Proximate and nutrition analysis of chocolate bar with addition of mangrove leaves powder during the shelf life

Sumartini1,*, P W Ratrina1 and K H Sitorus1

1Processing of Fisheries Product Study Program, Dumai of Marine and Fisheries Polytechnic, Dumai 28824, Indonesia

*Corresponding author: tinny.sumardi@gmail.com

Abstract. Exploration of the potential of mangrove leaves as an additive in food products has been widely carried out. Mangrove leaves contain primary and secondary metabolites that can be used to increase the nutritional value of food products, one of which is chocolate. The purpose of this part is must be done to increase the economic value of local chocolate during storage. This research aims to determine the nutritional characteristic of diversification chocolate by adding mangrove leaves powder Avicennia officinalis (AO), A. marina (AM), and Rhizophora apiculata (RA) extract as a natural agent that can increase the shelf-life of the product. Chocolate is made with four different varieties of mangrove leaves powder addition (AM, AO, RA, and CO) and storage for 14 days at ambient temperature. This research method is an experimental laboratory with a randomized block design. Based on the results of this study, the addition of Avicennia marina mangrove leaf powder was able to inhibit the decrease in the ash content of chocolate bars during 14 days of storage. In addition, the addition of A. officinalis leaf powder can suppress the decrease in water content and protein content. While the decrease in carbohydrate levels during storage in chocolate bars can be inhibited by the application of R. apiculata mangrove leaf powder.

Keywords: chocolate; mangrove leaves; nutrition; proximate analysis

1. Introduction

Mangrove plants have several parts such as roots, stems, and leaves. Several studies have mentioned that mangrove leaves contain bioactive compounds that are used in the health, beauty, and food fields. The phytochemical content of mangrove leaves contains antioxidant compounds. In previous studies, mangrove leaf powder was used as a natural preservative [1], [2], increasing the acceptability of wet noodles [3], increasing the acceptability of chocolate bars [4]. Api-api leaves are high in fiber and carbohydrates. The bioactive components detected in Api-api leaves are flavonoids, steroids, and reducing sugars [5]. In addition to mangrove leaves in Avicennia species, other species such as Rhizophora are known to have high antioxidants. Rhizophora mangrove leaf extract has antioxidant activity, namely 57.14 ppm - 77.32 ppm, which means that it belongs to the group of potent antioxidants in stick balm preparations [6]. Rhizophora mucronata has moisture content of 31.96%, ash content of 1.10%, fat content 0.86%, protein content 2.59% and carbohydrates 63.50% [7]. The dominant essential amino acid content in mangrove leaf flour Avicennia marina is leucine at 4.28 g/100g and the dominant non-essential amino acid found in Avicennia marina mangrove leaf flour is glutamic acid at 12.60 g/100g.[8]. The fat content of Avicennia marina leaves is 1.14% [9] the fat content of Rhizophora mangrove leaves is 0.7%[10].

One of the food products that people like the most is a chocolate bar. Chocolate in general has a sweet taste because it consists of constituent components such as sugar, sucrose, milk fat, and flavors. Chocolate is a type of food that is very rich in antioxidants (catechins, epicatechins, procyanidins, and polyphenols) [11]. In addition, chocolate also contains certain compounds, namely theobromine, and caffeine which work on the nerve center which in certain amounts can increase pleasure and mood [12]. According to previous research, the addition of mangrove plants can improve the quality of chocolate.
in terms of sensory characteristics [4]. In addition, the addition of mangrove leaf powder also improved the nutrition and proximate content of the chocolate bar [13]. Based on some of the findings above, that antioxidants can be used as agents to improve the sensory quality of food products and inhibit the growth of harmful bacteria, but there are not many findings that say that antioxidant compounds in mangrove leaf powder can improve the chemical composition of chocolate bars during storage. Therefore, the researcher wanted to know the effect of antioxidant mangrove leaves from Avicennia and Rhizophora species on the proximate characteristics of chocolate bars during storage.

2. Materials and Method

2.1. Materials and equipment
The materials used in this study were mangrove leaves of the *Rhizophora apiculata*, *Avicennia officinalis*, and *Avicennia marina* species. The ingredients used in the chocolate-making process are dark chocolate and emulsifiers. Chemicals used in testing proximate levels include equadest, kjeltab, H2SO4 solution (Merck, concentrated), NaOH (Merck), H3BO3 (Merck), 0.1 N HCl solution (Merck), 6N HCl (Merck), H3BO4 (Merck, 2%), and sodium carbonate buffer (Merck). The equipment used in this research is porcelain dish, oven, desiccator, test tube, Erlenmeyer flask, Sokhlet tube, Kjeldahl tube, distillation, burette, and furnace.

2.2. Sample preparation
Manufacture of mangrove leaf powder from *Rhizophora apiculata*, *Avicennia officinalis*, and *Avicennia marina* species. The manufacture of mangrove leaf powder refers to the sample of mangrove leaves that have been collected and then cleaned using clean water. The leaves are cut into small pieces and then dried using a black transparent cloth to dry. The dried mangrove leaves were mashed using a blender and then sieved to obtain a fine and uniform powder [1]. The procedure for making chocolate bars with the addition of 10% [10] mangrove powder by mixing mangrove leaf powder and chocolate mixture is stirred until homogeneous, then the tempering process is carried out, printed, and put into the refrigerator [4]. The study used an experimental method of Completely Randomized Design (CRD) with the addition of mangrove leaf powder from different species, namely *Rhizophora apiculata*, *Avicennia officinalis*, and *Avicennia marina*, and the control (without the addition of mangrove leaf powder) was repeated 3 times. Tests for proximate levels in chocolate were carried out on storage days 0, 7, and 14 at room temperature.

2.3. Moisture content analysis
The measurement of water content used the thermogravimetric method. The cup was dried in an oven at a temperature of 100-105°C until a constant weight is obtained, then cooled in a desiccator and weighed. The sample was weighed as much as 5 grams in a cup, then dried in an oven at a temperature of 100-105°C until a constant weight was obtained. The sample is cooled in a desiccator and then weighed. The principle of the water content analysis method is the evaporation of water contained in the sample. Weight reduction occurs due to the evaporation of water contained in the sample [14].

2.4. Ash content analysis
Measurement of ash content has used a furnace with a temperature of about 550°C with the dry ashing method. Determination of ash content is carried out by heating at a temperature of 550°C by oxidizing organic matter, then weighing the remaining substances [14].

2.5. Fat content analysis
Fat content analysis was carried out using the Soxhlet method. The principle of this analysis is to extract fat using hexane solvent. When heated, the hexane solvent will evaporate so that the fat content can be calculated. Measurement of fat content begins with drying the fat flask using an oven at 105°C for 30 minutes, then cooled in a desiccator for 15 minutes and weighed. A total of 5 grams of the sample was wrapped in filter paper and then put into a fat sleeve, then covered with fat-free cotton and doused with
hexane solvent. The next procedure is distillation until the hexane solvent evaporates. The extracted flask was then heated in an oven at 105ºC until the weight was constant. The dried sample was then cooled in a desiccator and weighed [14].

2.6. Protein content analysis
The method of measuring protein content was carried out using the Kjeldahl method. The principle of analysis of this method includes destruction, distillation, and titration. The principle of protein content analysis using the Kjeldahl method is to determine protein from carbon-containing materials and convert nitrogen into ammonia. Ammonia reacts with the acid to form ammonium sulfate, then ammonia is absorbed in boric acid solution (Merck). The HCl titration step can determine the amount of nitrogen contained in the sample [14].

2.7. Carbohydrate content analysis
Calculation of carbohydrate content in the proximate analysis was calculated using the by difference method. Calculation of carbohydrate analysis is 100%-(water content + ash content + fat content + protein content). Carbohydrates are obtained by subtracting the number 100 with a percentage of water content, ash content, fat content, and protein [14]

2.8. Statistical analysis
Data analysis using SPSS 22 software with analysis of variance (ANOVA) 95% confidence level. Further tests were carried out using Duncan's test to determine the significant differences between each variable.

3. Results and discussion
3.1. Moisture content
Determination of water content in this study using the method of thermogravimetry. Moisture content in chocolate bars was observed during the storage period, namely on days 0, 7, and 14. Moisture content is one of the main factors in food storage because the process of biological, physiological, and chemical damage to materials during storage requires water as a medium [15]. The results of testing the water content in this study still do not meet the quality requirements because the results of the water content test in all treatments are above 2%, where the quality requirements of SNI 3749-2009 regarding mass chocolate products, the moisture content of chocolate bars is a maximum of 2% [16]. During the storage process, the water content in all treatments decreased. This may be due to the evaporation process of the water content in the food ingredients into the surrounding environment [17].

Based on figure 1, the value of the brown bar moisture content shows that the difference in the addition of mangrove leaf species shows a significant difference in the decrease in water content. The decrease in water content in AM treatment for 14 days was 2.70%, AO treatment 2.27%, RA treatment 3.27%, and control 3.79%. Based on the results of the water content analysis, the addition of *Avicennia officinalis* mangrove leaf powder had the lowest reduction rate, while the control treatment experienced the largest decrease in water content. It is because the mangrove leaf powder contains alkaloid-derived compounds, where these compounds under acidic conditions can carry out the Wagner-Merwein reaction, namely rearrangement followed by a shift in the double bond and the release of one water molecule [18]. In addition, other allegations cause the high water content of chocolate products with the addition of mangrove leaf powder, including because the material contains a lot of water-soluble vitamins such as Vitamin B and Vitamin C [19].

3.2. Ash content
Analysis of the ash content in chocolate was carried out during the shelf life, namely days 0.7, and 14. Ash content can determine the mineral content contained in a food ingredient. The method used in testing the ash content is using the heating method at a temperature of 550 C by oxidizing organic matter, then weighing the remaining substances. The principle of ash content testing is to determine the amount
of ash content in a material related to the mineral content of the material. Ash is an inorganic substance leftover from the combustion of organic material. Ash content is a mixture of inorganic or mineral components contained in a food ingredient. Foodstuffs consist of 96% inorganic materials and water, while the rest are mineral elements. The ash content can show the total minerals in a food ingredient. Organic materials in the combustion process will burn but the inorganic components will not because that is called the ash content [20]. The results of testing the ash content of chocolate with the addition of different mangrove leaf powders are presented in figure 2.

Based on the graph of the ash content, shows that during the 14 days of storage, the ash content showed an increase during storage, but the ash content in the control showed a lower ash content value than the addition of mangrove leaf powder. The high ash content in chocolate with the addition of mangrove leaf powder was because mangrove leaves have a high mineral content so that they contribute a higher ash content value than the control. The content of Na, K, Ca, and Mg is the content found in Avicennia and Rhizophora mangroves. The macromineral content in *Avicennia sp.* is P total 0.16%; Total K 0.86%; Total Ca 0.58%; S 0.23%; Na 1.53% and a small portion of micro minerals such as Fe, Zn, Cu, and Mn [21]. The results of the ash content in all treatments in this study were following the quality standard, a maximum of 14%. The standard for ash content in chocolate bar products according to SNI 3749-2009 is a maximum of 14% [16].

### 3.3. Fat content

Fat has an important function for the human body because fat is a high energy source. The fat content test in this study aimed to determine changes in fat content in chocolate bars with the addition of mangrove leaf powder on storage days 0, 7, and 14. Determination of fat content in this study used the Soxhlet method. The fat content in chocolate will affect the quality of the chocolate itself. Chocolate is a food that has a high-fat content. Although it contains polyphenol compounds from cocoa, cocoa undergoes an oxidation process like any other product. This process is especially noticeable in chocolates that contain lower amounts of cocoa, such as white chocolate and milk chocolate. Fats in foodstuffs will be very sensitive to oxidation if they contain unsaturated fatty acids. If these fatty acids

![Figure 1](image-url)
are oxidized, hydroperoxide compounds are formed which decompose into aldehydes, ketones, and alcohols, causing rancidity [22].

Based on the results of fat content testing (figure 3), there is an increase and decrease in fat content in chocolate bars. The decrease in fat content can also be caused by a decrease in water content and protein content during storage. Changes in protein content during growth are proportional to changes in fat content in a food ingredient. The increased protein content in the food will decrease the fat content. Fat content decreases at low temperatures and increases at high temperatures [23]. The results of the fat content test showed that the addition of Avicennia marina leaf powder gave the lowest fat content compared to other treatments. It is because it does not contain many fat-soluble vitamins such as vitamins A, D, E, and K. In addition, the fat content in Avicennia marina itself also affects the results of this study, which is 1.14% [9]. Api-api leaves have also been shown to have antioxidant activity and are able to inhibit the oxidation process in oil emulsions, but are still relatively weak [5].

**Figure 2.** Ash content of chocolate bar with the addition of different mangrove leaf powder during the shelf-life KO: Control, AM: *Avicennia marina, Avicennia officinalis, Rhizophora apiculata.*

**Figure 3.** Fat content of chocolate bar with the addition of different mangrove leaf powder during the shelf-life KO: Control, AM: *Avicennia marina, Avicennia officinalis, Rhizophora apiculata*
Protein content

Protein is the main component of the food consumed by humans. The main function of protein is to meet the needs of amino acids and nitrogen, then the body's protein synthesis and other substances that contain nitrogen. Protein deficiency can make the body's resistance to a disease decrease and the body's metabolic processes are disrupted [24]. Protein is an important component of the human diet that is needed for tissue replacement, energy supply and is a versatile macromolecule in living systems that has important functions in all biological processes such as catalysts, transportation, various other molecules such as oxygen, as immunity and conducts nerve impulses [25]. Protein testing in this study used the Kjeldahl method, destruction, distillation, and titration. The results of testing the protein content of

Figure 4. Protein content of chocolate bar with the addition of different mangrove leaf powder during the shelf-life KO: Control, AM: *Avicennia marina*, *Avicennia officinalis*, *Rhizophora apiculata*.

Figure 5. Carbohydrate content of chocolate bar with the addition of different mangrove leaf powder during the shelf-life KO: Control, AM: *Avicennia marina*, *Avicennia officinalis*, *Rhizophora apiculata*.

3.4. Protein content

Protein is the main component of the food consumed by humans. The main function of protein is to meet the needs of amino acids and nitrogen, then the body's protein synthesis and other substances that contain nitrogen. Protein deficiency can make the body's resistance to a disease decrease and the body's metabolic processes are disrupted [24]. Protein is an important component of the human diet that is needed for tissue replacement, energy supply and is a versatile macromolecule in living systems that has important functions in all biological processes such as catalysts, transportation, various other molecules such as oxygen, as immunity and conducts nerve impulses [25]. Protein testing in this study used the Kjeldahl method, destruction, distillation, and titration. The results of testing the protein content of
chocolate bars with the addition of mangrove leaf powder during 14-day shelf life are shown in Figure 4.

The picture shows that the addition of mangrove leaf powder can protect the protein content of chocolate bars during the shelf life compared to without the addition of mangrove leaf powder. The nutritional content of api-api leaves, the most prominent nutrients are the protein of 3.67 g, crude fiber 4.12 g, and vitamin B 2.64 mg compared to other types of plants such as spinach and other vegetables [3]. The addition of mangrove leaves can suppress the decrease in protein levels, where the AM treatment decreased protein levels by 0.94%, AO 0.76%, RA 0.84%, and control 0.89%. The best treatment was obtained by giving mangrove leaf powder of *Avicennia officinalis* species. The role of mangrove leaf powder in the manufacture of mangrove chocolate is as a source of antioxidants, where antioxidants have been widely used as agents to ward off damage to macromineral components such as protein and lipid damage. Antioxidants can inhibit protein degradation for 14 days of shelf life. Proteins can be damaged by the influence of heat, chemical reactions and acids or bases, shocks, and other causes [11].

3.5. Carbohydrate content

Determination of carbohydrate content in this study using the by difference method based on mass weight. The test was carried out by calculating the percentage reduction of the total material minus the proximate percentage other than carbohydrates. Determination of carbohydrate content using the by difference method is a rough determination of carbohydrate content in a food ingredient [13]. This test is affected by the size of the solid mass components contained in the product, including water, ash, protein, and fat content, the higher the level, the lower the carbohydrate content in a product [16]. In addition, the role of carbohydrate content for the human body is that food substances containing carbon elements can be used as energy-forming materials, namely carbohydrates, fats, and proteins [26]. The results of testing the carbohydrate content of chocolate bars with the addition of mangrove leaf powder for a 14-day shelf life are shown in Figure 4.

Based on Figure 5, the results of the carbohydrate content test showed that during the 14-day storage period the carbohydrate content showed a decrease in the carbohydrate trend, but chocolate bars with the addition of an antioxidant source of mangrove leaf powder were able to suppress the decrease in carbohydrate content during the shelf life. The decrease in carbohydrate content during 14 days of storage in AM treatment was 3.92%, AO 6.18%, RA 3.02%, and control 6.26%. The best treatment for carbohydrate testing is the addition of *Rhizophora apiculata* leaf powder. Correlation of the relationship between the amount of antioxidant content of water and carbohydrates. The longer the storage, the higher the antioxidant content. The higher the number of antioxidants, the higher the carbohydrate content in the sample. On the other hand, increasing the amount of antioxidant content will decrease the amount of water content in the sample. The smaller the water content can extend the shelf life because it can stabilize microbial activity and restrain the rate of increase in the number of ingredients [27].

4. Conclusion

Based on the results of this study, the addition of *Avicennia marina* mangrove leaf powder was able to inhibit the decrease in the ash content of chocolate bars during 14 days of storage. In addition, the addition of *Avicennia officinalis* leaf powder can suppress the decrease in water content and protein content. While the decrease in carbohydrate levels during storage in chocolate bars can be inhibited by the application of *Rhizophora apiculata* mangrove leaf powder.

References

[1] Anggraini R, Hendri M and R 2018 Potensi larutan bubuk daun mangrove *Bruguiera gymnorrhiza* sebagai pengawet alami *Masperi J.* **10** 51–62.
[2] Mangalisu A and Armayanti A K 2020 Pemanfaatan daun mangrove (*Rhizophora mucronata*) sebagai pengawet alami telur ayam ras *J. Agrominansia* **5** 28–35.
[3] Zahara R and Zuraidah Y 2018 Pengaruh penambahan daun Api-api (*Avicennia marina*) terhadap
daya terima mie basah Wahana Inov. 7 86–92.
[4] Askama S, Wahyuni S and Mashuni 2017 Pengaruh penambahan bubuk daun tanaman mangrove (Cerioops sp.) terhadap karakteristik organoleptik produk cokelat batang J. Sains dan Teknol. Pangan 2 729–35.
[5] Jacoeb A, Purwaningsih S and Rinto 2011 Anatomi, komponen bioaktif dan aktivitas antioksidan daun mangrove api-api (Avicennia marina) J. Pengolah. Has. Perikan. Indones. 14 143–52.
[6] Faiqoh M, Fitri T, Utami Y and Pertwi Y 2020 Uji antioksidan sedian stick balm ekstrak daun Rhizophora mucronata dengan metode DPPH J. Ilm. Jophus Journal Pharm. UMUS 2 51–8.
[7] Muchali 2017 Nilai nutrisi tepung daun mangrove Rhizophora mucronata terfermentasi ragi tempe dengan lama waktu fermentasi yang berbeda Repository Brawijaya Univ. 2–3.
[8] Suganthy N and Devi K P 2016 Nutritional evaluation of asiatic mangrove Rhizophora mucronata-its proximate composition, amino acid profiles and physico-chemical properties Int. J. Pharm. Sci. Res. 7 2537–45.
[9] Vionita N N T and Insafirin I 2020 Analisis proksimat daun dan propagul mangrove (Avicennia marina dan Avicennia lanata) di ekowisata mangrove wonorejo surabaya Jav. IIm. Kelaaut. dan Perikan. 1 47–57.
[10] Apsari P A, Nur D, Sari E, Kusuma A P, Indrati O, Farmasi P S, Matematika F and Alam P 2018 Formulasi tablet effervescent ekstrak biji melinjo (Gnetum gnemon L.) menggunakan PEG 6000 sebagai lubrikan dan asam sitrat-asam tartrat sebagai sumber asam Eksakta J. Ilmu Ilmu Pangan IPA 18 30–41.
[11] Pratiwy A, Kusumaningrum I and Aminullah 2019 Pemanfaatan ekstrak rempah serai cymbopogon citratus terhadap kandungan antioksidan dan sifat sensori produk dark chocolate J. Pertan. 10 80–92.
[12] Ristanti E Y, Suprapti S and Anggraeni D 2016 Karakteristik komposisi asam lemak pada biji kakao dari 12 daerah di Sulawesi Selatan J. Ind. Has. Perkeb. 11 15–22.
[13] Ratrinia P W and Sumartini 2021 Komposisi nutrisi coklat dengan penambahan variasi jenis serbuk daun mangrove J. Ris. Teknol. Ind. 15 90–102.
[14] AOAC 2005 Official Methods of Analysis(AOAC) ed W Horwitz and G Latimer (Maryland: AOAC International)
[15] Dumadi S R 2011 The moisture content increase of dried cocoa beans during storage at room temperature jite 1 45–54.
[16] Negara H, Lelana I and Ekantari N 2014 Pengkayaan β-karoten pada cokelat batang dengan penambahan Spirulina platensis J. Fish. Sci. 16 17–28.
[17] Solhinh, Muhtarudin and Sutrisna R 2015 Pengaruh lama penyimpanan terhadap kadar air kualitas fisik dan sebaran jamur wafer limbah sayuran dan umbi-umbian J. Ilm. Peternak. Terpadu 3 48–54.
[18] Heliawati L 2018 Kimia Bahan Organik Alam (Bogor: Pascasarjana-UNPAK)
[19] Wibowo C, Kusmana C, Suryani A, Hartati Y and Oktadiyani P 2009 Pemanfaatan pohon mangrove Api-api (Avicennia spp.) sebagai bahan pangan dan obat Prosiding Seminar Hasil-Hasil Penelitian IPB (Institut Pertanian Bogor) pp 158–66
[20] Nurhidayah, Soekendarsi E and Erviani A E 2019 Kandungan Kolagen Sisik Ikan Bandeng (Chanos-chnanos) dan Sisik Ikan Nila (Oreochromis niloticus) J. Ilm. Peternak. Terpadu 3 48–54.
[21] Rizaldi M R 2019 Pengaruh pemberian pakan daun mangrove Api-api (Avicennia marina) dan rumput lapangan terhadap Pertambahan Bobot Badan Harian (PBBH) kambing kacong (Capra aegagrus) (Universitas Sumatera Utara)
[22] Fauzi A, Surti T and Rianingsih S 2016 Efektivitas daun teh (Camellia sinensis) sebagai antioksidan pada fillet ikan bandeng (Chanos-chnanos ) selama penyimpanan diringin J. Peng. dan Biotek Has. Pi 5 1–10.
[23] Hidayati F, Darmanto Y and Romadhon R 2017 Pengaruh perbedaan konsentrasi ekstrak Sargassum sp. dan lama penyimpanan terhadap oksidasi lemak pada fillet ikan patin (Pangasius sp.) J. Ilmu Lingkung. 15 64.
[24] Bakhtra D D A, Rusdi and Mardiah A 2016 Penetapan kadar protein dalam telur unggas melalui analisis nitrogen menggunakan metode kjeldahl J. Farm. Higea 8 143–50.

[25] Fredrick W S, Sadeesh Kumar V and Ravichandran S 2013 Protein analysis of the crab haemolymph collected from the trash Int. J. Pharm. Pharm. Sci. 5 304–8.

[26] Riansyah A, Supriadi A and Nopianti R 2013 Pengaruh perbedaan suhu dan waktu pengeringan terhadap karakteristik ikan asin sepat siam (Trichogaster pectoralis) dengan menggunakan oven Fishtech 2 53–68.

[27] Juwitaningtyas T and Khairi A N 2018 CHEMICA: Jurnal Teknik Kimia Chem. J. Tek. Kim. 5 21–7