THESIS

KARAKTERISTIK FISIKOKIMIA NIB KAKAO (*Theobroma cacao* L.) DENGAN PERLAKUAN BAHAN BAKU DAN METODE SANGRAI BERTEKANAN

PHYSICOCHEMICAL CHARACTERISTICS OF COCOA (*Theobroma cacao* L.) NIBS WITH RAW MATERIALS TREATMENT AND PUFFING GUN METHOD

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AGRICULTURAL PRODUCT TECHNOLOGY STUDY PROGRAM
AGRICULTURAL TECHNOLOGY DEPARTMENT
FACULTY OF AGRICULTURE
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This thesis was written to fulfill one of the requirements to accomplish a Bachelor's Degree in Agriculture Product Technology Study Program at the Faculty of Agriculture, Sriwijaya University

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SUMMARY

DWI INDAH PERMATA SARI. Physicochemical Characteristics of Cocoa Nibs (*Theobroma cacao* L.) with Raw Material Treatment and Puffing Gun Method (Supervised by **UMI ROSIDAH** and **EKO HERI PURWANTO**).

This study aimed to determine the physical and chemical characteristics of cocoa nibs by analyzing the effect of raw material treatment (cocoa bean fermentation method) and pressurized roasting method (puffing gun pressure) to obtain the optimal cocoa bean processing method while maintaining nutritional content, functional properties, with sensory characteristics favored by the panelists. This research was carried out at the Integrated Laboratory of the Indonesian Industrial and Beverages Crops Research Institute, Sukabumi, West Java.

This study used a Factorial Completely Randomized Design (CRD) with two treatment factors, namely variations in puffing pressure (A) which consisted of 3 levels and types of raw materials (B) consisting of 3 levels so that 9 treatments were obtained. Each treatment was repeated 3 times and the analysis was done in duplicate. The parameters observed included chemical characteristics (moisture content, ash content, protein content, fat content, carbohydrate content, total polyphenols and antioxidant activity) and physical characteristics (color, aroma and taste).

The results showed that puffing pressure had significant effect on water, carbohydrates, fat, and total polyphenols contents, antioxidant activity, taste and aroma. The higher the puffing pressure, the higher the carbohydrate content, while the water, fat, total polyphenols, antioxidant activity, taste and aroma decreased. The type of raw material had significant effect on the ash, fat and carbohydrates contents, the color intensity of L * (lightness) and a * (redness), antioxidant activity, taste and aroma. The non-fermented cocoa nib gave the highest color value of L * (lightness), ash content, and total polyphenols, but the lowest values of color a * (redness), and the lowest score of taste and aroma. Meanwhile, spontaneous fermented cocoa nib had the highest antioxidant activity, and highest score of taste and aroma. The highest fat content, antioxidant activity and organoleptic properties were produced by spontaneous fermented cocoa nib which was roasted at a pressure of 3 Bar (A1B1).

RINGKASAN

DWI INDAH PERMATA SARI. Karakteristik Fisikokimia Nib Kakao (*Theobroma cacao* L.) dengan Perlakuan Bahan Baku dan Metode Sangrai Bertekanan (Dibimbing oleh **UMI ROSIDAH** dan **EKO HERI PURWANTO**).

Penelitian ini bertujuan untuk mengetahui karakteristik fisikokimia nib kakao dengan menganalisis pengaruh perlakuan bahan baku (metode fermenasi biji kakao) dan metode sangrai bertekanan (tekanan *puffing gun*) sehingga didapatkan metode pengolahan biji kakao yang optimal dengan tetap mempertahankan kandungan gizi, sifat fungsional, dan cita rasa yang disukai panelis. Penelitian ini dilaksanakan di Laboratorium Terpadu Balai Penelitian Tanaman Industri dan Penyegar, Sukabumi, Jawa Barat.

Penelitian ini menggunakan Rancangan Acak Lengkap (RAL) Faktorial dengan dua faktor perlakuan yaitu tekanan *puffing* (A) yang terdiri dari 3 taraf dan jenis bahan baku yang terdiri dari 3 taraf. Setiap perlakuan diulang sebanyak 3 kali dan analisis dilakukan duplo. Parameter yang diamati meliputi karateristik kimia, yaitu kadar air, kadar abu, kadar protein, kadar lemak, kadar karbohidrat, total polifenol dan aktivitas antioksidan, dan fisik meliputi warna, aroma dan rasa.

Hasil penelitian menunjukkan bahwa tekanan *puffing* berpengaruh nyata terhadap kadar air, kadar karbohidrat, lemak, total polifenol, aktivitas antioksidan, rasa dan aroma. Tekanan puffing yang semakin tinggi memberikan kadar karbohidrat yang semakin meningkat, sedangkan kadar air, lemak, total polifenol, aktivitas antioksidan, rasa dan aroma akan semakin menurun. Jenis bahan baku berpengaruh nyata terhadap kadar abu, karbohidrat, intensitas warna L* (*lightness*) dan a* (*redness*), kadar lemak, aktivitas antioksidan, rasa dan aroma. Nib kakao non fermentasi memberikan nilai warna L* (*lightness*), kadar abu, dan total polifenol paling tinggi, namun nilainya paling rendah untuk warna a* (*redness*), rasa dan aroma. Nib kakao fermentasi spontan mempunyai aktivitas antioksidan, rasa dan aroma paling tinggi. Kadar lemak, aktivitas antioksidan serta nilai sifat organoleptik paling tinggi dihasilkan oleh nib kakao fermentasi spontan yang disangrai pada tekanan 3 Bar (A1B1).

APPROVAL SHEET

PHYSICOCHEMICAL CHARACTERISTICS OF COCOA (*Theobroma cacao* L.) NIBS WITH RAW MATERIALS TREATMENT AND PUFFING GUN METHOD

THESIS

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Declare that all data and information contained in this thesis are the result of my own research activities under the supervision of my advisors, unless the sources are clearly mentioned. If in the future found any element of plagiarism in this thesis, then I am willing to accept academic sanctions from Sriwijaya University.

Thus, I make this statement consciously and without coercion from any party.

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BIOGRAPHY

The writerr was born in Muara Enim city, South Sumatra province on January 10, 1999. The writer is the second of 3 children of Mr. Muhammad Yani, S. Pd and Mrs. Herlina Krisnawati.

The writerr started her education at State Elementary School number 18 Muara Enim City for 6 years and graduated in 2011. After that, the writer continued her study at State Junior High School 1 Muara Enim for 3 years and graduated in 2014. Then the writer went to high school in 2014 until graduated in 2017 at SMAN South Sumatra. In August 2017, the writer was enrolled as a student of the Agricultural Technology, focusing on Agricultural Product Technology Study Program at Sriwijaya University through the National Selection of State Universities (SNMPTN).

The writer was also active in campus organization activities, namely as general secretary of the Sriwijaya University Agricultural Technology Student Association in 2019. The writer participated in the Student Entrepreneur Program activities in 2019.

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Hopefully this thesis can contribute useful thoughts for all of us in the development of science.

Indralaya, June 2021

Writer

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CHAPTER 1
INTRODUCTION

1.1. Background

Cocoa is one of the commodities that contributes to the country's third largest foreign exchange earnings in the plantation sub-sector. Cocoa contributed US$ 1.24 billion in foreign exchange earnings (Directorate General of Plantation, 2019). In 2010, Indonesia was ranked third in the world for cocoa bean exporters after Ghana and Ivory Coast (ICCO, 2012). However, Indonesia's cocoa export volume has tended to decline every year in the last 10 years with an average decline of 2.24% per year (Yuliawati, 2019). This is partly due to the government's policy of stipulating export duties on dried cocoa bean exports. This stipulation aims to make cocoa beans more domestically processed so that they can be sold in the form of semi-finished materials that have more added value. Thus, this is a good thing if accompanied by the development of post-harvest technology and processing of processed cocoa bean products.

One of the obstacles to increasing added value and diversifying cocoa products is the low quality of dried cocoa beans produced by Indonesian farmers (Putra *et al*., 2010). The low quality of Indonesian cocoa beans is caused by several factors, one of which is cocoa bean processing that is still not in accordance with good handling practices (GHP) standards. According to Putra *et al.* (2010), most farmers do not ferment cocoa beans because the process takes a relatively long time. Meanwhile, the limited economic situation of farmers requires them to trade their crops quickly. In fact, fermented cocoa beans have a higher selling value because during the fermentation process a variety of aroma precursor compounds are formed in the final product that are more favorable to consumers.

Processed cocoa bean products are mostly consumed for their favorable flavors and functional compounds that are beneficial to health. The preferred flavor is the result of proper processing, especially in the fermentation and roasting processes. Wijanarti (2018) stated that the fermentation process of cocoa beans produces aroma precursor compounds and will react through the Maillard reaction during roasting to produce the preferred aroma and flavor of cocoa. However, the processing process can reduce one of the functional compounds of cocoa beans, namely antioxidant compounds. Ariyanti (2017) in her journal stated that the fermentation process can reduce the polyphenol content and antioxidant activity of cocoa beans. Cocoa polyphenols are compounds that act as antioxidants and are very important for human health. Several studies report that cocoa bean polyphenols have a role in the prevention of coronary heart disease and cancer (Othman *et al.,* 2007 in Diyantika *et al.,* 2017). However, the benefits of these polyphenols may be reduced during post-harvest processing such as fermentation, drying and roasting. Therefore, Hu *et al.* (2016) conducted a study to optimize the polyphenol content of fermented cocoa beans using the pressure roasting (puffing) method. The results showed that the loss of bioactive components and antioxidants in fermented cocoa beans can be minimized if processed with the puffing gun method. In addition to reducing exposure to high temperatures during processing, unfermented cocoa beans have higher antioxidants than spontaneously fermented cocoa beans. However, unfermented cocoa beans have a more astringent flavor and are less palatable to consumers. This poses a challenge to the industry in optimizing the processing of non-fermented cocoa beans while maintaining a taste that is preferred by consumers.

Purwanto *et al.* (2019) has conducted research to optimize the quality of cocoa beans by conducting lab-scale fermentation using cassava yeast starter. The results showed that the fermentation of cocoa beans carried out with the addition of cassava yeast took place faster, namely on the third day of degradation of the cocoa pulp substrate and many primary metabolites were formed. By looking at this potential, the quality of non-fermented cocoa beans produced by Indonesian farmers can be improved by fermenting using cassava yeast which does not take too long. Processing modifications such as fermentation using cassava yeast and pressure roasting using a puffing gun are expected to optimize the functional properties and taste of cocoa beans. In addition to processing modifications, puffing cocoa nibs can also be an alternative to increase added value and diversify cocoa products.

Cocoa nibs are dried cocoa beans that have been roasted and then reduced in size into small pieces. Puffed cocoa nibs can be consumed directly or processed into chocolate products. Therefore, this study was conducted with the aim to determine the physicochemical characteristics of cocoa nibs (*Theobroma Cacao* L.) with raw material treatment and puffing gun method to obtain a processing method that produces optimal physical and chemical characteristics.

1.2. Objectives

This study aimed to obtain the optimal cocoa bean processing method while maintaining the nutritional content, functional properties and taste preferred by panelists by analyzing the effect of cocoa bean fermentation and puffing gun method (pressure puffing) on the physical and chemical characteristics of cocoa nibs.

1.3. Hypothesis

Differences in pressure in the puffing process and raw material treatment significantly affect the proximate content, total polyphenols, antioxidant activity, color and taste of cocoa nibs.

CHAPTER 2
LITERATURE REVIEW

2.1. Cocoa

The cacao fruit is a dicotyledonous plant of the genus Theobroma (Table 2.1) that originated in the tropical forests of southern Mexico. Cocoa was first planted in Indonesia in 1560 in North Sulawesi. The fruit that was planted came from the Philippines, with the Criollo variety, which was thought to have originated from Venezuela. The Criollo variety of cocoa was then planted in East Java (Soehardjo *et al.,* 1996). Cocoa plants generally have three types of varieties, namely Criollo, Forastero, and Trinitario. The skin of young Criollo cocoa pods is red and orange when ripe, while Forastero is green, and the color of Trinitario is quite heterogeneous between green and red (Figure 2.1). Criollo varieties dominated the cocoa market until the mid-18th century, but today only a few Criollo trees remain. The Forastero variety is the largest group of varieties cultivated and farmed while the Trinitario variety is a cross between the Forastero and Criolo types. The cocoa plant can grow at latitudes 10^{o} S - 10^{o} N and at altitudes of 0 - 600 m above sea level (Sidabariba, 2015). The original habitat of the cocoa plant is in tropical forests with potential yields varying from 50-120 fruits/ tree/ year. Cocoa plants can be grown from seedlings that will germinate and then be taken from pots in no more than 15 days (Pusdatin, 2007).

Table 2.1. Classification of Cocoa

Subdivision	Name
Indonesian Name	Cocoa
Scientific Name	*Theobroma cacao* L.
Kingdom	Plantae (Plants)
Subkingdom	Tracheobionta(Vascular plants)
Division	Spermatophyta (Flowering plants)
Subdivision	Angiospermae

Class	Magnoliopsida (Dicotyledons)
Nation	Malvales
Tribe	Sterculiaceae
Genus	Theobroma
Type	Theobroma cacao

Source: Bhattacharjee and Akoroda (2018)

Source: Industry.co.id

Picture 2.1 Cocoa Fruit

Ripe cocoa beans are then harvested and categorized based on their maturity class. Quality criteria for cocoa beans include physical, flavor and cleanliness at the production stage. Non -simultaneous ripeness of cocoa affects the flavor and processing of cocoa beans. Usually, optimally immature cocoa pods are being wrapped so that they ripen quickly to homogenize the ripeness of the pods and facilitate the removal of the beans from the cocoa pods. Ripening activities by wrapping the cocoa can take 5 to 7 days in a place that is not directly exposed to sunlight. The cocoa pod consists of the fruit, pulp, placenta and seeds. Pulp is the part of the cocoa fruit that contributes water, albuminoids and astringents, iron oxide, potassium salts and Cu salts to the cocoa fruit (Porbowaseso, 2005). In its seeds, cocoa is known to contain high polyphenols as much as 120-180 g/kg (Wollgast and Anklam, 2000 in Utami, 2018).

A common processed product of cocoa fruit is chocolate bar. In producing chocolate bars, the dried cocoa pods are further reduced in size to cocoa nibs.

Cocoa nibs are obtained by roasting the dried cocoa beans and crushing them into small pieces. Cocoa nibs have functional value and a distinctive aroma. Cocoa nibs contain various types of antioxidants, such as catechins, epicatechins, procyanidins which are types of polyphenols (Ramlan *et al.,* 2018). Polyphenols are secondary metabolites that give the characteristic astringent taste of chocolate through the mechanism of protein precipitation in saliva and give the typical bitter taste of chocolate along with alkaloids, some amino, peptides and pyrazine (Misnawi, 2003 in Purbowaseso, 2005).

2.2. Cocoa Bean Antioxidants

Antioxidants are a type of compound that plays a major role in inhibiting, delaying or preventing oxidation reactions in food. In biological systems, antioxidants can counteract free radicals in the body thus preventing various diseases such as cancer, arteriosclerosis, inflammation, cardiovascular disease, and dental caries (Ito *et al*., 2003; Prior and Gu, 2005 in Utami, 2018). Oxidation reactions are generally undesirable as free radicals trigger oxidative chain reactions between oxygen and certain compounds such as unsaturated fatty acids, proteins, carbohydrates and/or vitamins. Free radicals are compounds in the form of atoms or molecules that have unpaired electrons in the outermost orbitals so that they are reactive to find a partner (Utami, 2018). This oxidation reaction can occur easily if there are no antioxidant compounds. If a food ingredient has been oxidized, it will generally form new compounds that are biologically harmful to health and form new compounds with an unpleasant aroma and taste if it occurs in food ingredients.

Cocoa has become one of the commodities highlighted for its high antioxidant content. Antioxidants in cocoa are contained in the form of polyphenolic compounds, particularly flavonoids. Vinson *et al.* (1999) in Ramiro-puig and Castell (2009) reported that the polyphenol content of cocoa powder can reach 70 mg per gram of powder in the form of catechins. However, the content of these antioxidants varies depending on the type of cocoa variety, growing location and processing method of cocoa powder such as roasting, fermentation, and temperature in other processing such as in the process of felting, polishing and others. Raw cocoa beans are reported to contain 12-18% polyphenolic compounds (Tomas-Barberan *et al.,* 2007 in Oracs and Nebesny, 2016).

The antioxidant activity of cocoa beans, which is mostly produced by polyphenolic compounds, was reported to have an effect on reducing the antihyperglycemic effect in rats in an experiment conducted by Amin *et al.* (2004) in Maleyki and Ismail (2008), inhibit breast cancer cell proliferation in humans (Ramljak *et al.,* 2005 in Maleyki and Ismail, 2008), and have effects on innate immunity and improve immune function (Ramiro-Puig and Castell, 2009). In addition to their role in health, polyphenols also serve as compounds that form the distinctive flavor of cocoa when reacting with compounds such as proteins, polysaccharides and other polyphenolic compounds. The high antioxidant content of raw cocoa beans is based on the presence of flavone-3ols compounds, which consist mainly of catechins and epicatechins (Table 2.2.) as well as phenol compounds such as anthocyanins, flavonoids, and phenolic acids (Niemenak *et al.,* 2006 in Oracs and Nebesny, 2016).

Table 2.2. Changes in polyphenols in cocoa beans during fermentation.

Parameter	Cocoa Bean		Change	
	Not fermented	Fermented	Unit	
Total polyphenols (g/kg)	135 ± 4.17	63.0 ± 1.71	-72.1	-53.5
Total tannin :				
1) Concentration (g/kg)	79.3 ± 1.55	48.6 ± 1.19	-30.7	-38.7
2) Peecentage	58.7 ± 0.90	77.2 ± 3.99	+18.5	Na
(-) Epicatechin (g/kg)	40.0 ± 0.25	17.4 ± 3.02	-22.6	-56.5
(+) Catechin (g/kg)	7.7 ± 1.00	1.2 ± 0.50	-6.5	-84.5
Total anthocyanins (mg/kg)	483.1 ± 16.97	190.8 ± 13.40	-292.2	-60.5
Fermentation Index	0.57 ± 0.02	1.58 ± 0.01	+1.01	+177.2

No: +, increase ; -, decrease ; na, not analyzed

Source: Misnawi and Selamet (2003)

2.3. Cocoa Bean Fermentation

The fermentation process of cocoa beans plays an important role in determining the final quality of processed cocoa bean products. Basically, the fermentation process is carried out with the aim of destroying the fruit pulp so that it is separated from the cocoa beans before the cocoa bean drying process is carried out. During the fermentation process, many reactions occur that produce several precursor compounds that form the aroma, color and taste of cocoa. In addition, the fermentation process also causes several changes in cocoa beans, such as fermentation of sugar in the pulp layer into alcohol which is then converted back into acetic acid, temperature increase, oxidation by bacteria, causing seed death, loss of germination, diffusion of color substances from cell bags, and deconstruction of anthocyanin color substances (Hatmi and Rustijarno, 2012). These changes provide the taste, aroma and color that consumers prefer in processed cocoa bean products. Cocoa beans that do not go through the fermentation process will not experience these compound changes that make the product less favorable to consumers. Most of the cocoa beans harvested by Indonesian farmers (around 85%) are still processed traditionally and not fermented. This resulted in the price of cocoa beans and products harvested by Indonesian farmers decreased by 10 to 15% in the international market (Davit *et al.,* 2013).

The role of fermentation in cocoa beans lies not only in the formation of flavor precursors, but also in the decomposition of the pulp by microbes. Both processes are carried out by three types of microbes, namely yeast, lactic acid bacteria and acetic acid bacteria. Schwan and Whealts *et al.* (2004) in Purwanto *et al.* (2019) explained that yeast will degrade sugar-rich pulp into ethanol and release heat, lactic acid bacteria will convert sugar and citric acid into lactic acid and acetic acid, and acetic acid bacteria will oxidize ethanol into acetic acid and release greater heat.

The expected changes during fermentation occur due to the role of microbial activity during the fermentation process. This is what makes fermented cocoa beans more favorable to consumers. However, most local farmers do not do it because of the long fermentation time. Several studies on the use of starter in cocoa bean fermentation to shorten fermentation time have been conducted. Kristanto *et al.* (2017) in their journal explained that yeast from several species such as *H. Guilliermondii, P. Kudriavzevii, S. Cerevisiae* are consistently found active in fermented cocoa products around the world. Thus, the use of yeast as a starter in cocoa fermentation is feasible. Purwanto *et al.* (2019) in their research evaluated the use of cassava yeast for cocoa bean fermentation, this study studied the dynamics of microbial populations during fermentation, the degradation of sugar, ethanol, and organic acids produced in the pulp, the acidity of the beans, and the degree of fermentation of cocoa beans. The results showed that the addition of cassava yeast to cocoa fermentation resulted in rapid microbial succession and complete fermentation index test results on the third day. The study concluded that the fermentation of cocoa beans with the addition of cassava yeast can accelerate the fermentation process from 6 days to only 3 days.

The real difference between fermented cocoa beans and non-fermented cocoa beans is in the aroma, taste and color of the final product. This is because in fermented cocoa beans, free amino acids and reducing sugars are formed which will react during the roasting process or called the Maillard reaction. On the other hand, the process of fermenting cocoa either spontaneously or using a starter also reduces the antioxidant content of cocoa beans. The difference in chemical quality of fermented and non-fermented cocoa beans is shown in Table 2.3.

Table 2.3. Chemical quality of fermented and non-fermented cocoa beans and primary products

Parameter	Cocoa Bean		Cocoa Paste		Cocoa Powder	
	F	NF	F	NF	F	NF
Fat (%)	51.28	42.43	57.87	52.77	37.87	27.95
Water Content (%)		-	1.57	1.57	4.38	7..94
Protein (%)		-	7.52	16.42	16.62	19.57
Carbohydrat	-	-	29.82	26.06	36.62	40.27
pH	5.15	6.35	-	-	5.35	6.30
Ash (%)	-	-	3.22	3.40	4.60	4.23
Total Acid	1.98	0.94	-	-	-	-
Content Reducing	0.84	0.55	-	-	-	-

Note: F = Fermented; NF = Non-Fermented

Source: Towaha *et al*., (2012) in Tarigan and Iflah, (2017)

2.4. Roasting of Cocoa Beans

Cocoa roasting can be divided into several methods, namely batch or continuous systems, direct or indirect heating, and dry or wet roasting. Cocoa roasting technologies widely used by the chocolate processing industry in Europe are PDAT *(pasteurizing, deacidifying roasting)* and NARS *(nibs alkalizing roasting and sterilizing)* (Sri Mulato, 1997 in Azizah, 2005). Roasting machines in general are cylinder type whose heat is sourced from kerosene or LPG gas. The heat generated then flows through the cylinder wall and causes the water content of the cocoa beans to evaporate. The evaporation of water from the cocoa bean nucleus remains in the cylinder so that the environment in the cylinder becomes humid and hot. This makes the humidity and temperature in the cylinder roasting machine controllable so that the occurrence of over roasting beans can be prevented.

The precursor compounds of cocoa aroma, color and flavor formed during the fermentation process will then react through a browning reaction commonly called the Maillard reaction. This reaction is driven by the heat generated during the roasting process. The optimum combination of temperature and time during roasting can produce cocoa beans with a strong aroma, but in incomplete roasting (under-roasted), the aroma of chocolate is weak because the Maillard reaction does not run well (Wijanarti *et al.,* 2018). However, besides this, high temperatures during the roasting process can damage the nutritional content and bioactive compounds such as antioxidants contained in cocoa beans and can produce acrylamide which is a carcinogenic compound. The presence of acrylamide is due to the interaction between asparagine as one of the reactants with dicarbonyl compounds as co-reactants during the Maillard reaction during roasting (Farah *et al.,* 2012). High exposure to acrylamide can damage the nervous system as evidenced in animal experiments. This means that it can also potentially damage the human nervous system.

The roasting process is quite crucial if it is not done under optimal circumstances. However, even if the roasting process is carried out at a temperature and time that is considered optimal, it will still have shortcomings. Djikeng *et al.* (2018) stated that high temperatures during cocoa bean processing can also lead to fat oxidation and non-enzymatic browning which can reduce nutritional value due to loss of essential fatty acids, essential amino acids and digestible carbohydrates. Several studies have also reported that roasting can significantly reduce the polyphenol content and antioxidant activity of cocoa beans (Table 2.4). Djikeng *et al.* (2018) stated that phenolic compounds with low molecular weight can easily evaporate at high temperatures. During roasting, there is also evaporation of water-soluble compounds contained in cocoa beans, causing the moisture content of the bean matrix to decrease (Asep *et al.,* 2007). This condition will facilitate the diffusion of solvents through the internal cells of the cocoa bean when extracting cocoa butter. Despite the loss of many nutrients during roasting, consumer sensory acceptance of roasted cocoa beans (90-110°C) is high (Rocha *et al.,* 2017).

Table 2.4. Results of polyphenol, catechin, and pesticide residue analysis of cocoa powder

Parameter	Roasted Cocoa Powder	Unroasted Cocoa Powder
Polyphenols	6,89%	9,42%
Catechins	6,335 g/100g	6,626 g/100g
Pesticide Residues	TTD	TTD

Source: Wahyuni *et al*., 2019

2.5. Puffing Gun Technology

Puffing gun technology is an alternative way of roasting cocoa beans by utilizing temperature and pressure. In principle, puffing gun technology consists of accumulated hot steam that is released suddenly due to a change in internal pressure to atmospheric pressure and results in an expanded and porous grain matrix (Zapana *et al.,* 2020). Keesenberg (1978) developed a puffing gun technology composed of a rotating horizontal cylinder having a length of 1.2 m and an inner diameter of 200 m (Mishra *et al.,* 2014). The technology was initially used to cook milled grains. Today, puffing gun technology can be used to roast raw dry cocoa beans. Cocoa beans are placed in a closed, rotating chamber. When the chamber is closed, the outer chamber sides are heated so that the pressure in the chamber increases to about 8 bar and the temperature increases to 115ºC within 6 minutes (Hoke *et al.,* 2005). In this process, the pressure applied will evaporate the moisture in the cocoa bean matrix until the beans are cooked/roasted simultaneously. The application of puffing gun technology will also change the physicochemical characteristics of cocoa beans, forming a porous structure and changing the chemical composition of the ingredients (Hu *et al.,* 2015). In addition, Mu- yeol *et al.* (2017) reported their results stating that puffing-roasted cocoa beans have stronger acidity and darker color when compared to roasted cocoa beans. Several studies have also shown that the antioxidant activity, polyphenol and flavonoid content of cocoa beans decreased as the puffing pressure increased (Hu *et al.,* 2016; Mu-yeol *et al.,* 2017).

CHAPTER 3
RESEARCH METHODOLOGY

3.1. Place and Time

This research was conducted at the Integrated Laboratory, Research Center for Industrial and Refreshing Plants, Sukabumi, West Java from October 2020 to January 2021.

3.2. Tools and Materials

The tools used in this study were: 1) Beaker glass, 2) aluminum cup, 3) porcelain cup, 4) Hunterlab MiniScan EZ 4500 color reader, 5) desiccator, 6) Erlenmeyer flask, 7) grinder, 8) volumetric flask, 9) magnetic stirrer, 10) muffle furnace, 11) analytical balance, 12) oven, 13) drop pipette, 14) measuring pipette, 15) puffing gun, 16) Soxhlet circuit (Buchi solvent extractor), 17) centrifuge, 18) UV-Vis spectrophotometer, 19) test tube, 20) vortex, 21) Velp Kjeldahl apparatus.

The materials used in this study were: 1) distilled water/distilled akuades, 2) 95% alcohol, 3) acetic acid, 4) boric acid, 5) gallic acid, 6) lindak cocoa beans, 7) bromocresol green, 8) $CuSO_45H_2O$, 9) concentrated H_2SO_4, 10) HCl 0.01 N, 11) $K_2S_2O_4$, 12) filter paper, 13) DPPH solution, 14) methanol, 15) methyl red, 16) Na_2CO_3, 17) 30% NaOH, 18) hexane solvent, 19) Folin-Ciocalteu reagent, and 20) SeO_2.

3.3. Research Method

This study used a Factorial Completely Randomized Design (CRD) with 2 treatment factors, namely the pressure used during the puffing process (A) consisting of 3 treatment levels (3, 4, 5 Bar) and the type of raw material treatment before roasting (B) consisting of 3 treatment levels (fermentation, non-fermentation, and fermentation with yeast). The combination of puffing pressure and raw material treatment resulted in 9 treatments, namely:

A1 : 3 Bar puffing pressure B1: Spontaneously fermented cocoa bean raw material

A2 : 4 Bar puffing pressure B2: Non-fermented cocoa bean raw material

A3 : 5 Bar puffing pressure B3: Raw material of cocoa beans fermented with tape yeast

3.3.1. Data Analysis

The data obtained were processed using analysis of variance (ANOVA) with a confidence level of 95%. Treatments with significant effects were further tested using Duncan's test (DMRT) at the 5% level.

3.4. Procedures

3.4.1. Sample Preparation

3.4.1.1. Preparation of Non-fermented Cocoa Beans

Cocoa pods were peeled to extract the seeds. Cocoa beans that had been separated from the pod were dried in the sun until the moisture content reached ±7%. The dried non-fermented cocoa beans were stored in airtight plastic at room temperature.

3.4.1.2. Preparation of Spontaneous Fermentation Cocoa Beans

Cocoa pods were peeled to extract the seeds. Cocoa beans that had been separated from the pods were sorted and fermented in a wooden box with a capacity of 40 kg/batch for 5 days and dried in the sun until the moisture content reached ±7%. Dry fermented cocoa beans were stored in airtight plastic at room temperature.

3.4.1.3. Fermentation of Cocoa Beans with Tape Yeast Starter

The fermentation method used referred to the research method of Purwanto *et al.* (2019) with pilot plant scale modification in a wooden box. A total of ±400 cocoa pods were broken using a wooden beater. Wet cocoa beans were put into a fermentation box made of wood with a capacity of 40 kg/ batch. The tape yeast was crushed into powder and inoculated as much as 0.2% (w/ b) into the fermentation box. Fermentation was carried out for three days and then dried in the sun until the moisture content was ±7%. Dry fermented cocoa beans were stored in airtight plastic at room temperature.

3.4.1.4. Pressurised Roasting of Dry Cocoa Beans

The roasting process of cocoa beans was carried out using a traditional puffing gun machine. First, raw cocoa beans were loaded into the chamber and the heating process begins. The chamber was opened when the machine had reached the desired pressure (3, 4, and 5 Bar). After that, the cocoa beans were cooled. The roasted beans were then deshelled to obtain cocoa nibs, which were then stored and subjected to organoleptic analysis. Other cocoa nib samples were pulverized into powder using a grinder and stored for other analyses.

3.4.2. Parameter Analysis

3.4.2.1. Cocoa Beans Defatted

Cocoa nib defatting was conducted to analyze polyphenols and antioxidants of cocoa nibs, the method referred to Indiarto *et al.* (2019) with modifications, namely:

1. 1.A total of 250 g of crushed cocoa beans were added to 1250 ml of hexane.
2. Then extracted on a magnetic stirrer for 30 minutes.
3. Then centrifuged for 20 minutes at 1000 rpm.
4. After that, it was filtered to take the filtrate.
5. This process was repeated 3 times
6. Defatted cocoa beans were obtained.

3.4.2.2. Extraction of Cocoa Beans

Cocoa bean extraction referred to Indiarto *et al.* (2019) with slight modifications, namely:

1. 5 g each of fat-free cocoa powder was weighed and dissolved with 25 ml methanol PA (99.8%) in an Erlenmeyer flask.
2. The solution was homogenized with a magnetic stirrer for 1 hour.
3. Then extraction was carried out using a sonicator bath for 15 minutes.

4. The extracted solution was centrifuged.
5. Then the filtrate was filtered using Whatman no.1 filter paper.
6. The filtrate was obtained in the form of cocoa bean extract.

3.4.2.3. Total Polyphenol Analysis

Total polyphenol analysis of cocoa beans referred to Indiarto *et al.* (2019) with modifications, namely:

Preparation of Gallic Acid Curve

1. The standard curve was made by weighing 0.01 g of gallic acid and then diluted to 100 mL with distilled water.
2. A dilution series was made, each of which was 10 mL with a concentration of 0, 20, 40, 60, 80, 100 mg / L
3. Each standard was pipetted as much as 0.5 mL placed in a test tube
4. Added 0.5 Folin-Ciocalteu reagent, vortexed and incubated for 1 minute
5. Then added 1.5 mL of 20 percent Na_2CO_3 solution.
6. Next, vortexed and incubated for 2 hours at room temperature and dark place
7. After incubation, distilled water was added as much as 7.5 ml and then vortexed
8. Then read the absorbance value at a wavelength of λ 760 nm.

Total Polyphenol Analysis

1. Filtrate of cocoa bean extract was pipetted 0.5 ml then added 0.5 ml of Folin-Ciocalteu reagent.
2. Vortexed so that it is homogeneous and incubated for 1 minute.
3. Next, 1.5 ml of 20% Na_2CO_3 was added and vortexed.
4. Then added distilled water as much as 7.5 ml and vortexed
5. The sample was incubated in a dark room and room temperature for 2 hours
6. Read the absorbance value at a wavelength of λ 760 nm.

7. The results of total polyphenols were presented in mg GAE/gram of defatted cocoa beans obtained from the standard curve equation of gallic acid solution with a concentration of 1-100 mg/L (ppm).

3.4.2.4. Determination of Antioxidant Activity

Analysis of antioxidant activity by DPPH method was carried out according to Joyeux *et al*. (1995) which has been modified in the following way:

1. Take a sample of 0.2 ml in a test tube
2. Add 3.8 ml of DPPH solution (0.0020 mg in 80 mL of methanol) and homogenized with a vortex
3. Make a blank solution by adding 0.2 ml of methanol to 3.8 ml of DPPH solution that has been made earlier
4. All samples and blanks were incubated in a dark room and room temperature for 1 hour
5. 5.The absorbance value was measured with a spectrophotometer at a wavelength of 517 nm.
6. The absorbance value of % antioxidant activity was calculated by the following formula

$$\text{\% Antioxidant activity} = \frac{\text{Absorbance of blank (p0) - Absorbance of sample (p1)}}{\text{Absorbance of blank (p0)}} \times 100\%$$

3.4.2.5. Proximate Analysis

The method used in proximate analysis referred to SNI 01-2891-1992 on how to test food and beverages.

- **Water Content (Gravimetric Method)**
  1. The aluminum cup was put in the oven for 1 hour and cooled using a desiccator for 15 minutes, then the weight of the cup was weighed until constant (no more than 0.2 mg or 0.0002 g).
  2. Samples weighed as much as ± 2 g and put into a cup that has known weight

3. The aluminum cup containing the sample was dried in an oven at a temperature of 1050C until the weight was constant
4. The cup containing the sample was removed and cooled in a desiccator for 15 minutes, then the cup and sample were weighed again until the weight was constant.
5. 5.The moisture content of the sample was determined from the weight of the evaporated water. Percent moisture content could be calculated using the following formula:

$$\text{Moisture content (\%)} = \frac{\text{initial weight (g)} - \text{final weight (g)}}{\text{initial weight (g)}} \times 100\%$$

- **Ash Content (Gravimetric Method)**
  1. Samples that had been mashed weighed as much as ± 2 g
  2. The sample was then put into a porcelain cup that has known weight
  3. The sample was burned until the smoke is no longer visible.
  4. The cup containing the sample was put into a muffle furnace at a temperature of 5500C until the color of whitish ash
  5. 5.After the sample was white, the porcelain cup was taken with tongs, then put into a desiccator to cool down
  6. 6.Determination of sample ash content based on the weight of vaporized organic compounds. The calculation of ash content was the ratio of ash weight to wet weight, calculated using the following formula:

$$\text{Ash content (\%)} = \frac{\text{final weight (g)} - \text{empty cup weight (g)}}{\text{final sample weight (g)}} \times 100\%$$

- **Protein Content (Kjeldahl Method)**

  Deconstruction
  1. Weigh the sample as much as 0.25 grams and then put it into the deconstruction flask
  2. Add 5 ml of concentrated sulfuric acid
  3. Incubate overnight
  4. After incubation, the sample is heated/burned at 100°C for 1 hour
  5. Then added 2 ml of 30% HPO_4 solution

6. Reheat at 200°C for 1 hour 6
7. After 1 hour, 2 ml of 30% HPO_4 solution was added
8. The sample was heated again at 350°C for 1 hour
9. Cool the sample before diluting with distilled water to 50 ml.

Distillation and Titration

1. Take 5 ml of the extracted and diluted sample solution to the distillation flask.
2. Sample plus 30 ml of 40% NaOH solution
3. Add 2 drops of PP indicator
4. Distilled for 10 minutes
5. 15 ml of 1% boric acid solution was added to the collection glass.
6. Then the distilled sample was titrated with 0.01 N sulfuric acid solution.

Then the calculation is done with the following formula:

$$\text{Protein Content} = \frac{(V1 - V2) \times N \times 0{,}014 \times FK \times FP}{w} \times 100\%$$

- **Fat Content (Soxhlet Method)**

The working method for determining fat content is as follows:

1. The fat flask is dried in the oven at 100 - 110°C, then cooled and weighed.
2. Samples as much as ± 5 grams are weighed and wrapped in filter paper and then put into the Soxhlet which has contained a solvent (diethyl ether or hexane).
3. Reflected for 5 hours.
4. The flask containing the extracted fat was heated in an oven at 100°C until constant weight then cooled in a desiccator and weighed.
5. Calculation of percentage fat content can be done with the formula:

$$\%\text{Fat content} = \frac{\text{weight of flask and final sample (g) - weight of flask (g)}}{\text{initial sample weight (g)}} \times 100\%$$

- **Carbohydrate Content (Carbohydrate by Difference)**

  According to Winarno (1984), carbohydrate by difference is a way to determine carbohydrate content by calculation (formula) without the analysis process.

  The %carbohydrate formula is:

  % Carbohydrate = 100% - % (protein content + fat content + water content + ash content)

3.4.2.6. Color Analysis

Color measurement is done using a color reader.

According to (AOAC, 1995), the test is carried out as follows:

1. The color reader is calibrated with a white standard plate,
2. The optical head is affixed to the white plate, so that the white part of the plate faces the light source.
3. Then select the *L**, *a**, and *b** reading scale menu, and the START button is pressed so that the *L**, *a**, and *b** values are read
4. The optical head is then attached to the sample and the START button is pressed.
5. The measurement results will read the *L**, *a**, and *b** values of the sample.

3.4.2.7. Organoleptic Test

Assessment of the aroma and flavor of cocoa nibs was conducted organoleptically using the hedonic test. According to Pratama (2011), the hedonic test is used to determine the level of liking for the test sample. A total of 42 panelists (consumers) filled out questionnaires on samples placed in a container and were given a favorability assessment of aroma and taste by giving a score on the following scale:

1 = Very dislike 3 = Like

2 = Dislike 4 = Very like

CHAPTER 4
RESULTS AND DISCUSSION

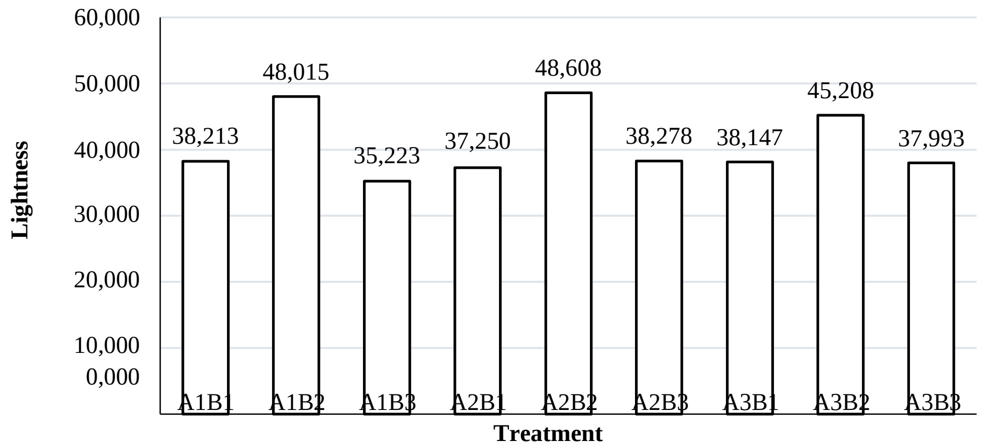
4.1. Physical Characteristics

4.1.1 Color

Measurement of the color of cocoa nibs is done because the color of the nibs will affect the quality of the final product. In addition to attracting consumers, color can also indicate the characteristics or quality of a food ingredient (Asmaraningtyas, 2014). In this study, color was measured using a color reader that showed the color of the material through the values of lightness, redness, and yellowness.

4.1.1.1 Lightness (L*)

Lightness indicates the degree of brightness of the material in a range of values between zero and one hundred on a color meter. An L* value close to 100 indicates the material has a high brightness and an L* value close to zero indicates a material that has a low or dark brightness (Nugrahani, 2014).



Note:

A1 = puffing pressure 3 Bar	B1 = spontaneously fermented cocoa beans
A2 = puffing pressure 4 Bar	B2 = non-fermented cocoa beans
A3 = puffing pressure 5 Bar	B3 = tape yeast fermented cocoa beans

Figure 4.1. Lightness value of pressure-roasted cocoa nibs

Figure 4.1. showed that the highest lightness value was obtained for non-fermented cocoa nibs roasted at 4 Bar pressure. The sample with the lowest lightness was fermented cocoa nibs with tape yeast and roasted at 3 Bar pressure, which meant that these nibs had a dark brown color in accordance with the typical color of roasted cocoa beans. The results of the analysis of variance showed that the treatment of raw material type and the interaction between puffing pressure and raw material type significantly affected the brightness of cocoa nibs, so a Duncan's further test was conducted which could be seen in Table 4.1. and Table 4.2.

Table 4.1. Duncan's further test results 5% effect of raw material on the value of lightness of cocoa nibs

Raw Material	Average *Lightness*
Fermentation Tape Yeast	37,165[a]
Spontaneous Fermentation	37,870[a]
Non-fermentation	47,277[b]

Note: numbers followed by the same letter meant not significantly different.

Table 4.2. Duncan's further test results of 5% interaction effect between two factors treatment on cocoa nib lightness.

Raw Material	Lightness on Puffing Pressure		
	3 Bar	4 Bar	5 Bar
Spontaneous Fermentation Cocoa Beans	38,217 [bX]	37,250 [bX]	38,150 [bX]
Non-Fermented Cocoa Beans	48,017 [aX]	48,613 [aX]	45,210 [aY]
Tape Yeast Fermented Cocoa Beans	35,227[cY]	38,283[bX]	37,997 [bX]

Note: numbers followed by the same lowercase letter in the column or the same capital letter in the row meant that they were not significantly different.

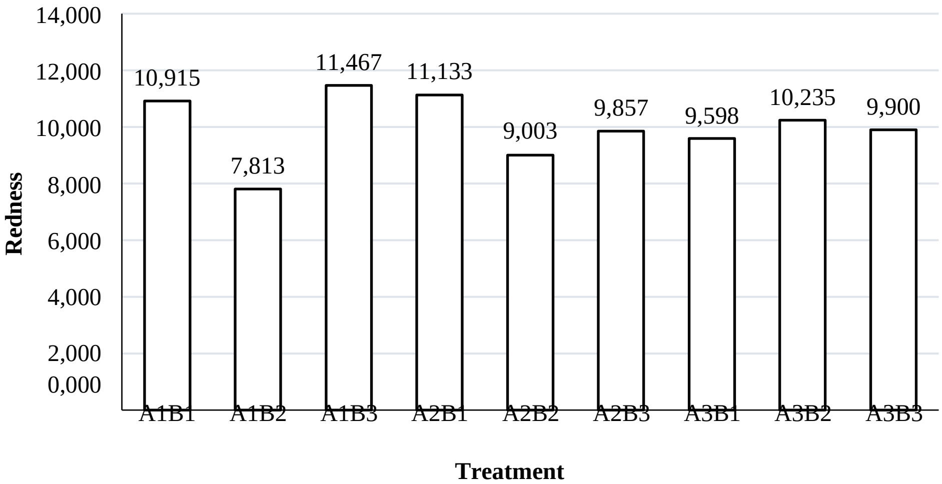
Further test results showed that non-fermented cocoa nibs obtained the highest value and were significantly different from the other two treatments. This meant that fermented cocoa nibs either spontaneously or using tape yeast gave a darker color than unfermented cocoa nibs. The interaction of puffing pressure and type of raw material also gave a significant difference between each treatment. Based on the results of the further test of the effect of treatment interaction, it could be seen that the most significantly different treatment interaction was fermented cocoa nibs with tape yeast roasted at 3 Bar pressure and non-fermented cocoa nibs roasted at 5 Bar pressure. Based on the results of color measurement, it could also be seen that spontaneously fermented cocoa nibs did not give a significantly different color from cocoa nibs fermented with tape yeast, while puffing pressure had no effect on color, but it would have an effect if it interacted with the raw material type treatment.

In general, the darker color of cocoa nibs along with the length of fermentation is due to the reduction of anthocyanins by hydrolysis enzymes (Afoakwa, 2014). The formation of dark brown color in cocoa beans is the result of the transformation of anthocyanin compounds during fermentation. Anthocyanins will polymerize with catechins and form complex tannins and change the color of cocoa beans to dark brown (Sulaiman & Yang, 2015). According to several researchers (Hawlader *et al.*, 2006; Hii *et al.*, 2009; Rocha and Morais, 2003 in Ndukwu and Udofia, 2016), the browner the color of the cocoa beans, the better the fermentation process and an increase in the degree of redness of the cocoa beans indicates a better browning reaction (Maillard reaction).

4.1.1.2. Redness (a*)

The a* or redness notation value expresses the degree of redness or mixed chromatic colors of red to green (Soewarno, 1990 in Souripet, 2015).

The average redness value of pressure-roasted cocoa nibs could be seen in Figure. 4.2.



Note:
A1 = puffing pressure 3 Bar
A2 = puffing pressure 4 Bar
B1 = spontaneously fermented cocoa beans
B2 = non-fermented cocoa beans

A3 = puffing pressure 5 Bar B3 = tape yeast fermented cocoa beans

Figure 4.2. Redness value of pressure-roasted cocoa nibs

The highest redness value was for fermented cocoa nibs using tape yeast and roasted at 3 Bar pressure while the lowest value was for non-fermented cocoa nibs roasted at 3 Bar pressure. When viewed based on the chromaticity diagram, the redness value ranging from 0 to 20 indicated a brownish-red color which if the value increases, the color saturation would also increase. The results of the analysis of variance showed that the treatment of raw material type and the interaction between puffing pressure and raw material type significantly affected the redness of cocoa nibs, so a Duncan's further test was carried out for the treatment of raw material type and the interaction of raw material treatment and puffing pressure.

Table 4.3. Results of Duncan's further test 5% effect of raw material on the value of redness of cocoa nibs

Raw Material	Average Redness
Tape Yeast Fermentation	9.017[a]
Spontaneous Fermentation	10.408[b]
Non-fermentation	0.[549b]

Note: numbers followed by the same letter meant not significantly different.

Further test results showed that unfermented cocoa nibs had the lowest redness value and were significantly different from the other two treatments. This meant that spontaneously fermented cocoa nibs or those fermented with tape yeast gave a more reddish color and the difference in fermentation method was not significantly different. The interaction of puffing pressure treatment and type of raw material also gave a significant difference between each treatment which could be seen in Table 4.4.

Table 4.4. Duncan's further test results 5% effect of interaction between two factors on the redness of cocoa nibs.

Raw Material	Redness on Puffing Pressure		
	3 Bar	4 Bar	5 Bar
Spontaneous Fermentation Cocoa Beans	10,917 ^{aX}	11,137 ^{aX}	9,603 ^{aY}
Non-Fermented Cocoa Beans	7,817 ^{bZ}	9,003 ^{cY}	10,240 ^{aX}
Tape Yeast Fermented Cocoa Beans	11,470 ^{aX}	9,860 ^{bY}	9,903 ^{aY}

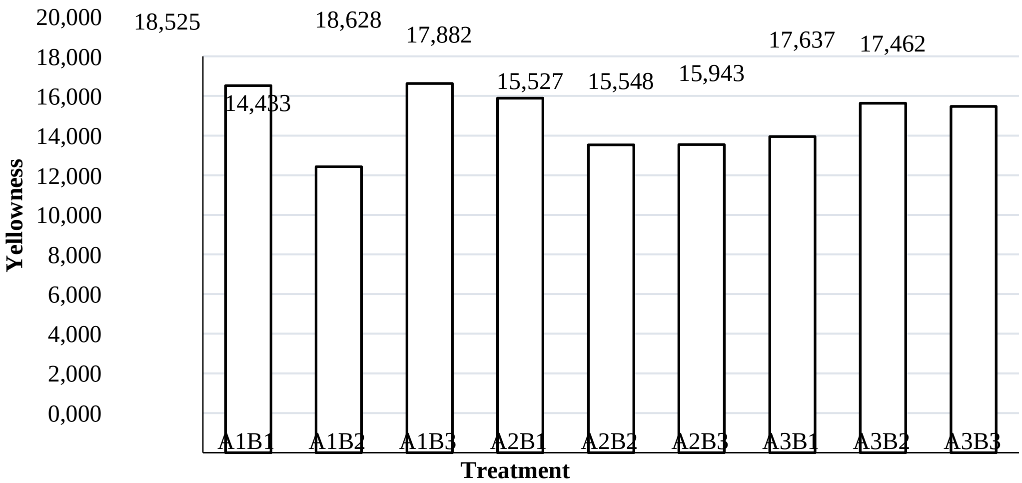
Note: numbers followed by the same lowercase letter in the column or the same capital letter in the row meant that they were not significantly different.

Based on the table, it could be seen that the type of unfermented raw material (B2), whether roasted at 3 or 4 Bar pressure, was the treatment interaction that most significantly affected the level of redness of cocoa nibs. Both treatment interactions also showed the lowest redness value compared to other treatment interactions. Table 4.4. also showed that the type of raw material for spontaneous fermentation gave a significantly different value to tape yeast fermentation only at 4 Bar pressure. This meant that to get the same color as spontaneously fermented cocoa beans, fermented cocoa beans with tape yeast were sufficiently roasted at 3 Bar puffing pressure.

This result was in line with the research of Hartuti *et al.* (2019), where fermented cocoa beans gave a higher reddish and yellowish color than non-fermented cocoa beans. This was related to the presence of polyphenol compounds that were oxidized during the fermentation process as a result of enzymatic oxidation by the enzyme polyphenol oxidase. Polyphenolic compounds, especially anthocyanins, would be oxidized to produce complex tannins that gave a dark brown color. In addition, the color formation also occurred due to the Maillard reaction during the heating process which made the color of the seeds darker brown (Puziah, 2005 in Hayati *et al.*, 2012).

4.1.1.3. Yellowness (b*)

The b* or yellowness notation value expresses the degree of yellow or blue-yellow mixed chromatic color with a positive notation from a value range of 0 to 70 for blue and a negative notation with a value range of 0 to - 70 for yellow color (Soewarno, 1990 in Souripet, 2015). The highest yellowness value was in fermented cocoa nibs using tape yeast and roasted at 3 Bar pressure while the lowest value was in non-fermented cocoa nibs roasted at 3 Bar pressure. When viewed based on the chromaticity diagram, the yellowness value ranging from 14 to 20 indicated a dark brown color, the greater the value, the more yellow. The average yellowness value of pressure-roasted cocoa nibs could be seen in Figure. 4.3.



Note:

A1 = puffing pressure 3 Bar	B1 = spontaneously fermented cocoa beans
A2 = puffing pressure 4 Bar	B2 = non-fermented cocoa beans
A3 = puffing pressure 5 Bar	B3 = tape yeast fermented cocoa beans

Figure 4.3. Yellowness value of pressure-roasted cocoa nibs

The results of the analysis of variance showed that the treatment of raw material type and puffing pressure had no significant effect on the yellowness value of cocoa nibs, while the interaction between puffing pressure and raw material type had a significant effect, so Duncan's further test was conducted for the interaction of raw material treatment and puffing pressure which was presented in Table 4.5.

Table 4.5. Duncan's further test results of 5% interaction effect between two factors on the yellowness of cocoa nibs.

Raw Material	Yellowness of Puffing Pressure		
	3 Bar	4 Bar	5 Bar
Spontaneous Fermentation Cocoa Beans	18,527 ^{aX}	17,887 ^{aX}	15,947 ^{aX}
Non-Fermented Cocoa Beans	14,437 ^{bY}	15,530 ^{aXY}	17,640 ^{aX}
Tape Yeast Fermented Cocoa Beans	18,630 ^{aX}	15,550 ^{aY}	17,463 ^{aXY}

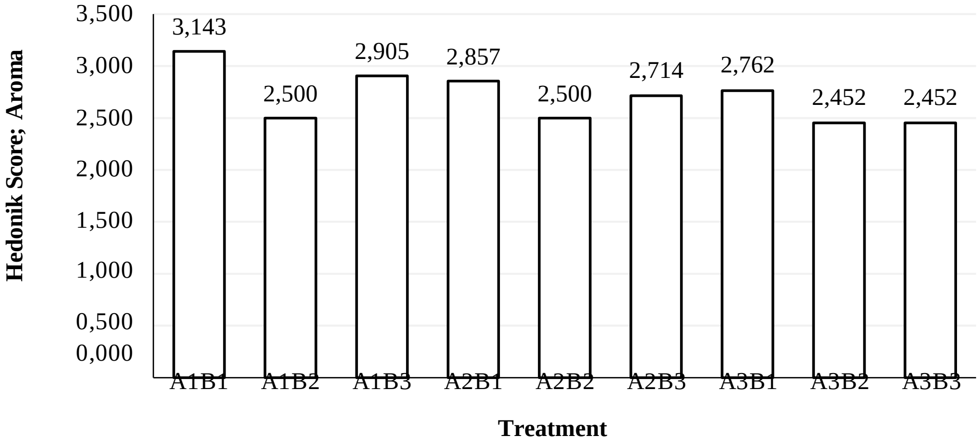
Note: numbers followed by the same lowercase letter in the column or the same capital letter in the row meant not significantly different.

Based on Table 4.5, it could be seen that the type of raw material that was not fermented and roasted at a pressure of 3 Bar was the treatment with the lowest value and the most significant effect on the level of yellowness of cocoa nibs, and fermented cocoa nibs with tape yeast roasted at a pressure of 3, 4, or 5 Bar did not show different yellowness values with spontaneously fermented cocoa nibs. Similar to the degree of brightness and redness, and in line with the research of Puziah (2005) in Hayati *et al.* (2012), that the degree of yellowness of cocoa nibs was also influenced by two factors, namely the process and length of time of fermentation and the length of drying and/ or heating process. Unfermented cocoa nibs would not give an optimal cocoa bean color because the original anthocyanins which were a mixture of red, blue and purple are not hydrolyzed into dark brown complex tannins. However, the formation of the dark brown color was still formed in non-fermented cocoa beans, namely during the roasting process due to the Maillard reaction between reducing sugars and amino acids whose content was not as optimal as in fermented cocoa beans. Therefore, the non-fermented raw material treatment was significantly different from spontaneously fermented cocoa beans and tape yeast only at 3 Bar pressure.

4.1.2 Organoleptic Characteristics

4.1.2.1 Aroma

Indonesian cocoa production is one that has a distinctive aroma and is not found in cocoa from other countries (Riffin, 2012 in Nurhafsah *et al.,* 2020). In this study, 42 panelists tested the aroma of cocoa nibs which was presented in Figure 4.4.



Note:

A1 = puffing pressure 3 Bar B1 = spontaneously fermented cocoa beans
A2 = puffing pressure 4 Bar B2 = non-fermented cocoa beans
A3 = puffing pressure 5 Bar B3 = tape yeast fermented cocoa beans

Figure 4.4 Panelists' favorability scores for the aroma of pressure-roasted cocoa nibs

The results of the favorability test in Figure 4.4. showed that the aroma of spontaneously fermented cocoa nibs roasted at 3 Bar pressure was most favorable because it showed the highest average preference score compared to other treatments. The lowest average score was obtained for tape yeast fermented cocoa nibs roasted at 4 and 5 Bar pressure. The result of analysis of variance showed a significant difference between treatments on the aroma of cocoa nibs. Therefore, Duncan's further test was conducted to determine whether there was a difference between each treatment.

Table 4.6. Duncan's further test results 5% treatment effect on panelists' favorability of cocoa nib aroma

Treatment Combination	Average Hedonik Score
A3B2	$2,452^{a}$
A3B3	$2,452^{a}$
A1B2	$2,500^{a}$
A2B2	$2,500^{a}$
A2B3	$2,714^{ab}$
A3B1	$2,762^{ab}$
A2B1	$2,857^{bc}$
A1B3	$2,905^{bc}$
A1B1	$3,143^{c}$

Note: Numbers followed by the same letter meant not significantly different.

A1 = puffing pressure 3 Bar B1 = spontaneously fermented cocoa beans
A2 = puffing pressure 4 Bar B2 = non-fermented cocoa beans
A3 = puffing pressure 5 Bar B3 = tape yeast fermented cocoa beans

Further test results (Table 4.6.) showed that spontaneously fermented cocoa nibs roasted at 3 Bar pressure had the highest favorability score and was significantly different from the other 8 treatments. The other two most preferred treatments were spontaneously fermented cocoa nibs roasted at 4 Bar pressure and tape yeast fermented cocoa nibs roasted at 3 Bar pressure, which were significantly different from the other 5 treatments. The fermentation process and puffing pressure affected the content of volatile compounds in cocoa nibs that contributed to the formation of flavor and aroma. However, the type of fermentation either spontaneous or using tape yeast at the same puffing pressure gave no significant difference to the panelists' favorability of aroma. During the fermentation process, precursors of typical cocoa aroma were formed (Hatmi and Rustijarno, 2012). These aroma precursors were associated with amino acids and reducing sugars that would develop into aroma-forming compounds after passing through the roasting stage. These compounds interacted with each other to produce aroma-giving compounds, including glyoxal and glycine, which were the most dominant in giving the sensation of roasted bean aroma (Wahyudi *et al.,* 2015 in Nurhafsah *et al.,* 2020). Increased pressure during the puffing process could minimize the loss of aroma-forming volatile compounds, where Kim (2017) reported that exposure to high pressure in closed conditions during puffing could prevent the loss of trigonelline volatile compounds in coffee beans. Another study stated that increasing a certain roasting temperature, some volatile compounds such as pyrazine (the most dominant volatile compound in cocoa beans) would increase but would decrease if it exceeded its optimal temperature (Sudibyo, 2008).

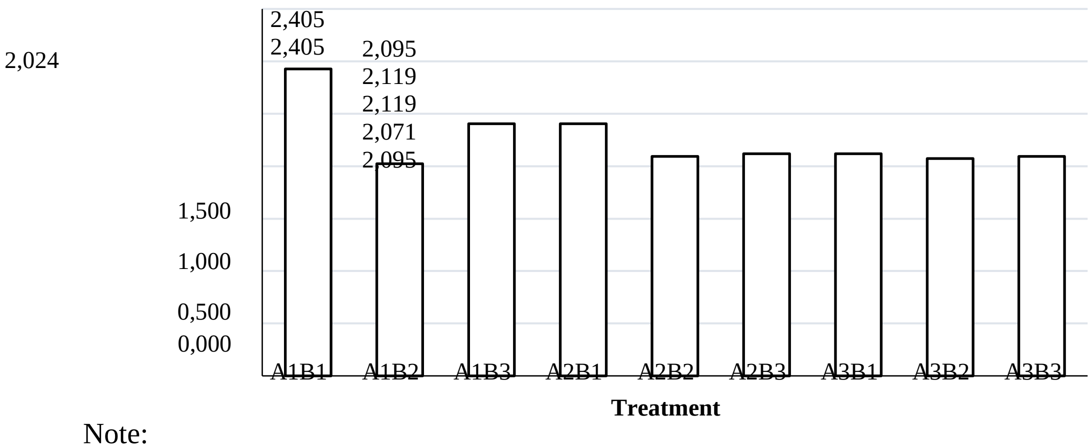
4.1.2.2. Flavor

Flavor is one of the characteristics that is also influenced by the fermentation and roasting process of cocoa beans (Purwo, 2012 in Nurhafsah, 2020). The average score of panelists' favorability for the of pressure-roasted cocoa nibs could be seen in Figure 4.5.

3,500 3,000 2,500 2,000

2,929

Hedonik Score; fFavor



Note:

A1 = puffing pressure 3 Bar	B1 = spontaneously fermented cocoa beans
A2 = puffing pressure 4 Bar	B2 = non-fermented cocoa beans
A3 = puffing pressure 5 Bar	B3 = tape yeast fermented cocoa beans

Figure 4.5. Panelists' favorability scores for the flavor of pressure-roasted cocoa nibs

Figure 4.5. showed that spontaneously fermented cocoa nibs roasted at 3 Bar pressure obtained the highest favorability score. Analysis of variance showed that each treatment had a significant effect, so Duncan's further test was conducted, which was showed in Table 4.7.

Table 4.7. Duncan's further test results of 5% treatment effect on panelists' favorability of cocoa nibs flavor

Treatment Combination	Average Hedonik Score
A1B2	$2{,}024^{a}$
A3B2	$2{,}071^{ab}$
A2B2	$2{,}095^{ab}$
A3B3	$2{,}095^{ab}$
A2B3	$2{,}119^{ab}$
A3B1	$2{,}119^{ab}$
A1B3	$2{,}403^{b}$
A2B1	$2{,}403^{b}$
A1B1	$2{,}929^{c}$

Note: Numbers followed by the same letter meant not significantly different.

A1 = puffing pressure 3 Bar	B1 = spontaneously fermented cocoa beans
A2 = puffing pressure 4 Bar	B2 = non-fermented cocoa beans
A3 = puffing pressure 5 Bar	B3 = tape yeast fermented cocoa beans

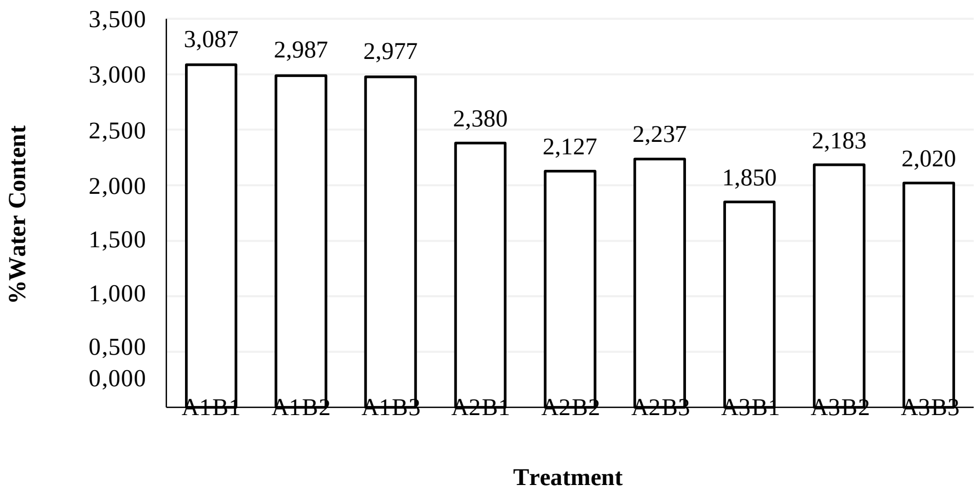
Based on Table 4.7, it could be seen that spontaneously fermented cocoa nibs roasted at 3 Bar pressure had the highest favorability score and was significantly different from the other 8 treatments. The panelists' favorability score for the next highest taste was for spontaneously fermented cocoa nibs roasted at a pressure of 4 Bar and tape yeast fermented cocoa nibs roasted at 3 Bar pressure, both samples were significantly different from the other two samples. The lowest score was obtained by non-fermented cocoa nibs roasted at 3 Bar pressure.

These results showed that panelists preferred fermented cocoa nibs, either naturally or with the addition of tape yeast compared to non-fermented cocoa nibs. This might occur because during the fermentation process, the formation of typical cocoa flavor candidates and the reduction of astringent and bitter flavors that were usually not preferred (Atmawinata *et al.,* 1998 in Oktarianti and Rohmah, 2017).

4.2. Chemical Characteristics

4.2.1. Moisture Content

The moisture content of the material is one of the characteristics that shows the amount of water contained in the material including free water and bound water and is expressed in percent (Najih & Nurhidajah, 2011). This characteristic is an important parameter and is quite influential on many other material properties. Moisture content of ingredients can also be used as one of the determinants of quality and shelf life of food.



Note:

A1 = puffing pressure 3 Bar	B1 = spontaneously fermented cocoa beans
A2 = puffing pressure 4 Bar	B2 = non-fermented cocoa beans
A3 = puffing pressure 5 Bar	B3 = tape yeast fermented cocoa beans

Figure 4.6. Average moisture content of pressure-roasted cocoa nibs

Based on the figure above, it was known that the average moisture content value of pressure-roasted cocoa nibs using the puffing method ranges from 1.85% to 3,09%. In general, the moisture content of cocoa beans roasted without pressure ranges from 1-2% (de Zaan, 2009 in Utami, 2018). The highest moisture content was found in spontaneously fermented and pressure-roasted cocoa nibs at 3 Bar, while the lowest average moisture content was found in spontaneously fermented and pressure-roasted cocoa nibs at 5 Bar. The moisture content decreased by 4% from before roasting using a puffing gun.

The result of analysis of variance showed that the puffing pressure variation treatment significantly affected the moisture content of cocoa nibs while the raw material type treatment did not significantly affect the moisture content of cocoa nibs. Therefore, Duncan's further test was conducted for the treatment of puffing pressure variation which could be seen in Table 4.8.

Table 4.8. Duncan's further test results of 5% effect of puffing pressure variation treatment factor on cocoa nibs moisture content.

Puffing Pressure	Average Water Content
5	2,018[a]
4	2,248[a]
3	3,017[b]

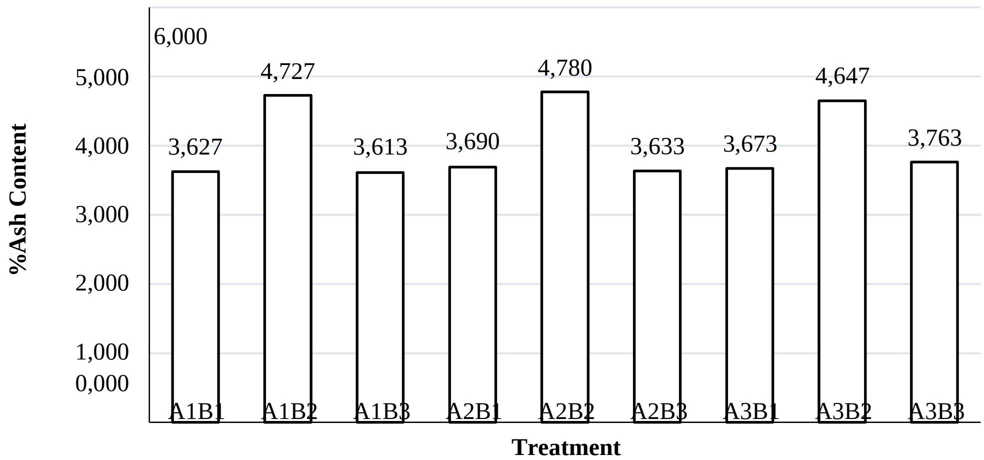
Note: numbers followed by the same letter meant not significantly different.

The results of the further test showed that the puffing pressure of 4 Bar was not significantly different from the puffing pressure of 5 Bar, but the puffing pressure of 3 Bar was significantly different from the other two treatments. The moisture content at a pressure of 3 Bar was also in accordance with the maximum limit of moisture content of roasted cocoa beans, which was around 7%. This meant that 3 Bar puffing pressure significantly affected the moisture content of cocoa nibs and met the maximum limit of roasted cocoa bean moisture content.

Based on the average moisture content, there was a decrease in moisture content from 3 Bar to 4 and 5 Bar pressure treatments. The decrease in water content was in line with the results of research conducted by Pamungkas *et al.* (2008) which stated that the higher the temperature, time and pressure used during the puffing process, the higher the constant drying rate of the material. The drying rate constant of a material was directly proportional to the diffusion coefficient of the material. This meant that the speed of water or water mass to diffuse out of the material when dried would be higher if the drying rate constant increased. Thus, the moisture content of cocoa nibs roasted at 4 and 5 Bar pressure would be lower than cocoa nibs roasted at 3 Bar pressure.

4.2.2. Ash Content

Ash is an inorganic substance that results from the residual combustion of an organic material and is related to the mineral content of the material (Sudarmadji *et al.,* 1996). Thus, the measurement of ash content means that it shows the amount of mineral contained. The measurement of ash content in this study aimed to determine the effect of different pressures and types of raw materials used on the ash content contained in roasted cocoa nibs using a puffing gun. The ash content of pressure-roasted cocoa nibs was showed in Figure 4.7.



Note:

A1 = puffing pressure 3 Bar B1 = spontaneously fermented cocoa beans
A2 = puffing pressure 4 Bar B2 = non-fermented cocoa beans
A3 = puffing pressure 5 Bar B3 = tape yeast fermented cocoa beans

Figure 4.7. Average value of ash content of pressure-roasted cocoa nibs

The average value of ash content of pressure-roasted cocoa nibs above showed that the highest average ash content was produced by non-fermented cocoa nibs roasted at 4 Bar pressure, while the lowest average ash content was produced by cocoa nibs fermented with tape yeast and roasted at 3 Bar pressure. The average ash content of the pressure-roasted cocoa nibs studied ranged from 3.61% to 4.78%.

The results of the analysis of variance showed that the treatment of raw material type significantly affected the ash content of cocoa nibs while the treatment of puffing pressure variation did not significantly affect the moisture content of cocoa nibs. Therefore, Duncan's further test was conducted for the treatment of raw material type as showed in Table 4.9.

Table 4.9. Duncan's further test results of 5% effect of raw material treatment factor on ash content of cocoa nibs

Raw Material	Average Ash Content
Spontaneous Fermentation Cocoa Beans	$3,663^{a}$
Tape Yeast Fermentation Cocoa Beans	$3,670^{a}$
Non-Fermentation Cocoa Beans	$4,718^{b}$

Note: numbers followed by the same letter in the same column meant that they were not significantly different.

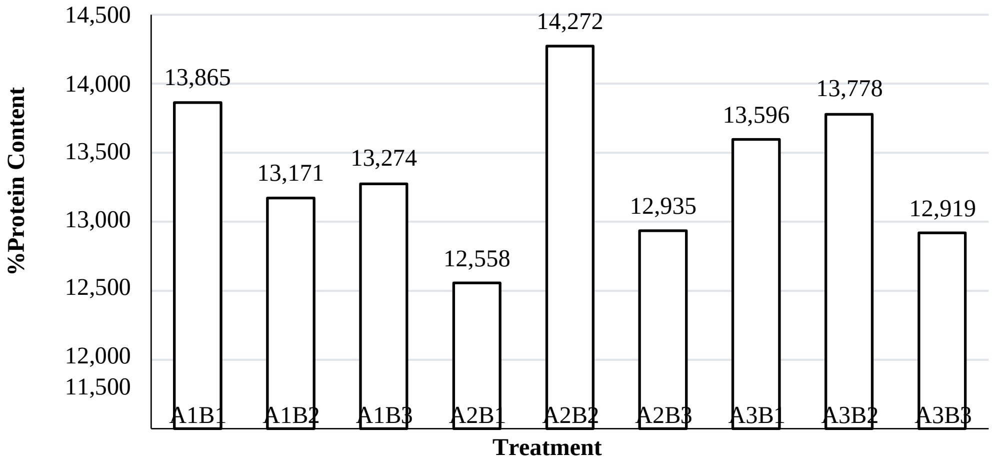
Based on the table of further test results, it could be seen that the type of non-fermented raw material was significantly different from the other two types of raw materials, while the type of spontaneous fermentation raw material was not significantly different from the type of raw material of tape yeast fermentation. This meant that non-fermented cocoa nibs had a significant effect on ash content when compared to spontaneously fermented cocoa nibs and tape yeast fermentation. When viewed from the mean value in the further test table above, the mean ash content of non-fermented cocoa nibs was higher than the ash content of spontaneously fermented cocoa nibs and fermentation using tape yeast.

The lower ash content of fermented cocoa nibs was thought to be due to the fermentation process triggering the release of compounds and water from the cocoa beans, resulting in mineral loss that reduced the ash content of the material (Hayati *et al.,* 2012). In addition, Muchtadi *et al.* (1992) in Hayati *et al.* (2012)

stated that the ash content of a food ingredient could be influenced by factors such as variety, crop condition, and treatment during processing or post-harvest processes such as curing, fermentation, drying, and heating. The fermentation process also produced heat from increased microbial activity. This caused complex organic compounds in cocoa beans to degrade into simpler ones and facilitates the release of water and fat as well as soluble components, including minerals (Situmorang, 2010). Therefore, the ash content of fermented cocoa nibs would be lower than unfermented cocoa nibs.

4.2.3. Protein Content

Protein is one of a group of macronutrients that play a role in biochemical processes and replacing damaged cells in the body of living things. Protein deficiency in living things can inhibit the growth process because 80-90% of all organic matter in animal tissues consists of protein (Sudarmadji *et al.,* 1996). Therefore, protein consumption is very important for humans. The average protein content of pressure-roasted cocoa nibs could be seen in Figure 4.8.



Note:

A1 = puffing pressure 3 Bar
A2 = puffing pressure 4 Bar
A3 = puffing pressure 5 Bar

B1 = spontaneously fermented cocoa beans
B2 = non-fermented cocoa beans
B3 = tape yeast fermented cocoa beans

Figure 4.8. Average value of protein content of pressure-roasted cocoa nibs

The average value of protein content of pressure-roasted cocoa nibs above showed that the highest average protein content was produced by non-fermented cocoa nibs roasted at 4 Bar pressure, while the lowest average protein content was produced by spontaneously fermented cocoa nibs roasted at 4 Bar pressure. The average protein content of the pressure-roasted cocoa nibs studied ranged from 12.56% to 14,27%.

The results of the analysis of variance showed that the raw material type treatment significantly affected the protein content of cocoa nibs while the puffing pressure variation treatment did not significantly affect the protein content of cocoa nibs. In addition, the interaction between puffing pressure treatment and type of raw material also significantly affected the protein content of cocoa nibs. Thus, Duncan's further test was conducted for the treatment of raw material type and interaction between treatments as showed in Table 4.10 and Table 4.11.

Table 4.10. Results of Duncan's further test of 5% effect of raw material treatment factor on cocoa nib protein content.

Raw Material	Average Protein Content
Tape Yeast Fermentation Cocoa Beans	12,919^{a}
Spontaneous Fermentation Cocoa Beans	13,596^{ab}
Non-Fermentation Cocoa Beans	13,778^{b}

Note: numbers followed by the same letter meant not significantly different.

Table 4.11. Duncan's further test results of 5% interaction effect between two factors treatment on cocoa nib protein content

Raw Material	Protein Content on Puffing Pressure		
	3 Bar	4 Bar	5 Bar
Spontaneous Fermentation Cocoa Beans	13,863 ^{aX}	12,557 ^{bY}	14,367 ^{aX}
Non-Fermented Cocoa Beans	13,170 ^{aX}	14,273 ^{aX}	13,890 ^{aX}
Tape Yeast Fermented Cocoa Beans	13,273 ^{aX}	12,933 ^{bX}	12,543 ^{bX}

Note: numbers followed by the same lowercase letter in the column or the same capital letter in the row meant not significantly different.

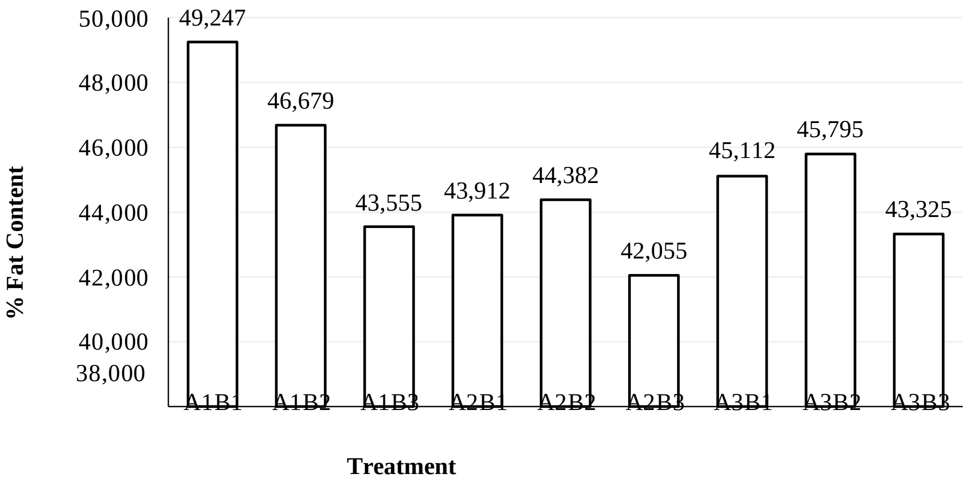
The table showed that the treatment of tape yeast fermented raw materials was significantly different from the treatment of non-fermented raw materials, but the treatment of spontaneous fermented raw materials was not significantly different from the other two treatments. This meant that cocoa nibs fermented with tape yeast provided a significant difference in protein content compared to non-fermented cocoa nibs.

The fermentation process of cocoa beans with the addition of tape yeast had microorganisms that played a role since the beginning of the fermentation process and their activities took place faster than microorganisms in spontaneous fermentation (Purwanto *et al.,* 2019). These microorganisms degraded the sugar in the pulp as their substrate to grow and produce metabolite compounds from these activities. Sugar degradation in fermentation with tape yeast left 1.4 percent sugar on the third day, while spontaneous fermentation still left as much as 1.7 percent sugar. This meant that microorganisms in fermentation with tape yeast produced more metabolites in the first three days of the fermentation process. Metabolite compounds such as organic acids and ethanol diffused and induced enzymatic activity in the cocoa bean cotyledons, one of which was a protease enzyme that could degrade proteins (Vuyst and Weckx, 2015; Haliza *et al.,* 2019). The protein degradation produced peptide bonds and amino acids. Amino acids could be lost after the roasting process due to reactions with reducing sugars to form aroma compounds (Ho *et al.,* 2014). Muthmainna *et al.* (2016) also stated that protein degradation during the fermentation process will form dipeptide bonds and so on can become NH_3 or NH_2 compounds which are lost through evaporation, thus reducing the protein content of the ingredients.

The interaction between the puffing pressure treatment and the type of raw material also significantly affected the protein content of cocoa nibs. Based on the results of further tests, non-fermented cocoa nibs roasted at 4 Bar pressure were significantly different from spontaneously fermented cocoa nibs and fermented tape yeast roasted at 4 Bar pressure and showed a greater mean than the two treatments. This meant that non-fermented cocoa nibs produced higher protein content at the same roasting pressure. Tape yeast fermented cocoa nibs roasted at 5 Bar pressure also significantly differed from spontaneously fermented and non-fermented cocoa nibs roasted at 5 Bar pressure and showed a lower mean protein content compared to both treatments. The interaction between spontaneously fermented cocoa nibs roasted at 4 Bar pressure was significantly different from spontaneously fermented cocoa nibs roasted at 3 and 5 Bar pressure and showed the lowest mean protein content compared to the two treatments. Thus, the treatment interaction that gave the most significant effect on protein content was non-fermented cocoa nibs with roasting pressure of 4 Bar and gave the highest protein content. This might be due to the fact that the protein content in fermented cocoa beans was degraded to form amino acids and reacted during roasting to form aroma and flavor compounds. Non-fermented cocoa nibs did not give the typical aroma and flavor of cocoa beans because there was no protein breakdown during fermentation so that the formation of volatile compounds during roasting was not maximized and the protein was not reduced as much as fermented cocoa nibs.

4.2.4. Fat Content

Fat in the field of nutrition is a nutrient that is needed by the body because it can provide high enough calories, which is 9 kilo calories/ gram. Fat can also provide essential fatty acids namely linoleate and linolenic and can dissolve vitamins A, D, E, and K for the human body (Sudarmadji *et al.,* 1996). The fat component plays an important role in determining the overall physical characteristics of food ingredients, such as aroma, texture, flavor and appearance (Angelia, 2016). The average fat content of cocoa nibs roasted using a puffing gun could be seen in Figure 4.9.



Note:

A1 = puffing pressure 3 Bar B1 = spontaneously fermented cocoa beans

A2 = puffing pressure 4 Bar	B2 = non-fermented cocoa beans
A3 = puffing pressure 5 Bar	B3 = tape yeast fermented cocoa beans

Figure 4.9. Average value of fat content of pressure-roasted cocoa nibs

Based on the diagram, it could be seen that the average fat content of the pressure-roasted cocoa nibs studied ranged from 42.06% to 49.25%. The highest average fat content was produced by spontaneously fermented cocoa nibs roasted at 3 Bar pressure, while the lowest average fat content was produced by cocoa nibs fermented with tape yeast and roasted at 4 Bar pressure.

The result of analysis of variance showed that the treatment of raw material type and puffing pressure variation significantly affected the fat content of cocoa nibs. Therefore, Duncan's further test was conducted for the treatment of raw material type and puffing pressure variation as shown in Table 4.12 and Table 4.13.

Table 4.12. Duncan's further test results 5% effect of pressure variation treatment factor puffing pressure variation on cocoa nib fat content

Puffing Pressure	Average Fat Content
4	$43{,}450^{a}$
5	$44{,}744^{ab}$
3	$46{,}494^{b}$

Note: numbers followed by the same letter meant not significantly different.

Table 4.13. Duncan's further test results 5% effect of raw material treatment factor on cocoa nib fat content

Raw Material	Average Fat Content
Tape Yeast Fermentation Cocoa Beans	$42{,}978^{a}$
Non-Fermentation Cocoa Beans	$45{,}618^{b}$
Spontaneous Fermentation Cocoa Bens	$46{,}090^{b}$

Note: numbers followed by the same letter meant not significantly different.

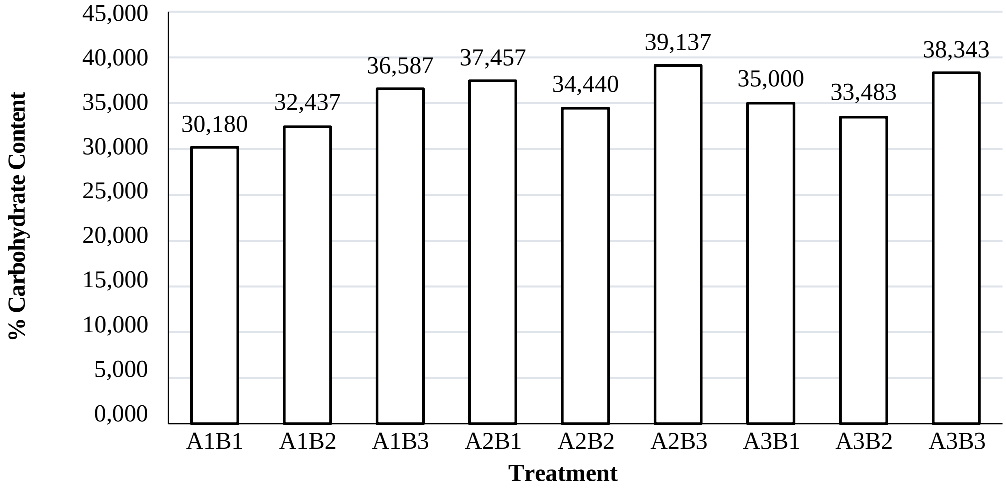
Based on the results of further tests of puffing pressure variations, it could be seen that the 3 Bar puffing pressure was significantly different from the 4 Bar puffing pressure treatment, but the 5 Bar puffing pressure was not significantly different from the other two treatments. As for the type of raw material, the spontaneous fermentation treatment was not significantly different from the non-fermentation treatment, but the tape yeast fermentation treatment was significantly different from the other two treatments.

The fat content of cocoa nibs roasted at 3 Bar pressure showed a higher value compared to cocoa nibs roasted at 4 Bar pressure. This was thought to be due to the temperature increasing along with the increase in pressure during the puffing process. The increase in temperature accelerates the movement of fat molecules so that the distance between molecules became large and facilitated the fat removal process (Winarno, 1997 in Sipayung *et al.,* 2014). In addition, the decrease in fat content during the roasting process also occured due to the conversion of fat into acidic aroma compounds (Hu *et al.,* 2016).

The lowest average value of fat content in the raw material treatment was obtained from fermented cocoa nibs with tape yeast which also showed significant differences from the other two treatments. Basically, the fermentation process would increase the fat content of cocoa beans. Silfia *et al.* (2017) stated that during the fermentation process of cocoa beans, glucose catabolism occurs which produces acetyl Co-A, thus increasing fat content through the process of lipogenesis. However, the addition of tape yeast allows the work of microorganisms that are increasingly active and help the speed of fermentation, so that organic acids are formed that can break down or hydrolyze fat and cause the fat content of cocoa beans to decrease (Sitompul, 2018). The shorter fermentation process also causes lower fat content when compared to spontaneous fermentation (without starter). This is because the shorter the fermentation takes place, the microorganisms that grow under anaerobic conditions and degrade compounds such as polyphenols, proteins, and sugars into fat will not be as much as when the spontaneous fermentation process lasts longer (Silfia *et al.,* 2017).

4.2.5. Carbohydrate Content

Carbohydrate content in this study was calculated using the by difference method, which was a way to determine carbohydrate content by calculation (formula) without the analysis process (Winarno, 1984). The average carbohydrate content of pressure-roasted cocoa nibs could be seen in Figure 4.10.



Note:

A1 = puffing pressure 3 Bar	B1 = spontaneously fermented cocoa beans
A2 = puffing pressure 4 Bar	B2 = non-fermented cocoa beans
A3 = puffing pressure 5 Bar	B3 = tape yeast fermented cocoa beans

Figure 4.10. Average value of carbohydrate content of pressure-roasted cocoa nibs

The results in Figure 4.10 showed that the average value of carbohydrate content of pressure-roasted cocoa nibs ranged from 30.18% to 39.14%. The highest carbohydrate content was produced by fermented cocoa nibs with tape yeast roasted at 4 Bar pressure, while the lowest carbohydrate content was produced by spontaneously fermented cocoa nibs roasted at 3 Bar pressure. The results of the analysis of variance showed that the treatment of raw material type and variation of puffing pressure had a significant effect on the carbohydrate content of cocoa nibs, so Duncan's further test was conducted for both treatments.

Table 4.14. Duncan's further test results 5% effect of puffing pressure variation treatment factor on carbohydrate content of cocoa nibs

Puffing Pressure	Average Carbohydrate Content
3	$33,068^{a}$
5	$35,609^{b}$
4	$37,011^{b}$

Note: numbers followed by the same letter meant not significantly different.

Table 4.15. Duncan's further test results 5% effect of raw material treatment factor on carbohydrate content of cocoa nibs

Raw Material	Average Carbohydrate Content
Non-Fermented Cocoa Beans	33,453[a]
Spontaneous Fermented Cocoa Beans	34,212[a]
Tape Yeast Fermented Cocoa Beans	38,022[b]

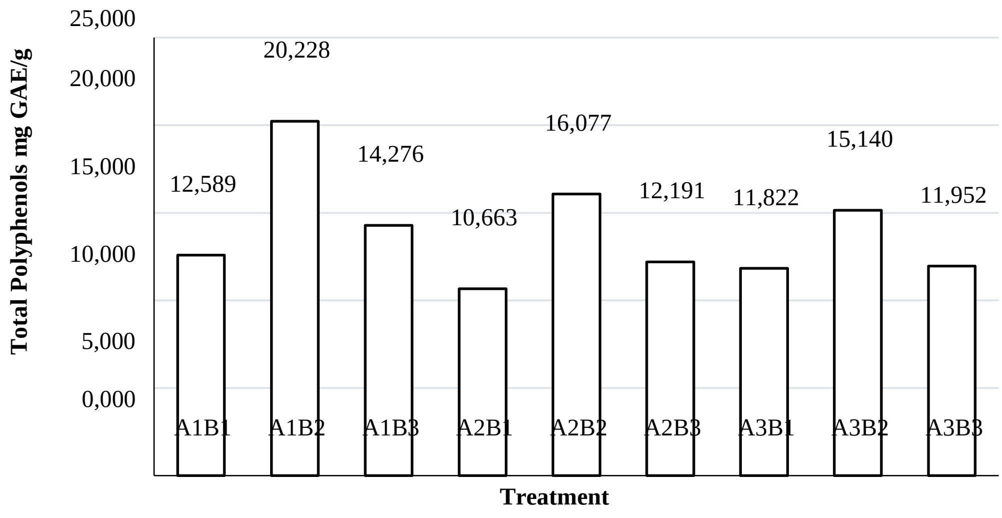
Note: numbers followed by the same letter meant not significantly different.

The results of further tests in Table 4.14. and Table 4.15. showed that 3 Bar puffing pressure was significantly different from the other 2 treatments, but 4 Bar puffing pressure was not significantly different from 5 Bar puffing pressure. Cocoa nibs fermented using tape yeast showed the highest value and significantly different from the other two treatments, and non-fermented raw materials were not significantly different from the spontaneous fermentation treatment. In fermentation with the addition of tape yeast, the added microorganisms will hydrolyze the starch in the pulp and produce higher ethanol, lactic acid and acetic acid. Some of these compounds are diffused into the beans which will then initiate enzymatic reactions in the cocoa beans to be more optimal (Nurhayati *et al.,* 2017). One of the enzymatic reactions occurring in the bean is activity by the enzyme glycosidase, which converts anthocyanins into cyanidins and sugars that can affect the color and flavor of cocoa beans. The breakdown of these compounds also will eventually increase the carbohydrate content of the material (Yuniar *et al,* 2018).

Cocoa nibs roasted at 3 Bar pressure showed the lowest average carbohydrate content compared to cocoa nibs roasted at 4 and 5 Bar pressure. This was related to the decreasing moisture content with increasing puffing pressure. The decreasing water content as the puffing pressure increases will reduce the degree of starch gelatinization in the ingredients. The higher the degree of gelatinization, the easier it is for starch hydrolysis to occur, which will then be dispersed in the water content of the material and will evaporate during the heating process (Huang *et al.,* 2018). Therefore, cocoa nibs at 3 Bar pressure will lose the highest carbohydrate content compared to other treatments. In addition, the low carbohydrate content in the 3 Bar pressure treatment and the high carbohydrate content in the tape yeast fermentation raw material treatment were also influenced by other proximate levels, such as the high fat content in the 3 Bar pressure treatment and the lowest fat content in the tape yeast fermentation treatment. This is because the calculation of carbohydrates was done without analysis but by means of a rough calculation (proximate analysis) or also called carbohydrate by difference (Winarno, 1984).

4.2.6. Total Polyphenols

Polyphenols are compounds that have a number of phenol groups and have more than one ring. In cocoa beans, polyphenols are secondary metabolites that have various health benefits but give cocoa its characteristic astringent taste along with alkaloid, amino, peptide and pyrazine compounds (Misnawi, 2003 in Purbowaseso, 2005). The content of these polyphenolic compounds can change during cocoa bean processing such as fermentation and roasting, so it is necessary to analyze total polyphenols for roasted cocoa nibs showed in Figure 4.11. Based on Figure 4.11, it could be seen that non-fermented cocoa nibs roasted at 3 Bar pressure had the highest total polyphenol content, while the lowest total polyphenols were found in spontaneously fermented cocoa beans roasted at 4 Bar pressure.



Note:

A1 = puffing pressure 3 Bar
A2 = puffing pressure 4 Bar
A3 = puffing pressure 5 Bar

B1 = spontaneously fermented cocoa beans
B2 = non-fermented cocoa beans
B3 = tape yeast fermented cocoa beans

Figure 4.11: Average value of total polyphenols of pressure-roasted cocoa nibs

Analysis of variance showed that both puffing pressure and raw material type significantly affected the total polyphenols of cocoa nibs. Duncan's further test results are showed in Table 4.16. and Table 4.17.

Table 4.16. Duncan's further test results 5% effect of puffing pressure variation treatment factor on total cocoa nib polyphenols

Puffing Pressure	Average Total Polyphenols
5	12,971[a]
4	12,977[a]
3	15,698[b]

Note: numbers followed by the same letter meant not significantly different.

Table 4.17. Duncan further test results 5% effect of raw material treatment factor on total cocoa nib polyphenols

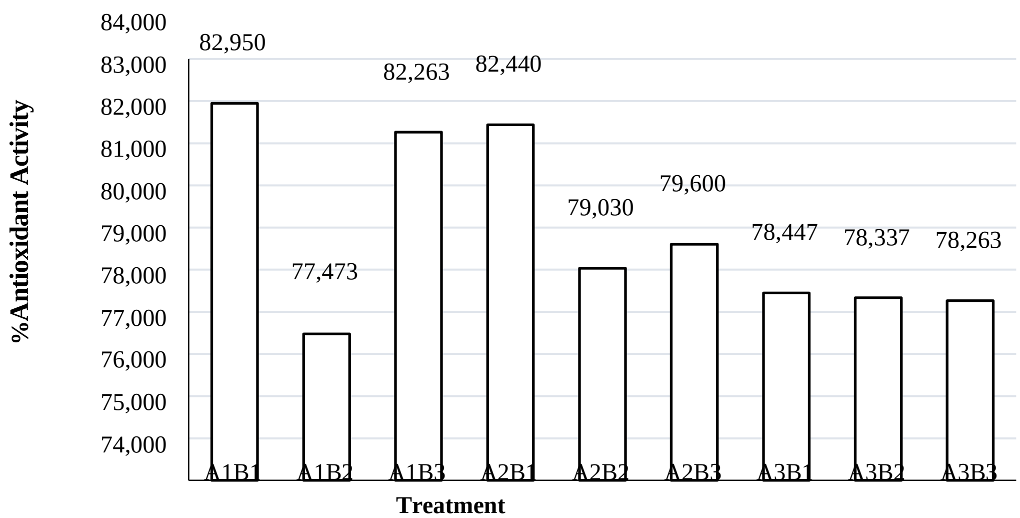
Raw Material	Average Total Polyphenol
Spontaneous Fermentation	11,691[a]
Tape Yeast Fermentation	12,806[a]
Non-Fermentation	17,148[b]

Note: numbers followed by the same letter meant not significantly different.

The results of further tests showed that cocoa nibs roasted at a pressure of puffing pressure of 3 Bar had the highest total polyphenols and was significantly different from other treatments. For the type of raw material, cocoa nibs with the highest total polyphenols and significantly different from the other treatments were non-fermented cocoa nibs. Both statements were in line with the conclusion in the previous study which stated that total polyphenols would decrease as the puffing pressure increases. This can occur due to degradation and oxidation of polyphenol compounds, followed by polymerization and formation of insoluble compounds with high molecular weight during the puffing process (Hu *et al.,* 2015). In addition, the fermentation process also reduces the polyphenol content of cocoa beans. The decrease is due to the diffusion of polyphenols out of the cotyledons during fermentation, polymerization and complexation with proteins during fermentation (Bonvehl and Coll, 1997 in Utami, 2018). Another reason is also because during fermentation acetic acid is formed which also plays a role in activating the enzyme polyphenol oxidase. This enzyme will help oxidize polyphenols in cocoa beans and reduce the content of these compounds. The acetic acid formed will also diffuse into the vacuoles of cocoa bean cells, causing polyphenols to leave the cells and be hydrolyzed (Atmaja *et al.,* 2016).

4.2.7. Antioxidant Activity

Antioxidants are compounds that are quite high in cocoa beans. However, these compounds are quite sensitive to heat and are expected to decrease in content during the processing of cocoa beans into consumable products. The average antioxidant activity of cocoa nibs roasted using a puffing gun could be seen in Figure 4.12. The average value of antioxidant activity showed that spontaneously fermented cocoa nibs roasted at 3 Bar pressure had the highest antioxidant activity, while non-fermented cocoa nibs roasted at 3 Bar pressure had the lowest antioxidant activity. The antioxidant activity of pressure-roasted cocoa nibs ranged from 78.26% to 82.95%.



Note:

A1 = puffing pressure 3 Bar
A2 = puffing pressure 4 Bar
A3 = puffing pressure 5 Bar

B1 = spontaneously fermented cocoa beans
B2 = non-fermented cocoa beans
B3 = tape yeast fermented cocoa beans

Figure 4.12. Average value of antioxidant activity of pressure roasted cocoa nibs

Analysis of variance showed that both puffing pressure and raw material type significantly affected the antioxidant activity of cocoa nibs. Therefore, a 5% Duncan's further test was conducted for both treatments. The results of the further test of the effect of pressure treatment factors and raw materials could be seen in Table 4.18 and Table 4.19.

Table 4.18. Duncan's further test results of 5% effect of puffing pressure variation treatment factor on antioxidant activity of cocoa nibs

Puffing Pressure	Average Antioxidant Activity
5	78,349[a]
4	80,357[b]
3	80,896[b]

Note: numbers followed by the same letter meant not significantly different.

Table 4.19. Duncan's further test results of 5% effect of raw material treatment factor on antioxidant activity of cocoa nibs

Raw Material	Average Antioxidant Activity
Non-Fermentation	78,280[a]
Tape Yeast Fermentation	80,042[ab]
Spontaneous Fermentation	81,279[b]

Note: numbers followed by the same letter meant not significantly different.

Further test results showed that 5 Bar puffing pressure gave the lowest antioxidant activity and was significantly different from the other two treatments. In addition, the average antioxidant activity of cocoa nibs also decreased as the puffing pressure increased. This was related to the total polyphenols which also decreased as the puffing pressure increased because polyphenols were the main components in cocoa beans that played a role in antioxidant activity (Steinberg, 2002 in Utami, 2018). Several studies have also proven that polyphenols contained in cocoa beans had the ability to capture DPPH radicals.

The raw material treatment of spontaneously fermented cocoa nibs was not significantly different from tape yeast fermented cocoa nibs, but significantly different from non-fermented cocoa nibs and showed higher antioxidant activity. This contradicts some research results which showed that processing including fermentation would reduce the antioxidant activity of cocoa beans (De Brito et al., 2000; and Albertini *et al.,* 2015 in Rosniati *et al.,* 2019). Basically, the spontaneous fermentation process will reduce the polyphenol content of cocoa beans which should also reduce the antioxidant activity of cocoa beans. However, Hu *et al.* (2016) reported that antioxidant activity is more influenced by total flavonoids than total polyphenols. This is related to the different structures of cocoa flavonoid compounds that have different effects on antioxidant activity. Flavonoid compounds with certain structures such as the presence of catechol structures and the presence of hydroxyl groups in certain positions will reduce their antioxidant activity (Utami, 2018). Thus, high total polyphenols in non-fermented cocoa nibs may not necessarily give higher antioxidant activity than spontaneously fermented cocoa nibs.

CHAPTER 5
CONCLUSIONS AND SUGGESTIONS

5.1. Conclusions

The conclusions obtained from the results of this study were as follows:

1. Puffing pressure had a significant effect ($p<0.05$) on moisture content, carbohydrate content, fat, total polyphenols, antioxidant activity, taste and aroma, but had no significant effect on color value, ash content and protein.
2. The higher the puffing pressure used, the carbohydrate content will increase, while the water content, fat, total polyphenols, antioxidant activity, flavor and aroma will decrease.
3. The treatment of raw materials had a significant effect ($p<0.05$) on ash content, carbohydrates, fat, color intensity values L* (lightness) and a* (redness), antioxidant activity, taste and aroma.
4. The physicochemical characteristics of cocoa nibs from non-fermented cocoa beans gave the highest L* (lightness) color value, ash content, and total polyphenols, but the lowest values for a* (redness) color, taste and aroma, while the fat and carbohydrate content were not significantly different from cocoa nibs from spontaneously fermented cocoa beans.
5. The physicochemical characteristics of cocoa nibs from spontaneously fermented cocoa beans had the highest antioxidant activity, taste and aroma while the color values of L* (lightness), a* (redness), b* (yellowness), ash content and total polyphenols were not significantly different from those of nibs from tape yeast fermented cocoa beans.
6. The physicochemical characteristics of cocoa nibs from tape yeast fermented cocoa beans had the highest carbohydrate content and the lowest fat content, while the protein content and antioxidant activity were not significantly different from those of nibs from non-fermented cocoa beans.
7. The interaction of raw material treatment and puffing pressure significantly affected the L* (lightness), a* (redness), b* (yellowness), and protein content.

8. The combination of spontaneous fermentation cocoa bean raw material treatment and pressure of 3 Bar (A1B1) produced the highest fat content, antioxidant activity and panelists' favorability scores for the flavor and aroma of cocoa nib puffs.

5.2. Suggestions

It is necessary to analyze polyphenol or flavonoid compounds using HPLC/LC-MS to determine a more detailed profile of polyphenol compound content in cocoa nibs after puffing. In addition, further research is needed on the relationship between total polyphenols, total flavonoids, antioxidant activity and IC50 of spontaneously fermented, non-fermented and fermented cocoa nibs using tape yeast.