, and total carotenoids. In a similar study by Sowmya *et al*. (2022), they found cookies produced with basil leaves had greater amount of chlorophyll a, chlorophyll b, and total chlorophylls when compared with the control cookies. Dachana *et al*. (2010) discovered that the cookies added with dried moringa leaves powder enhanced the level of total carotene and β-carotene compared to the cookies without the incorporation of moringa powder. In contrast, the cookies containing CRF did not have as much phenolic compound content as cookies containing WL. The inclusion of 6% WL in cookies showed the largest amount of phenolic content compared with other cookies. Even though chloroplast is a rich source of phenolic compounds, the cell wall in plants also contains pectin, cellulose, hemicellulose, and phenolic compounds (i.e., lignin, hydroxycinnamic acid, ferulic acid) (Wallace and Fry, 1994; Heredia *et al.*, 1995).

The cookies produced at substitution levels of 0%, 3% WL, 6% WL, 3% CRF, and 6% CRF derived from holy basil leaves were tested for their radical scavenging activity using the DPPH assays. The DPPH results are shown in Table 4; the data showed that the addition of freeze-dried WL and CRF resulted in increased antioxidant activity in the cookies. The cookies with the presence of 6% CRF (21.93%) had the highest antioxidant activity compared to other cookies. The phenolic compounds can be commonly found in basil leaves (Kwee and Niemeyer, 2011; Aburigal *et al*., 2017). Generally, the phenolic compound has an antioxidant activity. Thus, the sample containing more phenolic compounds should also have more antioxidant activity. The result obtained in this study showed that the cookies made with the presence of CRF had greater radical scavenging activity than the cookies made with the inclusion of holy basil WL even though they had lower content of phenolic compounds (Table 4). It was possible that the antioxidant activity was influenced by the amount of chlorophyll and carotenoids rich in the CRF sample. Even though both chlorophylls and carotenoids are not phenolic compounds, they have antioxidant properties (Pérez-Gálvez *et al*., 2020).

The results from this study were consistent with the previous studies (Lim and Lee, 2016; Najjar *et al*., 2022). They found that cookies formulated with date

Table 4. Phytonutrients and antioxidant capacity (on dry weight) of cookies with freeze-dried whole leaf and freeze-dried chloroplast-rich fraction from holy basil leaves.

Cookie	Chlorophyll a (μg/g, DW)	Chlorophyll b (μg/g, DW)	Total chlorophyll (μg/g, DW)	Total carotenoid (μg/g, DW)	Phenolic content (mg GAE/g, DW)	DPPH inhibition (%)
Control	7.24±0.60^{d}	3.59±0.73^{d}	10.83±0.47^{d}	2.35±0.54^{e}	1.01±017^{d}	0.48±0.07^{c}
3% WL	52.57±2.11^{c}	42.37±2.86^{c}	94.94±4.90^{c}	12.94±0.63^{d}	1.68±0.21^{ab}	18.45±1.27^{b}
6% WL	67.57±4.27^{b}	48.06±2.37c	115.63±6.62^{b}	17.13±1.19^{c}	1.79±0.27^{a}	18.92±1.60^{b}
3% CRF	72.53±2.80^{b}	54.16±3.63^{b}	126.69±5.11^{b}	19.58±0.90^{b}	1.36±0.09^{abc}	18.38±1.72^{b}
6% CRF	135.31±6.16^{a}	69.57±5.25^{a}	204.87±10.53^{a}	40.82±2.01^{a}	1.25±0.28^{bc}	21.93±1.91^{a}

Values are presented as mean±SD from three separated samples. Values with different superscripts within the same column are statistically different (p<0.05) analysed using post-hoc analysis of variance (ANOVA) with Duncan test. Control: cookie without WL or CRF, WL: whole leaf, CRF: chloroplast-rich fraction, DW: dry weight.

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seed powder (Najjar *et al*., 2022) and persimmon leaf powder (Lim and Lee, 2016) increased antioxidant capacity compared with the control samples.

4. Conclusion

This study demonstrated that the freeze-dried CRF derived from holy basil had higher levels of macro and micronutrients compared with WL, except for carbohydrates and fibre. The partial replacement of wheat flour with holy basil WL and CRF powders enhanced the nutritional values of cookies, which presented more protein, fat, crude fibre, ash, chlorophyll, carotenoid, and phenolic compounds than the cookies that contained only wheat flour. Regarding the colour characteristics, the addition of a greater amount of WL and CRF powders made the cookies turn greener than those with control cookies, especially the use of CRF. Moreover, the texture of cookies containing a higher concentration of WL made the cookies tougher compared with cookies with the absence of WL or CRF powder. Our findings on the best formulation of cookies for cookie colour, spread ratio and hardness were cookies substituted with 3% CRF. There was evidence of increasing phytochemical compounds (i.e., phenolic compounds, chlorophyll, carotenoids) and antioxidant activity of the cookies with the presence of freeze-dried holy basil WL and CRF. The butter cookies substituted with 6% WL showed the highest concentration of phenolic; the cookies substituted with 6% CRF had the highest content of chlorophyll, carotenoid, and DPPH inhibition. Therefore, the results showed the possibility of utilising freeze-dried holy basil WL and CRF as food ingredients to improve the nutritional properties of butter cookies. However, tests of sensory analysis and acceptability with potential consumers should be performed in the future.

Conflict of interest

The authors declare no conflicts of interest.

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