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Effect of microalgae extract enriched coconut oil on the antioxidant activity, pigment stability, and microstructure of cookies

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Abstract

Microalgae are abundant in lipid-soluble substances that may be extracted with coconut oil, hence promoting antioxidant activity for the prevention of chronic illness and the enhancement of cookie appearance. This study was designed to determine the impact of incorporating microalgae extract-enriched coconut oil from the species *Chaetoceros* sp., *Chlorella vulgaris* and *Skeletonema costatum* on the physicochemical properties, antioxidant activity, and microstructure of cookies. The three different microalgae were extracted with coconut oil and microalgae extract-enriched coconut oils were added to cookie dough. Treated cookies were supplemented with 15% microalgae extract-enriched coconut oils and untreated were fortified with 15% coconut oil. The cookies were then analyzed for physicochemical quality including proximate, water activity, color, pigments content (chlorophyll and carotenoid), antioxidant activity and microstructure analyses. Microalgae extract-enriched coconut oil fortification causes a significant increase in pigments, protein, mineral content and antioxidant activity. It has a high total phenolic content, pigments content (chlorophyll and carotenoid) and antioxidant activity. Cookies containing microalgae extract-enriched coconut oils performed better in terms of free radical scavenging than controls. All microalgae extract-enriched coconut oils are high in chlorophylls (2.5-4.7 µg/g) and carotenoids (0.046-0.39 µg/g) concentration. In particular, *C. vulgaris* extract-enriched coconut oil improved the physicochemical, nutritional quality, and bioactive compounds of cookies.

1. Introduction

Microalgae are microscopic organisms containing valuable nutrients and bioactive compounds that can be exploited as functional food materials and fortifiers (Plaza *et al.*, 2008; Andrade, 2018; Uribe-Wandurraga *et al.,* 2019). They have high contents of protein, vitamins, minerals, polyunsaturated fatty acids (PUFAs), chlorophylls (chls), carotenoids (cars) and polyphenols (phl) (Singh *et al.,* 2005; Becker, 2007; Vaz *et al.*, 2016). They can be considered innovative and promising food ingredients because of their high nutritional value and bioactive contents (Gouveia *et al.*, 2010). In particular, *Chaetoceros* sp. (CA), *Chlorella vulgaris* (CH), and *Skeletonema costatum* (SC) are considered important food ingredients.

CA is a potential microalga for the production of bioactive compounds with economic value. Such compounds include vitamins (1.6%), chls a (1.04%), protein (27.68%), carbohydrates (23.32%), lipids (9.27%), eicosapentaenoic acids (EPA, 5.0%), and docosahexaenoic acids (DHA, 0.5%) (Tsitsa-Tzardis *et al.*, 1993). CH is a chlorophyte microalga that comprises 45.23% total protein, 23.43% carbohydrates and 18.12% lipids (Prabakaran *et al.*, 2018). It also contains chls a (4.7 mg/g); chls b (4.2 mg/g); and high levels of cars (6.11 mg/g), such as lutein, beta carotene, neoxanthin, violaxanthin and zeaxanthin (Hynstova *et al.*, 2018; Prabakaran *et al.*, 2018; Derwenskus *et al.*, 2019). SC is a popular diatom rich in chls a and c; α- and β-carotene; xanthophyll; and protein (21.63% to 32.05%).

The demand for cookies is increasing (Jnawali *et al.*, 2016) due to their good taste, easy availability and affordability (Ekin *et al.*, 2021). The total production of the cookie industry reached USD 6.6 billion in 2016 and was projected to expand by 5.3% from 2019 to 2025 (Market Analysis Report, 2018).

Cookies are made with flour, sugar, fat, and water and are shaped into small, sweet, and crispy pastries

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characterized by high sugar, high fat, and low moisture contents (Xu *et al.*, 2020). High levels of fats in cookie formulations are important in obtaining the desired textural properties (Dinç *et al.*, 2014; Ekin *et al.*, 2021). Shortening is traditionally used in baked goods, such as cookies, to impart a tender texture, mouthfeel, and stability. However, the use of shortening as a food ingredient has reduced in recent years because it has a high content of saturated fatty acids (SFAs), contributes trans fatty acids to baked goods and exerts adverse effects on human health when consumed (Jang *et al.*, 2015; Mert and Demirkesen, 2016). Therefore, alternative approaches to modifying the structure of liquid oil without the use of saturated and trans fats and without changing cookie characteristics are used (Mert and Demirkesen, 2016).

Several studies have been conducted to incorporate microalgae biomass into cookies (Gouveia *et al.*, 2010; Batista *et al.*, 2017; Hossain *et al.*, 2017). However, its incorporation has undesirable effects, such as a pungent odor, dark color and bitter taste (Lafarga, 2019). Microalgae extract is an alternative to microalgae in cookies. Commonly, microalgae bioactive compounds are extracted using an organic solvent, such as butane, acetone, or ethanol (Cerón-García *et al.*, 2018; de Carvalho *et al.*, 2020), or an enzyme (Tavanandi and Raghavarao, 2020). The safety of food-grade solvents must be ensured for human consumption. However, the organic solvents used in extraction have become a public concern due to their safety, adverse effects on humans, and environmental toxicity (Teramukai *et al.*, 2020). As a result, green extraction should be used to extract bioactive compounds for incorporation into foods. Plant or vegetable oils can replace solvents in the extraction of bioactive compounds (Yara-Varón *et al.*, 2017; Rahimi and Mikani, 2019; Teramukai *et al.*, 2020).

Plant oils can be applied to extract phytochemical compounds and pigments from vegetables, fruits, and seaweed for food production (Rahimi and Mikani, 2019; Teramukai *et al.*, 2020). Coconut oil is rich in medium-chain fatty acids, with lauric acids (C12:0) being the major fatty acid (Lockyer and Stanner, 2016; Filipini *et al.*, 2021). Lauric acids are beneficially effective in the reduction of adipose tissue and the improvement of lipid profiles (Filipini *et al.*, 2021). Coconut oil comprises 99.9% fatty acids, which are mainly composed of SFAs (91.6%), monounsaturated fatty acids (MUFAs), and polyunsaturated fatty acids (PUFAs) (Lockyer and Stanner, 2016). Although the use of coconut oil to increase the nutritional value of bread partially and fully (Folasade and Oluwaseun, 2017) has been studied, the addition of coconut oil to cookies has not been investigated.

Microalgae (rich in minerals, proteins, UFAs, pigments, and bioactive compounds), can be extracted using coconut oil, which enhances high antioxidant activity (AA) for the prevention of chronic disease and improves cookie appearance. Therefore, the objective of this study was to evaluate the effect of microalgae extract-enriched coconut oil (MECO) in cookies. It investigated phytochemicals that promote AA as well as innovative technology and product characteristics.

2. Materials and methods

2.1 Materials

Coconut oil and cookie ingredients were purchased from a local market in Semarang Indonesia. The microalgae (CA and SC) were purchased from a brackish water culture research center Jepara, Indonesia and *Chlorella* sp. was purchased from an online market. DPPH was purchased from E-merck (Darmstadt, Germany). Methanol and other chemical substances were obtained from a local chemical store.

2.2 Preparation of microalgae extract-enriched coconut oil

Microalga extraction was conducted in accordance with Teramukai *et al.* (2020) with modifications. CA, CV, and SC powders were diluted in coconut oil and immersed in an ultrasonic water bath for 15 s. The extraction was followed by 24 hrs of heating. Then, the MECO was filtered. The extracted samples were stored in amber bottles at −25°C before further use.

2.3 Preparations of cookies

Cookies were made with the following modified recipe, 45 g of MECO, 40 g of sugar, 5 g of salt, 185 g of wheat flour, and 2 g of baking soda (Table 1). Before the addition of flour, oil and castor sugar were beaten at high speed for 5 mins. The cookies were baked for 30 mins at 150°C. After being cooled to room temperature, the samples were packed in airtight containers and stored at −18°C for subsequent analysis.

Table 1. Cookie formulations (%, w/w).

Ingredients	CO (%)	CA (%)	CH (%)	SC (%)
Vegetable/microalgae oil	15	15	15	15
Wheat flour	65	65	65	65
Sugar	18	18	18	18
Salt	1.5	1.5	1.5	1.5
Baking powder	0.5	0.5	0.5	0.5
Total	100	100	100	100

CO: control cookie formulation, CA: *Chaetoceros* cookie formulation, CH, *Chlorella vulgaris* cookie formulation, SC: *Skeletonema costatum* cookie formulation.

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2.4 Dimensional analysis

Cookie thickness was determined by placing cookie samples on vernier calipers. The average of three values was taken for each set of samples. The average thickness was expressed in mm. Cookie diameter was determined by aligning four cookie samples. An average of three values was taken for each set of samples. The average diameter was expressed in mm. The average weight of cookies was 9.2 g individual cookies by using an analytical weighing balance.

2.5 Proximate analysis

The moisture content of cookies was determined gravimetrically by drying the cookies in an oven at 120°C until they reached a constant weight. Gravimetric analysis for total ash content was performed through incineration at 550°C in a muffle furnace. The Kjeldhal method was applied following the Association of Official Analytical Collaboration (AOAC) International (2000) and Indonesian National Standard (1992) to determine crude protein. The total protein content of MECO-based cookies was calculated by multiplying the nitrogen figure by 100/16, or 6.25. This nitrogen assumption was taken from protein, which contains 16% nitrogen. Meanwhile, the crude fat content of the cookies was evaluated using the Indonesian National Standard Method (1992).

2.6 Water activity analysis

The water activity (Aw) of MECO-based cookies was determined at 20.1°C by using a HygroPalm HP23-AW (Rotronic AG, Switzerland). Each sample in the crushed form was analyzed.

2.7 Texture analysis

Instrumental texture analysis was conducted using a TA.XT2.Plus texture analyzer (Stable Microsystems, UK). This analysis was performed to ascertain the cookie's hardness. In this analysis, the hardness of the material was determined in gram force (gf) units by dividing the maximum applied force (peak value) at the initial pressure or compression by the initial pressure or compression (gf). The hardness value was calculated using the software accompanying the instrument.

2.8 Extraction

Briefly, 5 g of cookie dough was extracted with 10 mL of 96% ethanol for 1 hrs at room temperature in a dimly lit room. The type of solvent used during the extraction was chosen based on Susanto *et al.* (2019). The mixtures were vortexed for 5 mins then centrifuged (Wina Instrument, Indonesia) at 36,000×*g* and room temperature for 10 mins. After centrifugation, the supernatant was collected into a volumetric tube. The extracts were kept in a freezer (−27°C) until further analysis, including the determination of total phenolic content (TPC), antioxidant activity and pigment content.

2.9 Total phenolic content

Folin–Ciocalteu (FC) reagent was used to determine the TPC of microalgae cookie extracts (Kuda *et al.*, 2006). The standard was gallic acid, and the results were expressed in mg of gallic acid equivalent (GAE) per 100 g of sample on a dry mass basis. Approximately 1 mL of cookie extract was combined with 4 mL of 6% (w/v) sodium bicarbonate solution and 5 mL of 10% FC reagent. After 1 hrs at room temperature, the mixtures were measured using a UV/Vis spectrophotometer mini 140 (Shimadzu, Japan at λ = 765 nm). Ethanol solution (96%) was used as the blank, and gallic acid was used as the standard to plot the calibration curve. A stock solution of gallic acid (0.5 mg/mL) was prepared in 96% ethanol. The calibration was prepared with five dilutions of gallic acid (GAE).

2.10 Antioxidant activity

The DPPH assay was conducted as described by Hossain *et al.* (2017) with slight modifications. Approximately 2 mg of cookies was dissolved in 2 mL of DPPH, and the concentration was determined. BHT was used as the control. A total of 2 mL of ethanolic DPPH was added, and the sample was vortexed thoroughly. The test tubes were allowed to rest for 30 mins at room temperature in the dark. The reduction of DPPH radicals in the cookie sample was determined by reading the absorbance at 517 nm by using a UV/Vis spectrophotometer mini V140 (Shimadzu, Japan). The scavenging activity of radicals is expressed as a percentage of DPPH free radical inhibition and calculated with the following formula:

$$\% \text{ Inhibition} = \frac{(\text{Abs Control} - \text{Abs Sample})}{\text{Abs Control}} \times 100\% \quad (1)$$

2.11 Pigment analysis

Pigment analysis (chls and cars) was carried out using a spectrophotometer as described by Braniša *et al.* (2016). In brief, the ethanolic extracts were dissolved in methanol at the concentration of 0.1 mg/mL, and the absorbance of chls and cars specific wavelengths was determined. Microalga pigment content was determined by using the following formulas:

chls

$$** \text{ Total chls (mg/g)} = (20.2A_{645} + 8.02A_{663})/(1000 \times W) \quad (2)$$

cars

$$** \text{ Total cars (mg/g)} = (7.6A_{480} - 1.49A_{510}V)/(1000 \times W) \quad (3)$$

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Here, A is the absorbance at a specific wavelength (ABS), V is the pigment extract (mL), and W is the sample extract (g).

2.12 Color analysis

The color intensity of the top and bottom surfaces of cookie samples (those that were in contact with the baking tray) was determined using a Konica Minolta Color Measuring System (Chroma Meter Conica Minolta CR400 LTD Japan). After conversion from RGB into HSV, the instrument was calibrated with a white tile to determine the values of lightness (L*), redness (a*), and yellowness (b*). The primary color was extracted using MATLAB R2017.

2.13 Microstructure analysis

The effect of different microalga oil extracts on the microstructure of the cookies was determined through SEM analysis. The micrographs of the control and treatment cookies were taken using a Jeol Benchstop Scanning Electron Microscopy JCM 7000 (Jeol, USA). Film samples were cryofractured for 3-5 days at 25°C by using liquid nitrogen and conditioned desiccators containing silica gel. The samples were mounted on the specimen holder using double-sided adhesive tape and then sputter-coated with gold under vacuum.

2.14 Statistical analysis

SPSS (1996) was used to perform statistical analysis. Means and standard deviation (SD) were used to represent data. One-way analysis of variance was used first, followed by Duncan's Multiple Range Test. The threshold of $p<0.05$ was considered statistically significant (Snedecor and Cochran, 1980). Graphs were generated using GraphPad Prism version 9.3.0 for Windows (GraphPad Software, San Diego, CA, USA).

3. Results and discussion

3.1 Characteristics of microalgae extract-enriched coconut oil

The MECO used in the cookies was subjected to color analysis, pigment analysis, and TPC and DPPH radical scavenging activity assays. The proximate analysis of cookies and extracts is shown in Table 2. The data indicated that the oil had high levels of chls, cars, and phenol, as well as high levels of energy due to its high proportion of lipids (Table 3). Accordingly, the oil was rich in pigments and had high TPC and AA. These results indicated that microalga oils have satisfactory quality attributes and could be used for further study.

Table 2. Proximate chemical composition (%), lipid energy (kcal 100/g) and total energy (kcal 100/g) of cookies (CO) and cookies with coconut oil enriched with *Chaetoceros* sp. (CA), *Chlorella vulgaris* (CH), or *Skeletonema costatum* (SC).

Parameter	CO	CA	CH	SC
Moisture (%)	0.16	0.11	0.14	0.13
Ash (%)	<0.02	<0.02	<0.02	<0.02
Protein (%)	<0.04	<0.04	<0.04	<0.04
Carbohydrate (%)	0	0	0	0
Lipid (%)	99.84	99.89	99.86	99.87
Lipid energy	898.56	899.01	898.74	898.83
Total energy	898.56	899.01	898.74	898.83

Values are presented as mean (n = 2-3).

3.2 Bioactive compounds of cookie dough

The pigments, TPC, and DPPH radical scavenging activity of cookie dough are presented in Table 3. In the case of pigments, the highest chls content was detected under the CA treatment. Whereas high cars contents were detected under the CH and CA treatments. Among the treated cookies, cookies with CA extract possessed the highest AA for scavenging DPPH. The oils used in the cookie dough contained different levels of bioactive compounds due to the use of different microalgae as sources. Commercial coconut oil, which may contain vitamin E, was used in this study. The vitamin E in commercial vegetable oil may act as an antioxidant. In addition, the recipe in this work used baking powder, which may act as a carrier of the antioxidant properties of ingredients (Carullo *et al.*, 2022).

3.3 Proximate composition of cookies

The proximate composition of a food product is very important because it describes the percentages of its nutrient contents (Suriya *et al.*, 2017). The proximate compositions of cookies with coconut oil (control) and MECO are shown in Table 4. All cookies had a moisture

Table 3. Pigments (µg/g), total phenol (mg GAE/100 g), and antioxidant activity (% inhibition) of cookies (CO) and cookies with coconut oil enriched with *Chaetoceros* sp. (CA), *Chlorella vulgaris* (CH), or *Skeletonema costatum* (SC).

Parameter	CO	CA	CH	SC
Chlorophylls (µg/g)	0^{a}	4.504 ± 0.173^{c}	4.005 ± 0.019^{d}	2.322 ± 0.086^{b}
Carotenoids (µg/g)	0.020 ± 0.0018^{a}	0.328 ± 0.004^{c}	0.447 ± 0.027^{d}	0.047 ± 0.005^{b}
Total phenolic content (mg GAE/100 g)	4.248 ± 0.05^{d}	4.008 ± 0.02^{bc}	3.932 ± 0.055^{b}	3.641 ± 0.174^{a}
Antioxidant activity (%)	71.585 ± 12.521^{a}	78.005 ± 3.313^{a}	73.087 ± 1.656^{a}	75.820 ± 10.67^{a}

Values are presented as mean±SD (n = 3). Values with different superscripts within the same column are statistically significantly different ($p < 0.05$).

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Table 4. Proximate chemical composition (%), lipid energy (kcal/100 g) and total energy (kcal/100 g) of cookie (CO) and cookies with coconut oil enriched with *Chaetoceros* sp. (CA), *Chlorella vulgaris* (CH), or *Skeletonema costatum* (SC).

Parameter	CO	CA	CH	SC
Moisture (%)	3.96±0.01[b]	3.49±0.03[a]	4.12±0.01[c]	4.17±0.03[c]
Ash (%)	1.97±0.01[a]	2.07±0.01[b]	2.13±0.02[c]	2.17±0.02[c]
Protein (%)	6.06±0.08[b]	5.79±0.08[a]	6.00±0.06[b]	5.98±0.03[ab]
Carbohydrate (%)	60.55±0.04[a]	59.7±0.07[a]	59.14±0.09[b]	60.72±0.11[c]
Lipid (%)	27.44±0.01[a]	27.92±0.07[b]	28.03±0.005[c]	28.62±0.01[d]
Lipid energy (kcal/100 g)	246.96±0.09[a]	252.31±0.04[b]	257.58±0.09[c]	251.28±0.63[d]
Total energy (kcal/100 g)	513.44±0.07[a]	517.36±0.51[c]	515.15±0.005[b]	517.72±0.17[c]

Values are presented as mean±SD (n = 3). Values with different superscripts within the same row are statistically significantly different ($p < 0.05$).

content of 3.49-4.17, which was considered typical of dried foods (Batista *et al.*, 2017).

Conversely, the protein content of cookies treated with MECO was lower than that of the control cookies (6.06%). The decrease in protein content could be attributed to the incorporation of coconut oil, which was rich in lipids but low in protein. A similar decrease in the protein content of cookies was observed after the addition of *Chlorella* sp. meal (Sahni *et al.*, 2019). By contrast, the protein content of cookies with the addition of microalga biomass was significantly increased compared with that of the control (Gouveia *et al.*, 2007; Shahbazizadeh *et al.,* 2015; Şahin, 2020) due to its protein-rich biomass.

Carbohydrates should be the major ingredients of cookies formulated with wheat flour, which contains a considerable amount of carbohydrates. A similar observation was found for cookies incorporated with microalgae (Batista *et al.*, 2020; Şahin, 2020). With the addition of MECO, the carbohydrate content of the cookies changed. Specifically, the CA and CH treatments decreased carbohydrate content, whereas the SC treatment increased the carbohydrate content (Table 4)

The results in Table 4 indicated that the addition of MECO increased the ash and lipid contents of the cookies significantly ($p<0.05$) with a noticeable effect. Significant changes ($p<0.05$) in the lipid content, but not in the ash content (1.97-2.17%), of the cookies were observed following the addition of various types of MECO (27.44-28.62%). The significant increase in total ash content after incorporation with MECO indicated that the mineral content of the cookies had increased as corroborated in another study that used biomass microalgae to produce cookies (Ashoush and Mahdy, 2019). The cookies containing 15% microalgae extract-enriched coconut oil had lipid content of 27.44-28.62% and ash content of 1.97-2.17%. Hence, the cookies incorporated with microalgae extract-enriched coconut oil were found to possess a better nutritive profile than the control cookies.

3.4 Water activity

The physical-chemical and microbiological stability of food is heavily dependent on water content and its interaction with food ingredients. A_w quantifies the availability of water molecules to participate in microbial, enzymatic, or chemical reactions. As a result, this parameter has been used to evaluate the potential for microbial growth and the chemical stability of foods after manufacture (Vieira *et al.*, 2020). The Aw of the control cookies and cookies treated with MECO are shown in Figure 1. The A_w values of the cookies treated with MECO were comparable with those of the control. The control cookies had an average A_w value of 0.36. A_w showed statistically significant ($p<0.05$) differences between treatments. Compared with the A_w under the control, that under the CH and SC treatments had significantly decreased ($p<0.05$), whereas that under the CA treatment had significantly increased ($p<0.05$). These results corroborated the findings of Batista *et al.* (2017). MECO had a greater effect on A_w values, which tended to increase, than the control treatment. The variation in A_w promoted the modification of cookie texture. Overall, the A_w values of all samples were less than 0.4 following processing. In addition, the cookie

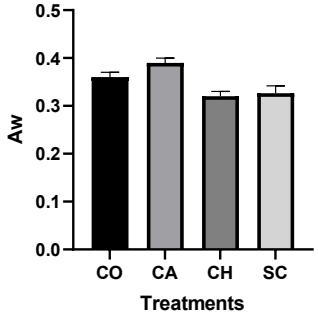

Figure 1. Water activity of untreated and treated cookies. CO: control cookie formulation, CA: *Chaetoceros* cookie formulation, CH, *Chlorella vulgaris* cookie formulation, SC: *Skeletonema costatum* cookie formulation.

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ingredients were mainly MECO and flour, which may have an effect on textural stability (Figure 1).

3.5 Color parameters

The color parameters, such as L*, a*, b*, and ΔE, of the untreated and treated cookies are summarized in Table 5. Cookie color is an important indicator of consumer preferences and aids consumers in judging the baking quality and market value of cookies (Oladunjoye *et al.*, 2021). It was determined by the physicochemical properties of the materials used, baking conditions, baking temperature and time, and the pigment contained in a cookie (Sahni *et al.*, 2019; Al-Saab and Gadallah, 2021). The color parameters of the coconut oil cookies (control) were L*(67.97), a*(1.14), b*(16.22), and ΔE (0). The control cookies had the highest L* value of 67.97. The L* value was significantly decreased after the inclusion of microalga oil extract. CA and CH presented the maximum and minimum L* values of 63.53 and 54.20, respectively. These results were in agreement with the findings of Różyło *et al.* (2017), who showed that the L* of bread decreased after the incorporation of different algae. The variation in color characteristics may be attributed to the differences in pigments in microalgae and the effect of baking (Batista *et al.*, 2017; Khemiri *et al.*, 2020; Oladunjoye *et al.*, 2021).

Table 5. Color analysis of cookie (CO) and cookies incorporated with coconut oil enriched with *Chaetoceros* sp. (CA), *Chlorella vulgaris* (CH), or *Skeletonema costatum* (SC).

	CO	CA	CH	SC
L*	67.97± 0.52^{d}	63.53± 0.25^{c}	54.20± 0.38^{a}	61.73± 0.18^{b}
a*	1.14±0. 09^{c}	1.41±0. 005^{d}	4.49±0. 03^{a}	−2.33± 0.01^{b}
b*	16.22± 0.01^{a}	18.11± 0.22^{c}	28.65± 0.28^{a}	16.26± 0.02^{b}
ΔE	0.00	4.83	18.85	7.14

Values are presented as mean±SD (n = 3). Values with different superscripts within the same row are statistically significantly different ($p < 0.05$).

The a* and b* of cookies treated with SC were significantly increased ($p<0.05$). The a* values ranged between −2.33 and 4.49, and the b* value ranged between 16.22 and 28.65. The a* and b* values obtained in this research were higher than those reported by Fradinho *et al.* (2020) for *A. platensis* biscuits. A wide variation in b* value can be observed under the CH treatment. A similar effect was found under the CV treatment (Gouveia *et al.*, 2007; Batista *et al.*, 2017; Khemiri *et al.*, 2020). The increase in a* and b* values (Table 5) was due to the high chls content of the cookies contributed by chlorophyte algae. Compared with other treatments, the CA and SC treatments presented less intensive color changes (lower a* and b* value) that reflected their lower chls and cars contents (Table 5). Therefore, microalga oil can be used as a natural coloring agent for cookies. The reduction in the a* and b* parameters was related to the pigment content of the cookies incorporated with microalgae and to baking effects (Batista *et al.*, 2017). Overall, the ΔE values indicated significant color differences ($p<0.05$) between the control and treated cookies. ΔE values >3 can be perceived by the human eye (Bodart *et al.*, 2008).

3.6 Bioactive compounds and antioxidant activity

Foods with high amounts of bioactive compounds, phls, and pigments have garnered considerable attention due to their antioxidant, anticancer, anti-inflammatory, and anti-obesity properties. The pigments and phls found in microalgae exhibit biological properties that are consistent with their AA (Renugadevi *et al.*, 2018; Monteiro *et al.*, 2020). The TPC, total chls, total cars, and AA of cookies incorporated with coconut oil enriched with microalga are presented in Table 6.

Microalga pigments include chls and cars which are considered natural antioxidants and have attracted interest from manufacturers and consumers because of their health benefits and potential use as natural pigments (Barkia *et al.*, 2019; Koyande *et al.*, 2019). Table 6 provides information on the pigments found in untreated and treated cookies. Chls were absent from untreated cookies because the coconut oil used in their preparation did not contain either pigment. Additionally, the chls content of the treated cookies had increased significantly ($p < 0.05$). The CH treatment yielded the highest chls content (5.2 µg/100 g), whereas the CA treatment yielded the lowest chls content (2.5 µg/100 g). Additionally, cars content was significantly increased ($p<0.05$) following the addition of microalga oil and ranged from 3.8 µg/100 g to 32.3 µg/100 g. This result demonstrated that microalga oil is high in cars, which acts as a potent antioxidant. Under the SC treatment, pigment loss occurred during baking due to the use of high temperatures (El Baky *et al.*, 2015; Singh *et al.*, 2020).

Additionally, the phl content of microalgae has been shown to be correlated with AA. The growing demand for foods containing bioactive compounds with unique health benefits necessitates the development of novel strategies (Batista *et al.*, 2017; Al-Saab and Gadallah, 2021). Alga-derived phls are currently one of the most popular functional foods for the prevention of cardiovascular diseases and diabetes (Murray *et al.*, 2018). The TPC assay results indicated that the control cookies had higher phenol content than the treated cookies with the exception of SC cookies, which had the highest phenol content. TPC was stable when baked at

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high temperatures (Tables 4 and 6).

The variation between the AA of the control and treated cookies could be related to the phls, pigments, fatty acids, and other bioactive compounds present in the cookies. The CH treatment presented the highest ($p<0.05$) AA (DPPH) (72.04%) followed by the cookies treated with CA (68.44%), C (40.57%), and SC (31.28%) treatments (Table 6). In addition to phenolic content, the CH sample contained high levels of pigments, mainly chls (Table 6). The higher level of AA under the CH treatment compared to that under other treatments may be attributed to the high content of pigments, vitamins, omega-3 PUFAs, phls, and other bioactive compounds in microalgae (Safi *et al.*, 2014; Martínez Andrade *et al.*, 2018; Gorgich *et al.*, 2021).

Additionally, the addition of microalga oil resulted in a significant ($p<0.05$) increase in the AA of all microalga oil-based cookies, except for SC cookies. However, the AA of SC cookies was higher than that of cookies enriched with CH biomass (Batista *et al.*, 2017). The radical scavenging activity of cookies added with the same concentration of MECO ranged between 31.18% and 72.04%, and the control possessed a high level of AA probably due to the presence of vitamin E in the coconut oil used. This study utilized commercial coconut oil, which may contain vitamin E, to aid lipid oxidation reduction. This effect could be attributed to the bioactive compounds found in various microalgae. This phenomenon indicates that MECO can be used as a functional ingredient to maintain health. A similar increase in the AA of cookies incorporated with microalga biomass has been reported by other researchers (Batista *et al.*, 2017; Şahin, 2020; Vieira *et al.*, 2020).

3.7 Physical measurement of cookies

The eating quality of cookies is directly related to their textural properties (Suriya *et al.*, 2017). Food texture is a physical stability factor that gives a product its identity (Carter *et al.*, 2015). Among the various textural parameters of cookies, hardness is considered an important characteristic and is measured as the peak force required to snap a cookie (Cheng and Bhat, 2016). Cookie texture was determined using penetration tests. The resulting hardness, which was expressed in terms of resistance to penetration work or the force required to break a cookie, was calculated from texturograms. The texture (hardness) of the control and microalga oil-containing cookies is shown in Table 7. Compared with the control treatment, the substitution of the same concentration of microalga oil had a significant ($p < 0.05$) effect on cookie texture. The texture of cookies incorporated with MECO was significantly lower than that of the control cookies and was lower than that of biscuits incorporated with *Isochrysis galbana* biomass (3059 gf) (Oliveira *et al.*, 2009). This result indicated that MECO significantly affected cookie texture. The diameters of the cookies ranged from 41.96 to 42.90 mm, and the thickness of the cookies, as a result of the incorporation of MECO, ranged between 9.33 and 10.6 mm.

3.8 Microstructure analysis

SEM was conducted to determine the effect of coconut oil enriched with various MECO on cookie structure. The micrographs in Figure 2 illustrate the morphology of the untreated and treated cookies. Figure

Table 6. Pigments (µg/g), total phenol (mg GAE/100 g) and antioxidant activity (% inhibition) of cookies (CO) and cookies with coconut oil enriched with *Chaetoceros* sp. (CA), *Chlorella vulgaris* (CH), or *Skeletonema costatum* (SC).

Parameter	CO	CA	CH	SC
Chlorophylls (µg/g)	0.0 ± 0.14^{a}	2.5 ± 0.8^{b}	5.2 ± 0.16^{c}	4.7 ± 1^{d}
Carotenoids (µg/g)	0.046 ± 0^{a}	0.11 ± 0.0^{b}	0.38 ± 0.1^{d}	0.39 ± 0.1^{c}
Total phenolic content (mg GAE/100 g)	4.13 ± 0.81^{a}	3.92 ± 0.21^{a}	3.84 ± 0.07^{ab}	3.51 ± 0.031^{b}
Antioxidant activity (%)	40.57 ± 0.022^{a}	68.44 ± 0.01^{b}	72.04 ± 0.009^{b}	31.28 ± 0.008^{a}

Values are presented as mean±SD (n = 3). Values with different superscripts within the same row are statistically significantly different ($p < 0.05$).

Table 7. Physical properties of cookie (CO) and cookies incorporated with coconut oil enriched with *Chaetoceros* sp. (CA), *Chlorella vulgaris* (CH), or *Skeletonema costatum* (SC).

Parameter	CO	CA	CH	SC
Weight (g)	8.14 ± 0.34^{ab}	9.03 ± 0.9^{b}	7.49 ± 0.64^{a}	8.31 ± 0.86^{ab}
Diameter (D; mm)	42.66 ± 0.2^{a}	42.23 ± 1.1^{a}	42.90 ± 0.5^{a}	41.96 ± 1.0^{a}
Thickness (T; mm)	9.33 ± 0.41^{a}	9.76 ± 0.47^{a}	10.16 ± 0.51^{a}	10.6 ± 0.7^{a}
Spread ratio (D/T)	4.57	4.32	4.22	3.95
Texture (*gf*)	1450 ± 102.51^{a}	757.73 ± 132.55^{b}	809.22 ± 129.04^{b}	835.11 ± 111.13^{b}

Values are presented as mean±SD (n = 3). Values with different superscripts within the same row are statistically significantly different ($p < 0.05$).

https://doi.org/10.26656/fr.2017.9(1).616

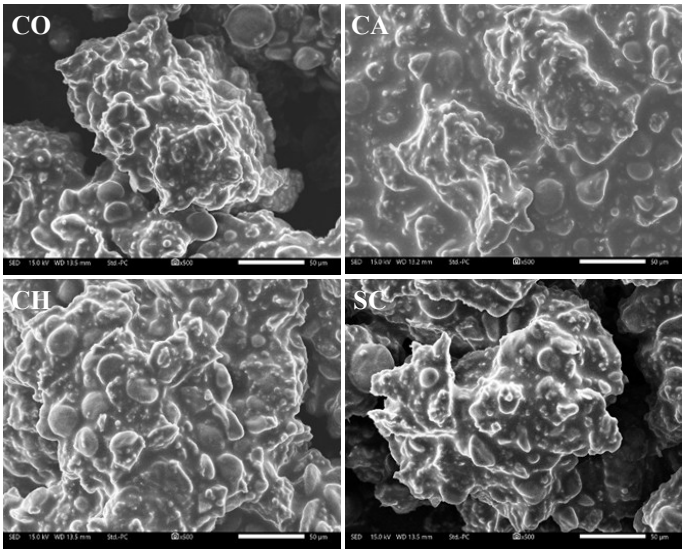

Figure 2. Microstructure of cookies: CO: control cookie formulation, CA: *Chaetoceros* cookie formulation, CH, *Chlorella vulgaris* cookie formulation, SC: *Skeletonema costatum* cookie formulation.

2 shows that the treated cookies had a homogenous structure and were thus soft. Structural differences were observed between the untreated and treated cookies. Round and irregularly shaped starch granules were observed. SEM analysis revealed that the dimensions of the starch granules varied significantly across all samples.

4. Conclusion

The findings of this study demonstrated that MECO from three microalgae, namely CA, CV, and SC, was rich in bioactive components and had a high amount of AA as evaluated by DPPH analysis. The treated cookies had significant levels of TPC and pigments, especially chls and cars, as well as AA. The results of the study suggest that MECO-based cookies may become functional foods in the future due to their rich lipid soluble bioactive compounds and high levels of AA, which can contribute to the natural bioactive compounds in human diets and thus benefit human health. Our findings also offer suggestions for enhancing the usage of MECO as a possible component of functional cookies.

Conflicts of interest

The authors declare no conflicts of interests.

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