

Effect of Different Cooking Methods on Chlorophylls and Total Carotenoids of Spirulina Powder-Enriched Snack

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Abstract

Chlorophylls and carotenoids are the source of bioactive compounds exerting a positive effect as antioxidants. In this study, the effect of four cooking methods (steaming, pan frying, microwaving, and boiling) on the chlorophylls and carotenoids content of Spirulina-enriched snack was investigated. Chlorophylls and carotenoids were spectrophotometrically determined in both raw and cooked snack samples. Results showed that pan frying and microwaving can maintain and even slightly increase the chlorophylls content in the cooked food compared to the uncooked food. The chlorophylls content significantly decrease after cooking by steaming and boiling. Our results also found that all cooking method can increase the total carotenoids in cooked snacks compared to the control due to the breakdown of cell wall to release the carotenoid into the snack. The results showed that pan-frying and microwaving can be considered as the appropriate cooking method to produce Spirulina powder-enriched snack as functional food.

Abstrak

Klorofil dan karotenoid merupakan sumber senyawa bioaktif yang memiliki efek positif sebagai antioksidan. Dalam penelitian ini, pengaruh empat metode memasak (mengukus, menggoreng, menggunakan microwave, dan merebus) terhadap kandungan klorofil dan karotenoid total pada makanan ringan yang diperkaya Spirulina diteliti. Klorofil dan karotenoid ditentukan secara spektrofotometri pada sampel makanan ringan mentah dan matang. Hasil penelitian menunjukkan bahwa menggoreng dan menggunakan microwave dapat meningkatkan kandungan klorofil pada makanan yang dimasak dibandingkan dengan makanan mentah. Kandungan klorofil menurun secara signifikan setelah dimasak dengan cara dikukus dan direbus. Hasil penelitian juga menunjukkan bahwa semua metode memasak dapat meningkatkan kandungan karotenoid total pada makanan ringan yang dimasak dibandingkan dengan kontrol karena adanya pemecahan dinding sel untuk melepaskan karotenoid ke dalam makanan. Hasil penelitian menunjukkan bahwa menggoreng, dan menggunakan microwave dapat dianggap sebagai metode memasak yang tepat untuk memproduksi makanan ringan yang diperkaya dengan bubuk Spirulina sebagai makanan fungsional.

INTRODUCTION

Spirulina is a type of cyanobacterium microalgae, that can grow in both seawater and freshwater [1]. Commercial Spirulina is mostly available in form of dried biomass and widely used as an additive in pharmaceutical and cosmetic products as well as a natural food colouring agent. The increasing consumer awareness regarding the importance of natural colouring agent for the nutritional and health-related benefits have led to an

increasing number of applications of the natural pigment from Spirulina in food industry.

Spirulina contains three types of photosynthetic pigments, including chlorophylls, phycobiliproteins, and carotenoids [2]. Chlorophylls in Spirulina consist of chlorophyll a and chlorophyll b. However, chlorophyll a is more abundant in Spirulina than chlorophyll b [3]. These pigments play an important role in the ability of Spirulina to capture light for photosynthesis and also have several bioactive properties that benefit for human health, especially antioxidant properties

[4]. Chlorophylls and phycobiliproteins are the dominant pigments in *Spirulina* and responsible for the green and blue colors, respectively [2]. However, phycobiliproteins is less stable during food processing than chlorophylls, because the proteins is denatured at the temperature above 40°C, while chlorophylls is still stable at the heating temperature up to 65°C. Therefore, chlorophylls in *Spirulina* are considered responsible for the green natural colorant when being added to a variety of food products to enhance their color.

Carotenoids are accessory photosynthetic pigments and play a role in protecting cell against photooxidative damage in *Spirulina* cells. Carotenoids are responsible for the yellow to red color in *Spirulina* and have strong antioxidant properties than other photosynthetic pigments [4]. Chlorophylls and carotenoids are natural lipid-soluble pigments, which are the source of bioactive compounds exerting a positive effect as antioxidants [5], that can neutralize free radicals and therefore lowering the risk of inflammation, heart disease, and type 2 diabetes as well as preventing cancer [6],[7].

Spirulina also have high nutritional value. The Food and Agriculture Organization (FAO) of the United Nations has designated *Spirulina* as the ideal food for mankind and a “super food” due to the high protein content (60 – 70% on a dry weight basis), vitamins, minerals, and essential fatty acids [8]. Therefore, the *Spirulina* enrichment into a snack has been investigated to increase the nutritional content (protein, lipid, ash content, carbohydrates, carotenoids, in vitro digestible protein) in the enriched snack [9]. Several studies have reported the *Spirulina* enrichment into various food products, e.g. cookies [10], juices [11], yogurts [12], extrudate snacks [9],[13], noodle [14], pasta [15], and sausage [16]. However, most of those studies only reported the nutritional value, physical and sensory acceptance of the enriched-foods. There has been no report regarding to the best cooking methods that are able to maintain the pigment stability as natural food colorants in cooked foods. [17] reported that the nutritional content of *Spirulina* began to experience a significant decrease in the 100°C heating process for more than 15 minutes. Therefore, this study aims to evaluate cooking methods (boiling, pan frying, microwaving, and steaming) on the total chlorophylls and carotenoids of cooked snack products enriched with *Spirulina*.

METHODS

Materials

Dried *Spirulina* biomass was purchased from (Spiruganik, Polaris Food, Indonesia). The flours used were wheat flour (Segitiga Biru, Bogosari, Indonesia). All chemicals used for analysis were obtained from Merck (Darmstadt, Germany).

Sample Preparation

The sample was formulated with wheat flour by addition of 5% *Spirulina* and 500 mL of distilled water, then homogenized. The sample was divided into five: one sample for control (uncooked snack) and four samples for cooked snack using different cooking methods (boiling, steaming, pan frying, and microwaving). For boiling, samples were cooked in boiling water with temperature ranging 80–90°C for 5 min. For steaming, samples were placed in a steamer with a lid for 15 min with cooking temperature of 56°C. For pan frying, samples were cooked in a frying-pan made from Teflon with the cooking temperature of 63.6°C for 5 min. For microwaving, samples were cooked by microwave with 450 Watt for 10 min. All treatments were repeated in triplicate.

Total Chlorophylls and Carotenoids

Chlorophylls and carotenoids were extracted from samples by homogenizing using mortar and pestle in methanol 96% (sample: methanol= 1:3). The homogenate was centrifuged at 4,000 rpm for 10 min. Supernatant (1 mL) was diluted to 5 mL with methanol 96%. Absorbance was read at 653, 666, and 470 nm. Results were calculated using this following formulae [3]:

$$\text{Chl a} = 15.65 A_{666} - 7.34 A_{653}$$

$$\text{Chl b} = 25.05 A_{653} - 11.21 A_{666}$$

$$\text{Total Chlorophylls} = \text{Chl a} + \text{Chl b}$$

$$\text{Car} = \frac{1000A_{470} - 2,86 (\text{Chl a}) - 129,2 (\text{Chl b})}{245}$$

Statistical Analysis

All cooking treatments were performed in triplicate. The obtained data were analyzed using the SPSS package program version 16.0 (SPSS Inc. Chicago, IL, USA) for one-way analysis of variance (ANOVA). Duncan’s multiple range test procedure was used to identify significant differences ($p < 0.05$).

RESULTS AND DISCUSSION

Effects of cooking methods on total chlorophylls and carotenoids content of snack samples were shown in Table 1. Cooking methods resulted in different values for concentrations of chlorophylls and carotenoids. Pan frying and microwaving exhibited no significant differences in total chlorophylls content with control. This indicated that both of these cooking methods can preserve the chlorophylls content in Spirulina-enriched snack during cooking process because the chlorophylls are stable at the temperature below 60–70 °C [18]. The elevating temperature can help to disrupt the cell wall and release the chlorophylls into the materials [20]. However, even though the cooking temperature in steaming was kept below 60°C, but the total chlorophylls were significantly

reduced compared to control. [21] reported that the steaming and microwaving method with cooking time of more than 7 min significantly reduced the chlorophyll content in broccoli. This was consistent to our result that the chlorophyll content in the samples undergo reduction after cooking time of 10 min (microwaving) and 15 min (steaming) due to the degradation process into different compound called pheophytin.

Chlorophylls are sensitive to high temperature [18],[19]. At a temperature exceeding 60 °C, the chlorophylls were degraded and result in reducing the amount of total chlorophylls in the materials [19],[23]. This can explain why the boiling method exhibited the lowest total chlorophylls content compared to another cooking method studied although the cooking time was only 5 min.

Table 1. Effect of cooking methods on chlorophyll a, chlorophyll b, total chlorophylls and total carotenoids contents in the snack

Cooking methods	Cooking methods		Chlorophyll a (mg/g dw)	Chlorophyll b (mg/g dw)	Total Chlorophylls (mg/g dw)*	Carotenoids (mg/g dw)*
	Temperature	Duration				
Control	-	-	153.35±17.90	40.18±11.68	193.54±6.21 ^{cd}	13.25±7.81 ^a
Pan Frying	63 °C	5 min	186.14±9.83	31.56±12.78	217.69±6.45 ^d	44.51±8.94 ^c
Steaming	56 °C	15 min	76.25±13.50	34.13±6.84	110.37±19.94 ^b	32.69±16.19 ^{bc}
Boiling	80 – 90 °C	5 min	50.30±15.72	8.06±6.98	58.35±21.12 ^a	19.15±8.59 ^{ab}
Microwave	~50 - 60 °C (450 W)	10 min	161.35±13.46	19.75±8.73	181.10±9.13 ^c	38.45±6.03 ^c

Notes: Means ± standard deviation (n = 3). *Data in the different letters in the same parameters (column) mean significant differences between samples based on one way ANOVA analysis (p<0.05).

Regarding to carotenoids, all of the cooking methods can increase the carotenoids content in the cooked snack. This indicated that carotenoids are more stable to heat than chlorophylls [18]. The increasing in total carotenoids after cooking was due to the breakdown of cell wall by heating process, and then release the carotenoids that previously trapped within the Spirulina cell. As a result, the total amount of the carotenoids available for measurement in the cooked snack may increase. However, our results indicated that there were significantly difference of carotenoids content after cooking using different four method. The highest losses of carotenoids was noted after cooking using boiling method. [22] reported that the moist-heat cooking methods (boiling and steaming) can result

in losses of carotenoids content due to leaching (dissolve into) the cooking water. This process reduces the carotenoid content in food itself. However, carotenoids are not water-soluble, so they do not transfer easily into water vapor during steaming process, so the losses of carotenoids in steaming method did not significantly occur.

Chlorophyll degradation can cause colour changes in cooked foods. The loss of chlorophylls can cause a colour change from bright green to olive-brown due to the degradation of chlorophyll to pheophytin by the replacement of magnesium in the chlorophyll by hydrogen [24]. The green color in this studied snack was mainly related to the chlorophylls content in Spirulina biomass (Figure 2). The snack color in control was bright blue-green

due to the phycobiliprotein contained in *Spirulina* biomass [2]. However, phycobiliproteins are susceptible to heat, and undergo degradation at a temperature exceeding 45°C [25], so the blue color in the snack disappearance after cooking.

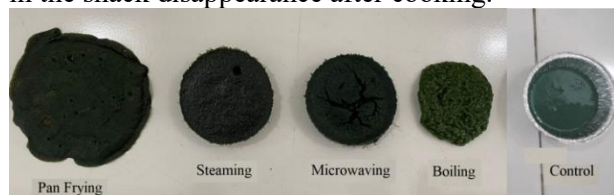


Figure 2. Snacks enriched with *Spirulina* were cooked using different methods: pan frying, steaming, boiling, and microwaving.

The color of food after cooking by pan-frying, microwaving, and steaming are dark green indicating the content of total chlorophylls is still high (exceeding 100 mg/g dw). On the contrary, the color of food after cooking by boiling are moss green indicating the severe degradation on the chlorophylls content. Color of green in *Spirulina*-enriched snack can be a symbol of snack quality processed as functional foods. Therefore, to produce functional foods rich in chlorophylls and carotenoids that are benefit for protecting the human body from several diseases, including obesity and cancer, the selection of processing methods is an important consideration.

CONCLUSION

The cooking methods evaluated in our study demonstrated the effect on the total chlorophylls and carotenoids contents. In the conclusion, among different cooking methods, boiling was the poor cooking method in terms of the loss of chlorophylls and carotenoids content during cooking process. The pan-frying, microwaving and steaming can be considered in processing the *Spirulina*-enriched snack depends on the type of snack produced, because the cooking method will affect the texture of cooked food. Thus, our study brings valuable information about the cooking methods that can maintain the valuable bioactive compounds in culinary enriched by *Spirulina* biomass powder.

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