

Enhancing Mineral Content in Cookies: Beetroot and Holy Basil Composite Flour with Butter and Margarine

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ABSTRACT

Cookies are a popular snack with many varieties available on the market. While the use of beetroot in cookies is relatively common, incorporating holy basil is rare, with limited research on its potential. This study explores composite flour made from beetroot and holy basil as a substitute for wheat flour, aiming to enhance the mineral content in cookies. The objective is to identify the preferred ratio of beetroot to holy basil flour (100:0, 90:10, 80:20, 70:30, 60:40) and the type of fat (butter or margarine) for improved mineral content, fat content, hardness, and spread ratio. The best formulation was an 80:20 ratio of beetroot to holy basil flour combined with butter. This formulation contained 160.01 ± 3.17 mg/100g of calcium, 5.85 ± 0.24 mg/100g of iron, and 46.62 ± 0.04 mg/100g of magnesium. Additionally, it had a fat content of $23.43 \pm 0.46\%$, a hardness of 2047.06 ± 35.06 g, and a spread ratio of 6.17 ± 0.41 .

Keywords: Beetroot, herb cookies, holy basil, mineral

INTRODUCTION

Cookies are convenient food products with various ingredients to substitute the flour or add ingredients as fortification. Cookies demand for healthier ingredient is increasing, other than the price is not relatively high and affordable, and many people eat cookies as a snack (Chopra et al., 2018). The high demand for a healthy lifestyle has led to the development of many healthy snacks. Herb cookies can be an alternative from conventional cookies for snacking, where some of the flour is substituted by herb (Hess et al., 2016; Njike et al., 2016). The application or utilization of cookies created with composite flour (CF) varies nowadays with a specific purpose, such as to increase fibre content to create herbal cookies and to increase the quality and

add more functionality to cookies (Sahni and Shere, 2016; Upadhyay et al., 2017).

Beetroot (*Beta vulgaris*) is a vegetable rich in phytochemicals and bioactive compounds, which is very useful for the human diet. Beetroot contains vitamin C (4.9 mg), Fe (0.8 mg), Ca (16 mg), Mg (23 mg), P (40 mg), thiamine (0.031 mg), riboflavin (0.04 mg), niacin (0.334 mg) in 100 g of raw beetroot (Mirmiran et al., 2020), aside from micronutrient beetroot also rich in source of phytochemical compound such as ascorbic acid, carotenoid, phenolic acid, and flavonoids. Beetroots contain a significant source of betalains that serve as anti-oxidant and anti-inflammatory agent (Masih et al., 2019).

Holy basil or Tulasi (*Ocimum tenuiflorum*) is a tropical herb with a strong



aroma and lemony taste. The dominant aroma component is eugenol. Most research studies indicate that the phenolic compounds in holy basil, including eugenol, rosmarinic acid, and vicenin, are effective antioxidants. Additionally, antioxidant minerals like zinc are abundant in holy basil, with manganese and zinc being the highest among the microminerals. An amount of Ca (25 mg), Fe (15.1 mg), and a trace amount of Mg also present in 100 g holy basil (Mannan et al., 2019; Vidhani et al., 2016). Several studies have been conducted on holy basil; however, most research has focused on its health benefits and use in medicine or supplements, while its application in food products remains limited (Joshi et al., 2017; Sowmya et al., 2022; Vidhani et al., 2016).

Micronutrients such as minerals is essential for the human body. Even though the requirement is only in small amounts, it is necessary for metabolism and human health. Prasetyo et al. (2018) shows that 52.4% of adults in Indonesia have Ca deficiency. Cookies offer convenient consumption in different locations and are relatively affordable while maintaining a stable shelf life. Studies regarding the utilization of beetroot itself vary, but the utilization combined with holy basil is minimal. In previous research conducted by Sahni and Shere (2016) and Ingle et al. (2017), beetroot cookies lacked acceptance because of the earthy flavour; therefore, the addition of holy basil, which contains a minty aroma profile, might have masked the earthy taste of beetroot (Shiradhonkar et al., 2014)

Margarine and butter are a type of fat that is common to be use in baking. Butter is made from dairy, while margarine is made from vegetable oil to imitate the function of butter. Margarine is a cost-effective alternative to butter, offering a longer shelf life. While serving as a butter substitute, margarine lacks the aroma, taste, and mouthfeel of butter, resulting in a different

texture. Butter contains more lauric acid compared to margarine, which produces buttery flavour. Various types of fat could affect the cookies' physical characteristics (texture and spread ratio) and sensory characteristics.

This study explores the combination of beetroot flour (BF) and holy basil flour (HBF) in specific ratios to create CF. The objective is to determine the most preferred wheat flour (WF) ratio to CF while enhancing the mineral content and overall functional value of cookies. Additionally, the study examines the influence of different fat sources (butter and margarine) and evaluates the sensory, physical, chemical, and functional properties to develop nutritionally improved cookie formulation.

MATERIALS AND METHODS

Tools and Materials

The tools used in this study included food dehydrator (Arieta), 80 mesh sieve, baking oven (Oxone Ox-899RC), glassware (Iwaki Pyrex), heater (Barnstead), rotary evaporator (Heidolph), mixer (Phillips), oven (Mettler UNB 500), desiccator (Duran), furnace (Thermolyne 48000), analytical balance (Ohaus), table balance (Tanika), evaporating dish, texture analyser (TA.XT Plus), Soxhlet flask, cylindrical flat ended 2mm probe (Perspex), chroma meter (Konica Minolta CR-400), digital vernier calliper, UV-Vis spectrometer, microwave digestion.

The materials needed in this research are fresh beetroot, fresh holy basil, low protein wheat flour, margarine, butter, free range chicken egg, sodium bicarbonate, sugar, hexane (p.a, Smart Lab), K₂SO₄ (Sigma Aldrich), H₂SO₄ 1.25% (p.a, Smart Lab), NaOH 1.25% (p.a, Smart Lab), HCl 1N (p.a, Smart Lab), NaOH 1N (p.a, Smart Lab), selenium (p.a, Smart Lab), 4% boric acid (p.a, Smart Lab).

Processing of BF and HBF

The making of BF followed the method described by Ingle et al. (2017) with modifications. Fresh beetroots undergo cleaning, skin removal, and cutting into small pieces or thin slices. The slice is then spread over a tray and dried using a food dehydrator at 50°C for about 10 hours. The dried beetroot is ground using a dry blender and sieved through 80 mesh.

The processing of HBF followed the method described by Parmar et al. (2018) with modification. HBF is obtained from fresh holy basil that is cleaned, separated the leaves from the twig, and then spread in a tray for drying in food dehydrator at 50°C for 5-5.5 hours. Dry leaves are refined in a blender to pass through 80 mesh sieves.

The resulting BF and HBF analysis include yield, moisture content, lightness, ash content, and mineral content. The yield analysis was performed based on Kharchenko et al. (2017). Moisture and ash content are determined using the oven method and dry ashing method, respectively, following the Association of Official Analytical Chemist (AOAC), (2005). The lightness (L^*) of BF and HBF was analysed using a chromameter with granular-materials attachment following the method described by Richirose and Soedirga (2023). The calculation of L^* value signifies the degree of lightness on a scale of 0 to 100 (0 = black; 100 = white). The yield, moisture content, and ash content of BF and HBF are determined using the following equations.

$$\text{Yield (\%)} = \frac{\text{Mass sample after sieving}}{\text{Mass of initial sample}} \times 100$$

$$\text{Moisture (\%)} = \frac{a-b}{c} \times 100$$

Where:

- a = initial weight of initial sample
- b = final weight of sample
- c = weight of wet sample

$$\text{Ash (\%)} = \frac{\text{weight of ash in the crucible}}{\text{weight of initial sample}} \times 100$$

The analysis of Ca, iron (Fe), and magnesium (Mg) employs microwave digestion and *Inductively Coupled Plasma-Optical Emission Spectrophotometry* (ICP-OES). The calibrated standard curve must be obtained before the mineral content can be calculated, and the mineral content was then calculated using the following equation.

$$\text{Mineral (ppm, mg/L, mg/Kg)} = \frac{\frac{(Aspl-a)}{b} \times V \times fp}{W_{Spl} \text{ or } V_{spl}}$$

Where:

- Aspl = Sample intensity
- a = Intercept from standard calibration curve
- b = Slope from standard calibration curve
- fp = Dilution factor
- V = End volume sample (mL)
- Wspl = Sample weight (gram)
- Vspl = Sample compression volume (mL)

Cookies Processing

The cookies processing adapted the method described by Herlina and Sinaga (2022) with some modifications. The obtained BF and HBF were then mixed in a ratio of 1:1 into CF. The CF then used to substitute WF in the ratios of 100:0, 90:10, 70:30, 50:50, and 60:40. The cookies formulation can be seen in Table 1.

The cookies processing starts from creaming, where the fat and sugar are mixed with egg yolk until they become homogenous. A dry mix consist of a homogenous WF, CF, and baking soda mixture. The combination of the creaming mixture and the dry mixture results in a homogenous blend, followed by spreading the dough on a flat surface and covering it for about 10-15 minutes. When the dough reaches a mouldable consistency, it will spread to a thickness of 0.5 cm, and a cookie cutter shapes it into a uniform form. The shaped cookies were put into the baking sheet and baked in the oven at 150-180°C for 20 min. Cookies are then put at room

temperature to let them cool before physical analysis (spread ratio and hardness) and chemical analysis (fat and mineral content).

The spread ratio analysis of cookies follows the method described by Mudgil et al. (2017). Diameter is measured using a digital vernier calliper, where six cookies are measured, then rotate 90° and measured again, and the average value of the cookie is calculated. The thickness of the six cookies was also measured and calculated. The spread ratio of cookies was measured from the ratio of the average diameter value and the average thickness value, as shown in the following equation.

$$\text{Spread Ratio} = \frac{\text{average cookies diameter (mm)}}{\text{average cookies thickness (mm)}}$$

The hardness of cookies is measured using a texture analyzer. This was achieved by measuring the peak force during penetration using a 2 mm cylinder probe. The specific settings, including a return to starting cycle, a pre-test of 1 mm/s, a test of 0.5 mm/s, a post-test speed of 10 mm/s, and a penetration distance of 5 mm.

The fat content of cookies will be analysed using Soxhlet extraction (Association of Official Analytical Chemist (AOAC), 2005) and calculated using the following equation. Meanwhile, the mineral analysis of cookies was determined using the same method and equation used to determine BF and HBF mineral content.

$$\text{Fat (\%)} = \frac{a - b}{c} \times 100$$

Where:

- a = final weight of flask
- b = initial weight of flask
- c = initial weight of sample used

Statistical Analysis

The data obtained will then be analysed using two-way ANOVA, and Duncan post hoc test with a completely randomised design with six levels of one factor and three repetitions. The experimental design employs

a completely randomized two-factor design. The factors that will be analyzed are the type of fat and the ratio of WF substituted with CF. The ratio of WF and CF consists of five levels; meanwhile, there will be two levels of type of fat: butter or margarine. The analysis includes three replications.

RESULTS AND DISCUSSION

Characteristics of BF and HBF

Table 2 displayed the characteristics of BF and HBF. The moisture of obtained BF is $11.35 \pm 0.05\%$. Dhawan et al. (2019) conducted an analysis using a cabinet dryer at 60°C for 24 hours, resulting in a moisture content of BF at $6.30 \pm 0.2\%$. The beetroot was dried using a food dehydrator at 50°C for 10 hours in this study. The difference in value occurs due to temperature and time, affecting the moisture content value. Longer time and higher temperatures result in lower moisture content (An et al., 2016). A higher moisture content of BF resulted in a lower yield than HBF.

Based on Table 2, L* value of HBF (36.55 ± 0.01) is higher than BF (25.47 ± 0.06) due to the native colour of beetroot darker than holy basil. Beetroot contain pigmen betalains. Betalains comprise 75-95% of betacyanins (red) and betaxanthins (yellow). Compared to betaxanthins, betalains are more unstable toward thermal processing. In this study, beetroot underwent a drying process using a food dehydrator at 50°C. Thus, there were some possibilities for betalains in beetroot to degrade their colour, leading to darker colour and lower lightness (Chandran et al., 2014). Wattanakul et al. (2024) produced freeze-dried HBF with L* of 58.19 ± 0.14 , which is higher than L* obtained in this study. This study used thermal drying, which resulted in a darker colour.

Table 2 displays that HBF contains higher ash content ($12.24 \pm 0.10\%$) than BF (9.12 ± 0.06). This result correlates with the

higher mineral content in HBF than in BF. Hamid and Mohamed Nour (2018) produced BF with $2.96 \pm 0.27\%$ of ash content, which is lower than the ash content of BF ($9.12 \pm 0.06\%$) in this study, as shown in Table 2. Hamid and Mohamed Nour (2018) used mesh size of 40, thus resulting in a bigger particle. Meanwhile, this study used 80 mesh size, which resulted in finer particle. According to Aliah et al. (2023), the ash content is influenced by particle size, where a smaller particle size will produce a high ash content and vice versa. When using smaller particles during ashing, combustion achieves completeness. Sample with larger particle sizes has a looser particle density. Thus, the ash content is low.

Conversely, the ash content of HBF in this study, as seen in Table 2, is lower ($12.24 \pm 0.10\%$) than the ash content of Pakistan holy basil ($14.21 \pm 1.50\%$) (Ashif and Shafqat Ullah, 2013) the difference in the ash content due to the difference in the drying process. The Pakistan holy basil used shade dried, while the HBF in this study used a food dehydrator. This result was in line with Sonkamble and Pandhure (2017) and Alara et al. (2018), where shade drying has higher ash and moisture content than cabinet or sun drying. Shade drying can maintain the morphological appearance of the leaf, while sun drying and cabinet drying can damage the morphological appearance due to the faster decrease in moisture content, which resulted in reduction of mineral content thus also affected to the ash content. Moreover, the difference in the analysis method also impacts the mineral analysis where Ashif and Shafqat Ullah (2013) use atomic adsorption spectrophotometry while the resulting HBF uses ICP-OES method.

Mineral Content of Cookies with Different Ratio of WF to CF and Different Type of Fats

Several minerals, namely Ca, Mg, and Fe, were analysed in this study. Ca is a vital nutrient commonly associated with the metabolism and formation of bone. Fe functions as several components in the human system, including enzymes and haemoglobin, and transports oxygen to tissues through the body for metabolism. Meanwhile, Mg is the fourth most common mineral in the human body and involves many functions and roles, most of them as enzymes. It involves a cofactor of more than 300 enzymes, such as protein synthesis, nerve function, and blood pressure regulation (Rizqiah and Romadhan, 2024; van Drongkelaar et al., 2018).

The RDA of Ca varies depending on age. Adult 19 to 50 years old, the recommended daily allowance for Ca is 1000mg/day. The RDA of Fe is approximately 16 to 18 mg/day for men and 12 mg/day for women, and the upper intake limit is 45 mg/day for adults (Pullakhandam et al., 2015). In addition, the RDA for Mg in adults 19-30 years is 400mg/day for men and 310 mg/day for women, and for 31-50 years is 420mg/day for men and 310mg/day for women (Schwalfenberg and Genuis, 2017).

Based on Table 3, there was a significant increase in Ca and Mg content as the higher the substitution of CF toward WF for every type of fat. The result in Table 3 also aligns with Ingle et al. (2017). Ingle et al. (2017) made cookies substituting beetroot powder and basil powder. The resulting cookies exhibit an increasing value in Ca content. It happened because beetroot and basil are food commodities with higher Ca, as shown in Table 2. Furthermore, Chinma et al. (2012) reported cookies prepared with unripe plantain flour contain 289.30 ± 0.91 mg/100g of Mg, which is higher than cookies prepared with WF (57.19 ± 0.84 mg/100g).

Table 3 shows that substituting CF to WF causes the decrease in the Fe content of cookies. The cookies prepared with WF or

without CF substitutions show the highest Fe content. It happened because the WF used in this research had already been fortified with 40% of Fe, thus suppressing the Fe content of BF and HBF itself. Cookies with a ratio of 90:10 and 70:30 show a significant difference where margarine use is higher than butter. In contrast, 80:20 and 60:40 show an opposite result: cookies that used butter have higher Fe content than butter. This phenomenon happens because the insoluble Fe increases after baking, followed by a decrease in ferric iron and the loss of elemental Fe in the flour (Diego Quintaes et al., 2017).

All the formulation cookies contain 10% Ca and 10% Mg needed based on RDA. Thus, all cookies produced in this study categorized as good source of Ca and Mg. In addition, all cookies in this study are also categorized as good source of iron because they contain more than 20% of the RDA for Fe.

Fat Content of Cookies with Different Ratio of WF to CF and Different Type of Fats

The statistical analysis showed that only the WF to CF ratio affected the significant difference ($p < 0.05$) of the fat analysis. In contrast, there was no interaction between ratios and type of fat toward the fat content of cookies. Moreover, the type of fat also did not show any significant difference ($p > 0.05$) because butter and margarine have similar fat content, which is approximately around 80% (Patel et al., 2016).

As seen in Table 4, the higher the ratio of CF, which consists of BF and HBF, the lower the fat content. The fat content in this research shows a similar result to Ingle *et al.* (2017), where higher substitution of BF in CF results in lower fat content in the cookies. Meanwhile, the substitution of HBF did not affect the fat content of the cookies. The BF have a lower fat content ($0.99 \pm 0.06\%$) compared to HBF ($3.88 \pm 0.38\%$); thus, the fat

content of BF will affect the fat content of cookies, resulting in a lower value of the fat content. The fat content of margarine and butter cookies is $22.41 \pm 1.49\%$ and $22.50 \pm 0.83\%$ respectively.

Hardness of Cookies with Different Ratio of WF to CF and Different Type of Fats

Hardness shows the force needed to deform a food substance by imitating the human bite and mastication through a machine (Peleg, 2019). As seen in Table 5, the higher the substitution of the CF, the higher the hardness. The hardness result obtained was in line with Ingle et al. (2017), the higher the substitution of beetroot powder, the harder the cookies.

The hardness increases due to the nature of BF and HBF, in which water adsorption is high. Fibre and high protein-containing flour can cause the increased value of hardness because of the total fibre in dried BF (7.68%), and HBF crude fibre (16.90%) is higher than the total fibre in WF ($0.36 \pm 0.07\%$); thus, the higher the substitution of CF the value of hardness also increasing (Heshe et al., 2016; Shende, 2020; Shokry, 2018).

Spread Ratio of Cookies with Different Ratio of WF to CF and Different Type of Fats

The spread ratio can be a parameter to determine the quality of a cookie; the higher the value of the spread ratio, the more desirable the cookies (Mudgil et al., 2017). Table 5 indicates that cookies made with margarine demonstrate a significant increase in spread ratio when CF replaces WF at a ratio of 90:10. However, this increase did not occur in cookies made with butter. A significant decrease in the spread ratio for cookies made with butter happened when the CF ratio increased from ratio of 20 (6.17 ± 0.41) to ratio of 30 (5.61 ± 0.52). Increasing WF: CF substitution at 80:20 and

70:30 also cause the spread ratio for cookies made with margarine to decrease significantly. However, the spread ratio showed no significant difference when the substitution of CF higher than 20 at 70:30 and 60:40 formulation, either margarine or butter.

The decreasing spread ratio happens because the amount of fibre increases for each formulation, where fibre causes an increase in water absorption, resulting in a lower spread ratio. This result correlates with the hardness analysis, indicating that more significant substitution of CF increases hardness value. The resulting data is in line with Sahni and Shere, (2016) and Ingle et al. (2017), where the higher substitution of beetroot powder causing the spread ratio to be decreasing. Both types of research indicate a high spread ratio when using beetroot powder at 5% and 10%.

CONCLUSION

Cookies made with a substitution ratio of 80:20 of WF: CF and butter as the fat used were selected as the best formulation. This formulation contained 160.01 ± 3.17 mg/100g of Ca and 46.62 ± 0.04 mg/100g of Mg. The increased Ca and Mg content in the selected ratio, compared to the 100:0 WF:CF ratio (which contained 110.98 ± 1.2 mg/100g of Ca and 18.86 ± 0.01 mg/100g of Mg), is primarily attributed to the addition of BF and HBF in the CF.

Meanwhile, cookies with the the best ratio contained 5.85 ± 0.24 mg/100g of Fe which lower than cookies made only with the WF (7.16 ± 0.09 mg/100g). WF used in this study was already fortified with 40% Fe, which may have suppressed the contribution of Fe from BF and HBF. Despite this, all cookies produced in this study are categorized as good sources of Ca, Fe, and Mg. Additionally, the best formulation had a fat content of $23.43 \pm 0.46\%$, a hardness value of 2047.06 ± 35.06 g, and a spread ratio of 6.17 ± 0.41 .

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Table 1. Formulation of cookies

Ingredient	Amount (g)
Flour*	100
Sugar	60
Fat (butter/margarine)	50
Egg yolk	15
Baking Soda	3

Note: *) based on treatment (ratio WF: CF = 100:0,90:10,70:30,50:50, 60:40)
 weight of other ingredients based on the weight flour
 Source: Herlina and Sinaga (2022)

Table 2. Characteristics of BF and HBF

Parameter	BF	HBF
Yield (%)	6.42 ± 0.002 ^a	8.27 ± 0.001 ^b
Lightness (L*)	25.47 ± 0.060 ^a	36.55 ± 0.010 ^b
Moisture Content (%)	11.35 ± 0.050 ^a	6.87 ± 0.030 ^b
Ash (%)	9.12±0.060 ^a	12.24±0.100 ^b
Ca (mg/100g)	98.23±1.560 ^a	1898.77±13.050 ^b
Fe (mg/100g)	2.29±0.030 ^a	8.27±0.020 ^b
Mg (mg/100g)	186.81±2.250 ^a	304.01±2.690 ^b

Note: Value with different superscript in the different column has a significant differences at 5%

Table 3. Mineral content of cookies with different ratio of WF:CF and different types of fat

WF:CF	Types of Fat	Mineral Content (mg/100 mg)		
		Ca	Fe	Mg
100:0	Margarine	110.98±1.2 ^a	7.16±0.09 ^d	18.86±0.01
90:10		157.91±0.28 ^c	7.41±0.07 ^c	29.64±0.32
80:20		195.16±0.84 ^e	4.97±0.01 ^a	45.41±0.21
70:30		155.53±2.90 ^c	5.68±0.85 ^c	45.24±0.28
60:40		162.12±4.62 ^c	4.97±0.08 ^a	55.78±1.01
100:0	Butter	129.66±1.92 ^b	8.75±0.08 ^f	13.19±0.01
90:10		179.25±0.81 ^d	5.23±0.14 ^b	33.58±0.57
80:20		160.01±1.19 ^c	5.85±0.14 ^c	46.62±0.04
70:30		208.81±4.31 ^f	4.87±0.00 ^a	52.49±0.24
60:40		280.01±5.42 ^g	7.01±0.18 ^d	65.78±0.08

Note: Value with different superscript in the same column has a significant differences at 5%

Table 4. Fat content of cookies with different ratio of WF:CF

WF:CF	Fat Content (%)		
	Butter	Margarine	Average Fat Content (%)
100:0	21.38	21.02	21.20±0.64 ^a
90:10	22.43	24.00	23.22±1.72 ^c
80:20	23.43	22.65	23.07±0.52 ^{bc}
70:30	22.42	21.43	21.93±0.72 ^{ab}
60:40	22.82	22.89	22.86±0.63 ^{bc}

Note: Value with different superscript in the last column has a significant differences at 5%

Table 5. Physical characteristics of cookies with different ratio of WF:CF and different types of fat

WF:CF	Types of Fat	Physical characteristics	
		Spread Ratio	Hardness (g)
100:0	Margarine	5.73±0.60 ^{ab}	1582.53±29.83 ^b
90:10		6.61±0.39 ^c	1849.4±72.25 ^{cd}
80:20		6.36±0.20 ^c	1952.17±12.82 ^{cd}
70:30		5.67±0.23 ^a	2189.98±20.11 ^{ef}
60:40		5.64±0.03 ^a	2780.02±252.47 ^g
100:0	Butter	6.46±0.12 ^c	1277.54±72.48 ^a
90:10		6.35±0.15 ^c	1834.71±47.32 ^c
80:20		6.17±0.41 ^{bc}	2047.06±35.06 ^{de}
70:30		5.61±0.52 ^a	2289.93±145.18 ^f
60:40		5.34±0.23 ^a	2732.45±160.34 ^g

Note: Value with different superscript in the same column has a significant differences at 5%