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Mineral Content and Antioxidant Capacity of Cookies Formulated with Spinach and Pangas Catfish

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Abstract

Spinach is a leafy green vegetable that contains various nutrients and antioxidant compounds. Meanwhile, Pangas catfish contains calcium and minerals that are very good for health. Spinach and Pangas catfish can be utilized as raw ingredients to manufacture functional food products in the shape of cookies, increasing the nutritional value and antioxidant content of the cookies to increase the fulfillment of food and public health needs. This research was conducted from February to July 2024. Cookies were prepared with three variations of spinach flour to Pangas catfish flour ratio designated as F1 (45:15), F2 (30:30), and F3 (15:45). Analysis of mineral, vitamin C, TPC, TFC, and antioxidant activity was performed on each cookie formula. The study results showed that the mineral content in terms of potassium and calcium was in the range of 323.538 to 671.186 mg/100g and 44.307 to 93.006 mg/100g, respectively. Meanwhile, the bioactive compounds in the form of vitamin C, TPC, and TFC were in the range of 9.92 to 20.02 mg/100g, 16.74 to 24.97 mg GAE/g, and 32.55 to 110.84 mg QE/g, respectively, and the antioxidant activity was 8.11-17.84%. The cookies prepared using the formula F1 showed significant content of minerals, i.e., potassium and calcium, vitamin C, TPC, TFC, and significant antioxidant activity. These cookies have the potential to be developed as a functional food product that is rich in nutrients and antioxidants to better meet the nutritional and public health needs.

Introduction

Spinach is a leafy vegetable that is very popular. The most consumed part of spinach is the characteristic green leaves. Various nutritional contents, including vitamin C, carotene, calcium (Ca), potassium (K), phosphorus (P), dietary fiber, antioxidants, and bioactive compounds of spinach have shown their potential benefits for health and disease control (Lasya, 2022; Munir *et al*., 2019). The mineral content, such as calcium, is reported to be more than 25%, while the potassium content is around 171.69 mg/100g (Galla *et al*., 2017). Dietary fiber from spinach may help reduce total cholesterol and plasma triglyceride levels while increasing HDL (Drisya *et al*., 2015). The antioxidant content, vitamin C, phenolics, and flavonoids act as anticancer agents (Jyoti *et al*., 2022).

Fish is a high-protein food source. Fish can be processed into various forms so that it can influence people's consumption levels of fish (Widyaningrum *et al*., 2022). Several types of fish, including Pangas catfish (Pangasius pangasius), can be processed into processed food products. Pangas catfish contains protein,

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fat, carbohydrates, ash, and water. Catfish have a higher fat content compared to other freshwater fish like snakeheads and goldfish (Yaqin *et al*., 2021). In addition, the meat of Pangas catfish is easily digestible by the intestines and contains calcium and minerals that are very good for health. The high nutritional content of Pangas catfish shows its potential to be developed as a functional food product in order to better meet food and public health needs. Spinach and Pangas catfish have been extensively researched and used as main ingredients of food products. Spinach has been used in products such as biscuits (Jyoti *et al*., 2022), sandwich biscuits, food bars (Ariyanti *et al*., 2022), cookies (Yudhistira *et al*., 2019), and cakes (Koc *et al*., 2019). Pangas catfish has also been used in products such as snack bars, biscuits, cookies, and nuggets (Putri *et al*., 2023).

Cookies are one of the most popular and extensively consumed food products on the market due to their variety of forms and flavors, long shelf life, and inexpensive pricing, occupying an important position in the snack food industry. Cookies are a sort of snack or dessert produced from a soft, high-fat dough consisting of wheat flour, vegetable or animal fat, and leavening agents. Cookies are often abundant in sugar and energy, but poor in minerals, fiber, antioxidants, and bioactive substances (ŠťAstná *et al*., 2021). Currently, consumers tend to choose to consume healthy foods, including cookies made from natural ingredients, because of the increasing public awareness of the health implications and understanding of the importance of nutritional content and bioactive compounds in food.

Using natural substances also avoids the overuse of synthetic additives, which have been linked with carcinogenic effects (Najjar *et al*., 2022). Wheat flour, which is the main ingredient in the manufacturing of cookies, can be replaced with other flours made from natural ingredients that are rich in antioxidant compounds, thus providing healthier product choices to the public as consumers (Antonic *et al*., 2021). Replacing wheat flour can also enrich the nutritional content, especially vitamins and minerals. It also aims to reduce the community's dependence on wheat flour (Ariesthi *et al*., 2021; Soesilowati *et al*., 2018). Several studies have examined the potential of natural ingredients in the development of functional food products. The use of spinach flour and Pangas catfish flour can increase the nutritional value and antioxidant content, so that cookies are not mainly high in carbohydrates and fat, but are also rich in potential antioxidant compounds for health. This study aimed to analyze the content of minerals, vitamin C, total phenolics content (TPC), total flavonoids content (TFC), and antioxidant activity of cookies formulated with spinach and Pangas catfish.

Methods

The main ingredients used in this research were spinach flour and Pangas catfish meal. A total of 300 g of spinach leaves were dried in a dehydrator at 60°C for approximately 19 hours. After that, mashed in a blender for 5 minutes and sifted through a No. 70 mesh sieve. One kilogram of fresh Pangas catfish was cleaned and flavored with lime juice and salt. They were then steamed for about 10 minutes until cooked. After that, the pangas catfish were crushed, the fat was squeezed out with a cloth, and the crushed Pangas catfish were dried in a dehydrator for 10 hours at a temperature of 40°C. The dried, crushed Pangas catfish was ground using a blender, then mixed with wheat flour until evenly mixed, then sieved with a No. 80 mesh sieve. The approval and ethical clearance from the Health Research Ethics Committee of Poltekkes Kemenkes Palangka Raya was attained upon commencement of the study [Reference No: 178/III/KE.PE/2024].

The main ingredients used were spinach flour and Pangas catfish meal, with three formulas labeled F1 (45 g spinach flour and 15 g Pangas catfish meal), F2 (30 g spinach flour and 30 g Pangas catfish meal), and F3 (15 g spinach flour and 45 g Pangas catfish meal). Other ingredients used, consisting of wheat flour, eggs, milk powder, margarine, baking powder, vanilla, salt, and sugar, were purchased from local markets. All ingredients were mixed according to the formulation and then stirred until evenly mixed. The mixture was then poured into the mold and baked in the oven at 100°C for 35 minutes. The cookies were stored in a tightly closed container. Analysis of calcium and potassium

mineral contents was performed using atomic absorption spectroscopy (AAS) (Bolang *et al*., 2022). The vitamin C content (mg/100 g) of the cookies produced with three different formulas was analyzed and determined by iodometric titration (Bahar *et al*., 2021; Dinnah *et al*., 2020). TPC was measured using UV-Vis spectrophotometry with the Folin-Ciocalteu reagent. TPC was reported as mg gallic acid equivalent (GAE) per gram dry weight of the sample (Azuan *et al*., 2020; Najjar *et al*., 2022). TFC was measured using UV-Vis spectrophotometry with the aluminum chloride colorimetric method. TFC was expressed as mg of quercetin equivalents (QE) per gram of dry sample weight (Nabil *et al*., 2020; Najjar *et al*., 2022). The antioxidant activity test was performed using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) method. The wavelength at which the absorbance was measured was 520 nm (Chikpah *et al*., 2023; Mahloko *et al*., 2019).

Result and Discussion

Healtay food is necessary to improve the quality of human life. Nutrients include minerals and naturally occurring bioactive substances with antioxidant activity that can be found in many healthful diets. Vegetables contain most of the natural polyphenols and are also a source of a variety of nutrients such as vitamins, minerals, and fiber. Fish, however, is high in minerals and protein (Dhalaria *et al*., 2020; Igbinosa *et al*., 2013). Minerals in food are crucial for human well-being. Minerals have anti-oxidant properties that can prevent and treat certain chronic diseases (Rahaman *et al*., 2023). The body's mineral balance can control membrane transport, osmotic pressure, acid-base balance, metabolic processes, and tissue development. Minerals indirectly contribute to growth. The roles of minerals in the body are interrelated (Haro *et al*., 2018). In this study, the contents of potassium, calcium, vitamin C, total phenolics, and flavonoids, and antioxidant activity of the cookies formulated with spinach and Pangas catfish were analyzed. Food contains minerals, which are essential nutrients that organisms require to grow and carry out a variety of vital tasks to survive (Ferreira, 2023; Weyh *et al*., 2022). Calcium and potassium are needed for the repair of damaged cells, the formation of bones and teeth, the formation of red blood cells, and body mechanisms (Cheng *et al*., 2015; Gebreslassie, 2016).

Analysis of potassium content showed that F1 cookies contained 671.186 mg of potassium per 100g of cookies, F2 cookies contained 382.672 mg of potassium per 100g of cookies, and F3 cookies contained 323.538 mg of potassium per 100g of cookies. Meanwhile, analysis of calcium content showed that F1 cookies contained 93.006 mg of calcium per 100g of cookies, F2 cookies contained 54.578 mg of calcium per 100g of cookies, and F3 cookies contained 44.307 mg of calcium per 100g of cookies (Table 1). The results of potassium (K) content analysis showed that the highest potassium content was in F1 cookies with 671.186 mg/100 g, which was higher than that of F2 cookies with 382.672 mg/100 g and F3 cookies with 323.538 mg/100 g. The potassium content in the cookies increased significantly in proportion to the addition of spinach flour ($p<0.05$). Spinach flour has a relatively high potassium content of 233.38 mg/100 g (Waseem *et al*., 2021). A study by Ariyanti *et al*. (2022) showed that the potassium content of food bar P1, which contained a 10% blend of oat and amaranth flour, was 52.66 mg/100g. The use of a 20% spinach flour as a substitute in the production of food products

TABLE 1. Minerals and Vitamin C Content of Cookies Formulated with Spinach and Pangas Catfish

Cookie	K (mg/100 g)	Ca (mg/100 g)	Vitamin C (mg/ 100 g)
F1	**671,186 ± 0,14***	**93,006 ± 0,10***	**20,02 ± 0,02***
F2	**382,672 ± 0,15***	**54,578 ± 0,15***	**14,54 ± 0,11***
F3	**323,538 ± 0,07***	**44,307 ± 0,08***	**9,92 ± 0,02***

*The data shows the mean ± standard mean error of readings taken in triplicate.
Source: Primary Data, 2024

resulted in a potassium content of 493.86 mg/100 g, while the use of 2.5% spinach flour as a substitute resulted in a potassium content of 452.01 mg/100 g (Waseem *et al*., 2021). The potassium mineral content of fresh and grilled Pangas catfish is known to be 239.70 and 319.44 mg/100 g, respectively (Ajai *et al*., 2019). In a study, the combination of 100% superior quality breadfruit flour, 0% wheat flour and fishmeal produced the highest potassium content, 582 mg/100 g, while the combination with a percentage of 86.53%: 6.79%: 6.68% produced the lowest potassium content, 377.50 mg/100 g (Bakare *et al*., 2020). These results suggest that the increase in potassium levels in the cookies was influenced by the good micronutrient profile of spinach flour.

The results of calcium (Ca) content analysis showed that the highest calcium content was in F1 cookies with 93.006 mg/100 g, followed by F2 cookies with 54.578 mg/100 g and F3 cookies with 44.307 mg/100 g. Spinach flour has a relatively high calcium content, 1,304 mg/100 g. The use of 20% spinach flour as a substitute in the production of food products resulted in high calcium content, which was 301.26/100 g, while the use of 2.5% spinach flour as a substitute resulted in the lowest calcium content, i.e., 72.71 mg/100 g (Waseem *et al*., 2021). The calcium contents of fresh and grilled Pangas catfish were 69.35 and 110.824 mg/100 g, respectively, according to Ajai *et al*. (2019). The combination of 100% superior quality breadfruit flour, 0% wheat flour and fishmeal produced the lowest calcium content, which was 100 mg/100 g, while the combination with a percentage of 60%: 0%: 40% produced the highest calcium content, 754 mg/100 g (Bakare *et al*., 2020). The calcium content in the cookies increased significantly in proportion to the addition of spinach flour ($p<0.05$). These results suggest that the increase in calcium levels in the cookies was influenced by the high calcium content of the spinach flour.

One kind of vitamin that is essential to the body's health is vitamin C, which also functions as an antioxidant. It is a crucial cofactor in the body's metabolic processes and helps in hormone synthesis (Dosedˇ *et al*., 2021; Koc *et al*., 2019). In order to limit the amount of vitamin C content lost during processing, it is crucial to determine the vitamin C content present in food products. The results of the analysis of vitamin C content in the cookies showed that F1 contained 20.02 mg of vitamin C per 100 g of cookies, F2 contained 14.54 mg of vitamin C per 100g of cookies, and F3 contained 9.92 mg of vitamin C per 100g of cookies (Table 1). The statistical analysis resulted in a significant difference ($p<0.05$) in vitamin C content between cookies made with different formulations. Based on the analysis of vitamin C levels, F1 cookies had the highest vitamin C level (20.02 mg/100 g), which were prepared using the 45 g of spinach flour and Pangas catfish flour 15 g. Meanwhile, F2 and F3 cookies had low vitamin C content, 14.54 mg/100g and 9.92 mg/100g, respectively, with the use of less spinach flour than F1, which was 30 g for F2 and 15 g for F3. The vitamin C content in the cookies increased significantly with the addition of spinach flour ($p<0.05$). Increasing the amount of spinach increased the vitamin C content of the cookies to increase because spinach has a relatively high vitamin C content, 61.29 mg/100 g of material (Singh & Harshal, 2016). One could argue that the product's vitamin C content increases with the amount of spinach flour added to the cookies (Koc *et al*., 2019). In this instance, though, the cookies' vitamin C level was minimal. The amount of vitamin C in food products, including cookies, is negatively impacted by food processing methods, including baking and drying. Food processing involves heating food in order to prepare it for consumption and extend the shelf life of food products (Galla *et al*., 2017; Lešková *et al*., 2006; Rakcejeva *et al*., 2011). Phenolic compounds are potential compounds containing hydroxyl groups. The level of phenolic chemicals in antioxidant activity plays a role in donating hydrogen ions from their hydroxyl groups to scavenge free radicals (Chong *et al*., 2022; Lim *et al*., 2023). Plant phenolic chemicals, which possess potent antioxidant qualities, have been demonstrated scientifically to avert a range of chronic diseases linked to oxidative stress, including cardiovascular ailments, cancer, and neurological disorders (Bhuyan & Basu, 2018).

The TPC of the cookies formulated with spinach and Pangas catfish are shown in Table

TABLE 2. TPC and TFC of Cookies Formulated with Spinach and Pangas Catfish

Cookies	TPC Value (mg GAE/g)	TFC Value (mg QE/g)
F1	24,97 ± 0,08*	110,84 ± 0,08*
F2	20,33 ± 0,10*	68,34 ± 0,11*
F3	16,74 ± 0,15*	32,55 ± 0,03*

*The data shows the mean ± standard mean error of readings taken in triplicate.
Source: Primary Data, 2024

2. The total phenolic contents of the cookies prepared with formulas F1, F2, and F3 were 24.97, 20.33, and 16.74 mg GAE/g, respectively. After analyzing the cookies made using the three recipes, the analysis revealed a significant difference ($p<0.05$), and the highest TPC was found in cookies prepared with the formula containing the most spinach, namely F1, followed by F2 and F3. This is because the total phenolic content in fresh spinach is quite high, 2088 mg GAE/kg, or ranging from 183.2 to 1344.7 mg/100 g, according to studies (Bunea *et al*., 2008; Turkmen *et al*., 2005). Increasing the amount of spinach had an effect on increasing the TPC in cookies because of the high bioactive compounds in spinach. The results of other studies showed that an increase in the total phenolic content in cookies occurred along with the addition of clove flour concentration, depending on the formula used. Clove flour has high bioactive properties (Aljobair, 2022). In this study, the process of processing spinach powder into cookies affected the TPC of the cookies. These results are consistent with studies by Mohd *et al*. (2023) and Ikram *et al*. (2022), which stated that heating is a processing method that can affect the levels of secondary metabolites, including TPC. When a phenol-containing plant is heated, its TPC value can increase, and the antioxidant activity becomes better because the phenolic compounds that accumulate in the cell membrane vacuoles are released as the cell components and cell membrane are damaged (Fei *et al*., 2018).

Flavonoid substances are secondary metabolites found in plants that feature an aromatic ring and at least one hydroxyl group. In addition to vitamin C and phenolic content, the content of flavonoid compounds in foods with antioxidant activity has attracted attention in the fields of nutrition, food, and health (Aryal *et al*., 2019). Flavonoids are believed to have anti-inflammatory, anticarcinogenic, diabetes mellitus, anticancer, antiallergic, antimicrobial, and antioxidant properties, and cardiovascular disease, and obesity (Kustiawan *et al*., 2022; Xhaxhiu *et al*., 2023). The TFC of the cookies formulated with spinach and Pangas catfish is in Table 2. The total flavonoid contents in the cookies prepared with formulas F1, F2, and F3 were 110.84, 68.34, and 32.55 mg QE/g, respectively. The results of the analysis showed that there were significant differences, and the highest total flavonoid content was found in cookies prepared with formula F1, followed by those prepared with formulas F2 and F3. The total content of flavonoids in the cookies prepared with formulas F1, F2, and F3 was high, 110.84, 68.34, and 32.55 mg QE/g, respectively. In addition to the increase of vitamin C and total phenolic content, the increase in the amount of spinach powder resulted in the increase of total flavonoids in the cookies. Sarker *et al*. (2020) discovered that spinach includes flavonoid molecules, which contribute significantly to spinach's high antioxidant activity. Quantitative investigations revealed that the total flavonoid concentration in spinach is approximately 1000-1200 mg/kg, or 128.54 mg QE/100 g (Hossain *et al*., 2017; Singh *et al*., 2018). The flavonoid content increases and is effective for extraction at temperatures ranging from 50 to 130 °C (Howard & Pandjaitan, 2008).

DPPH radical scavenging reflects a sample's antioxidant capacity. A sample's antioxidant activity is essentially measured by the total phenolic and flavonoid content produced during non-enzymatic browning processes (Mohd *et al*., 2023). The percentage of DPPH radical scavenging activities of the cookies prepared using formulas F1, F2, and F3 was 17.84%, 13.45% and 8.11%, respectively (Table 3). The statistical analysis resulted in a significant difference in the percentage of

TABLE 3. DPPH Radical Scavenging Activity of Cookies Formulated with Spinach and Pangas Catfish

Cookies	%DPPH Radical Scavenging
F1	17,84 ± 0,14*
F2	13,45 ± 0,34*
F3	8,11 ± 0,14*

*The data shows the mean ± standard mean error of readings taken in triplicate.
Source: Primary Data, 2024

DPPH radical scavenging activity ($p<0.05$) between the cookies prepared using the three formulas. The cookies prepared with formula F1 showed the highest activity compared to the cookies prepared with formulas F2 and F3. These findings suggest that the addition of spinach flour increased the antioxidant activity of the cookies.

Fresh spinach has an antioxidant activity of no more than 19.8% (Ligor *et al*., 2013). Hatamian *et al*. (2020) found that roasting soybeans significantly increased their antioxidant activity ($P < 0.05$). Melanoidin production may account for the increase in antioxidant activity observed during the roasting process. Melanoidin formation is contributed to by non-enzymatic browning reactions that occur during heating (Ahmed *et al*., 2020; Hatamian *et al*., 2020). These findings emphasize the potential of roasting as a method for improving the antioxidant capabilities of spinach and its potential as a key ingredient in the production of cookies, as spinach has demonstrated its potential as a food ingredient rich in antioxidants. Vitamin C and bioactive compounds such as phenolics and flavonoids are thought to be the primary contributors to antioxidant activity in plants, including spinach, which can be employed as a main ingredient in food products, such as cookies (Jing *et al*., 2015; Šťastná *et al*., 2021).

Conclusion

This research has shown that cookies prepared with formula F1 contain minerals, vitamin C, total phenolics, and flavonoids content in significant amounts and exhibit antioxidant activity. Increasing the amount of spinach has increased the content of minerals, vitamin C, total phenolics, and flavonoids in the cookies. It has also increased the antioxidant activity of the cookies because of the high bioactive compounds in spinach. Spinach flour and Pangas catfish meal can be recommended as main ingredients and functional food ingredients to increase the nutritional value and antioxidant content of the cookie product. Further study must be conducted to analyze the nutritional content of cookies, especially their relationship with the use of Pangas catfish as one of the main ingredients. In the future, the study can be continued in clinical trials to observe whether or not these cookies can have a good impact on public health.

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