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Phytochemical and Bioactive Compounds of Cocoa Beans as Supplement Ingredient Affected by Drying Methods

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***Abstract*— Cocoa beans are a common food ingredient recognized by the public because they can be made into various products and have health benefits such as antioxidant, anti-inflammatory, and anti-diabetic if appropriately processed. This study used the Forastero variety cultivated at Sukabumi region, Indonesia, as a cocoa bean sample. This study aimed to analyze phytochemical properties and the bioactive compounds content of cocoa beans using GC-MS affected by various drying methods. The methods used were sun drying, cabinet drying, and freeze drying. The observed parameters to determine the cacao bean quality were antioxidant capacity, phenol and flavonoid content, and bioactive compound. The result showed that the drying method affected dried cocoa beans' phenol and flavonoid content and antioxidant activity. However, the freeze-drying gave the best phytochemicals properties in phenol content (5,6785 mg GAE/g), flavonoid (64,33 mg QE/g), and antioxidant capacity (42,17% inhibition on DPPH 0,1mM). The result was in line with volatile compounds with proven high biological activity. Cabinet drying produced cocoa beans, which is better than sun drying. The freeze-drying maintains lactones, ketones, alcohols, aldehydes, and phenols. The freeze-dried cocoa beans had the highest content of γ-Tocopherol, 2,4-Di-tert-butylphenol, 5-hydroxymethylfurfural, neophytadiene, and palmitic acid as antioxidants. The freeze-drying method could be considered for producing dried cocoa beans as a supplement ingredient.**

***Keywords*—Antioxidant; cocoa; drying; phenol; GC-MS.**

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I. INTRODUCTION

The public has intended to consume traditional herbs as a healthy food in the long term to increase immune health. However, nowadays, people prefer to intake food supplements as a more practical healthy lifestyle. It is due to the bioactive compound being concentrated in the capsule. Consequently, dietary supplements are more effective in raising the body's immune system to prevent illness than traditional herbs. Polyphenols are known as bioactive compounds which has activity as the immune booster. There are three mechanisms of polyphenol action in autoimmune regulation: activating arachidonic acid-dependent metabolic pathways, then inhibiting cyclooxygenase and lipoxygenase. Moreover, polyphenol regulates the inhibitory proteins of κB (IκBs). In addition, polyphenol activates mitogen-activated protein kinases (MAPKs) pathway [1].

Several studies have been reported on using polyphenol dietary as therapeutic drugs to treat autoimmune disorders such as hepatitis, systemic lupus erythematosus, type-1 diabetes, vitiligo, and sarcoidosis [2]. In Covid-19 patients, polyphenols can boost the immune system through the mechanism of increasing antioxidants and the immune system [3]. Furthermore, polyphenol consumption has benefits as a potential agent in aging inhibition and increasing the telomere length [4]. Plant polyphenol is also proven as an antioxidant against different oxidative stress-induced diseases [5].

Polyphenols are substances commonly found in plants, such as Theobroma cacao L. The investigations on the consumption of cacao with high polyphenols in healthy people are reported can improve insulin secretion, its shown regulation of postprandial sugar levels [6]. In addition, the polyphenols in cocoa can also prevent cardiovascular problems, cerebrovascular accidents, and cancer avoidance [7].

The differences in planting area and genotype could affect the polyphenol content in cocoa beans [8]. Another study has reported that the mineral cadmium influences the total polyphenols content in cocoa beans in the soil [9]. In parallel, the polyphenol content in the cacao beans might be reduced due to improper processing [10]. The handling techniques for the drying process after harvesting the cacao bean is one of our focuses in this study, which aims to keep the polyphenol content in the cacao beans. Accordingly, this study aimed to analyze the phytochemical properties and profile the targeted bioactive compounds in cocoa beans affected by various drying methods by employing Gas Chromatography/Mass Spectroscopy (GC/MS) analysis. In this research, the unfermented cocoa beans were used to maintain the bioactive compounds activity in cocoa beans. The fermentation process could reduce the polyphenols and flavonoids content in cocoa beans [11].

II. Material and Methods

A. Materials

The Forastero variety cocoa beans (cultivated in Sukabumi, Indonesia) were used in this study. The chemicals used were of analytical grades, such as ethanol, methanol, Milli-Q pure water, Follin-Ciocalteu, Na_2Co_3 CH_3COONa, $AlCl_3$ DPPH (2,2-diphenyl-2-picrylhydrazyl), gallic acid, quercetin ascorbic acid and aquades.

B. Methods

1) Sample preparation: Unfermented fresh cocoa beans were used in this study. Three drying methods were employed in this study, namely (i) freeze drying, (ii) cabinet drying, and (iii) sun drying. In freeze drying method, the samples were lyophilized and stored in a glass tube container at -60 ºC for three days. For cabinet drying, the fresh cocoa beans were laid down in a tray and heated at 50 ºC for seven days. Fresh cocoa beans were spread in a tray under the sun for ten days for the sun drying method. The solar radiation heats up the cocoa beans as well as the surrounding air. The end of the drying process was set up when cocoa bean skin could be peeled. The dried cocoa beans were separated from the skin and crushed using a coffee grinder to obtain fine powder. The cocoa powder was sifted by a 60-mesh sieve, placed in plastic vacuum, and then stored in the refrigerator.

2) Cocoa extraction: Extraction was conducted by adding 20 mL of methanol into a tube containing 1 gram of cocoa bean powder. Afterward, the sonication method extracted the sample using a sonicator for 30 seconds extraction time, then centrifuged at 4 ºC, 3000 gravity for 10 minutes. Furthermore, the supernatant was taken and concentrated using a rotary evaporator. To maximize extraction quality, the extraction process was repeated three times. The filtrate was evaporated to dryness with a pressure of 100 atm and a temperature of 40 ºC. The residue was redissolved by adding 10 mL of hypergrade methanol to obtain a concentrated extract.

3) Total phenol determinations: The total polyphenol content was determined by the Follin-Ciocalteu method [12]. Briefly, 125 µL of the concentrate extract cocoa powder was diluted with 25 mL distillate water. Afterwards, 3 mL solution was put into the test tube, and 500 µL of Follin-Ciocalteu reagent and 2 mL of Na_2CO_3 were added. The solution was vortexed for 30 seconds and incubated at room temperature. After 30 minutes, the absorbance was measured using a UV VIS spectrophotometer (Shimadzu 1800, Japan) at 760 nm. Distillate water was used as a blank solution with a similar solvent for the sample solution. Total polyphenols were calculated using a standard gallic acid curve and expressed as gallic acid equivalents (GAE) in milligrams per gram of dry extract. The absorbance (y) was inputted into the gallic acid standard curve equation to obtain the polyphenol value (x value).

4) Total flavonoid determinations: The total flavonoid content from cocoa powder was determined by diluting 1 mL of concentrated extract in 10 mL of distilled water. Then, 500 µL of the solution was added with 1.5 mL of pro-analyzed ethanol, 100 µL of CH_3COONa, 100 µL of 10% $AlCl_3$, and 2.8 mL of distilled water [12]. The sample solution was vortexed for 30 seconds and incubated at room temperature for 30 minutes. The absorbance sample was measured using UV-VIS spectrophotometry (Shimadzu 1800) at 415 nm. The blank solution was distilled water with a similar reagent solution as a sample. Previously calibration curve of quercetin as a standard was prepared, and total flavonoid values were expressed as quercetin equivalent (QE) in milligrams per gram of dry extract. Absorbance (y) was entered into the quercetin standard curve equation to obtain the flavonoid value (x value).

5) Antioxidant activity determinations: Antioxidant activity was determined based on the ability of polyphenolic compounds to scavenge free radicals from DPPH (2,2-diphenyl-1-picrylhidrasil). Briefly, 25 µL concentrated cocoa powder extract was diluted in 5 mL of methanol. Then, 600 µL of the sample was put into a test tube and added with 2400 µL of 0.1 mM DPPH solution. The test tube was vortexed and left for 30 minutes. The absorbance was measured using a spectrophotometer at 516 nm. Antioxidant activity is calculated based on a percentage of inhibition by using formula (1) [12]:

$$Antioxidant\ activity: \frac{\text{Blank absorbance} - \text{Sample absorbance}}{\text{Blank absorbance}} \times 100\% \quad (1)$$

6) Profile of volatile compounds: Profile of volatile components was conducted using the Gas Chromatography/Mass Spectrometer (GC/MS, Shimadzu QP2010 Ultra, Japan) method. Briefly, filtered 350 µL concentrated cocoa extract was placed into a vial and injected into the GC/MS. The Rtx-5 MS capillary column (30 m, 0.25 mm i.d, 0.25 µm film thickness) was used as stationary phase and helium as the mobile phase was set at 6.0 mL/min for flow rate. The program mode was split injection at 1:1. The column temperature was set at 150 ºC for 1 minute as an initial temperature and rose slowly at flow rate 10 ºC until 300 ºC as the final temperature and left for 24 minutes. Identification of the volatile compounds in the sample was by comparing the mass spectra and volatile components obtained from the National Institute of Standards and Technology (NIST) library which has the highest similarity index. Linear Retention Index (LRI) was calculated by following the equation (2) below:

$$LRIx = \left[\frac{(tx-tn)}{(tn+1-tn)} + n\right] x\ 100 \qquad (2)$$

Remarks:

- LRIx: linear retention index value of component x
- tx: retention time of component x (minutes)
- tn: retention time of alkane standard, with n carbon, appeared before the x component (minutes)
- tn+1: retention time of alkane standard, with n carbon atoms coming after the x component (minutes)
- n: the number of standard alkane carbon that appears before component x.

III. Results and Discussion

Drying reduces fungal contamination during storage and extends the shelf life of dried cocoa beans. Various chemical and biochemical changes occur during drying, forming aroma and flavor precursors. Choosing the proper drying method determines the quality of the cocoa beans before further processing. Sun drying is used as a conventional method in cocoa processing. Technological developments allow artificial drying to accelerate the process and protect the active compounds in cocoa beans.

A. Total Phenol Content

Polyphenols are contained naturally in plants. One of the plant sources of polyphenols is cocoa. Polyphenol content in cocoa beans is the fourth largest after water, fat, and protein content [9]. The color, taste, and aroma quality is important in the presence of polyphenols in cocoa [13]. The polyphenols in cocoa are believed to protect from chronic disease and oxidative damage to the body. Variety, planting environment, level of maturity, processing, and storage affect the polyphenol content in cocoa beans. In this study, the polyphenol content was expressed as GAE. Different drying methods give different polyphenol content, as shown in Fig. 1.

The freeze-drying method gives the highest polyphenol value compared to other methods (567.85 mg GAE/g). Using low temperatures in freeze drying will protect heat-sensitive compounds, such as polyphenolic compounds, from damage. Low temperatures will also inactivate the enzymes so that the enzymatic breakdown of polyphenols can be avoided. During the drying of cocoa beans, polyphenol oxidase catalyzes the oxidation reaction of polyphenols into flavor and color-forming compounds [14]. The use of high temperatures and drying duration will decrease the polyphenol content. Heat energy from sunlight and cabinets causes thermal breakdown of the phenol compounds [15]. The longer the drying time, the greater the opportunity for oxidized polyphenols to react with polyphenols, which decreases the polyphenol content. Sun drying took longer than cabinet drying, which caused the greatest reduction in polyphenols. During drying, an enzymatic browning process occurs, water evaporation, and a significant decrease in polyphenol content. The drying method had a significant effect ($P<0.05$) on the polyphenol content. Freeze drying method gives better polyphenol content in dry samples also reported by Menon et al. [5].

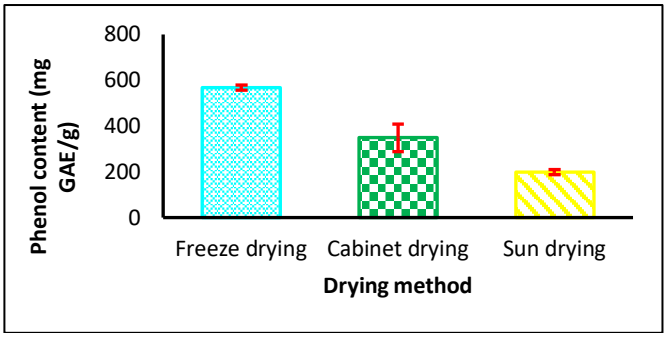

Fig. 1 Polyphenol content of cocoa bean in different drying methods

B. Total Flavonoid Content

Flavonoids are secondary metabolite products from plants with a polyphenolic structure. Factors that affect the chemical properties of flavonoids include the degree of hydroxylation and polymerization, the presence of substitution and conjugation, and class of structure. The biggest content of flavonoids in cocoa is flavanols. Changes in cocoa polyphenols and flavonoids during processing are a current research topic.

The content of flavonoids was determined by the colorimetric method and equated with the content of quercetin. The average content of flavonoids ranged from 24.44- 64.33 mg QE/g (Fig. 2). The effect of the drying method on the flavonoid content has a similar trend to the phenol content. The largest content of flavonoids is found in cocoa beans by freeze-drying. Flavonoids are one of the main components of polyphenols in cocoa beans besides tannins. Many flavonoid compounds are heat-sensitive [16]. Increased heat during drying will cause the degradation of flavonoid compounds. The statistical analysis results showed a significant effect of the drying method on the levels of flavonoids ($P<0.05$). Flavonoid content was positively correlated with phenol content (Pearson value 0.948).

C. Antioxidant Activity

Cocoa is popular as a source of antioxidants. The content of phenol and flavonoid compounds is cocoa's main source of antioxidants. High antioxidant content is useful in preventing metabolic syndrome from degenerative diseases. Previous studies have concluded that cocoa consumption can provide benefits as anti-inflammatory, anti-atherosclerotic, increase insulin sensitivity, and modulate blood pressure and immunity. Antioxidant activity can be done by measuring the mechanism of scavenging free radicals into stable compounds. The DPPH method is widely used because it is easy, inexpensive, fairly accurate, valid, and reproducible.

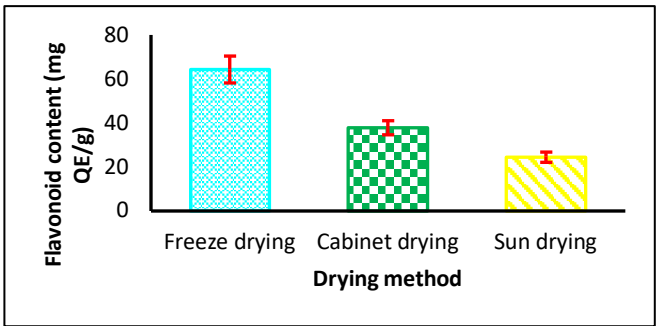

Fig. 2 Flavonoid content of cocoa beans in different drying method

Fig. 3 shows the percentage of antioxidant activity of cocoa beans with different drying methods. The percentage of inhibition indicates antioxidant activity, which can inhibit free radical activity, so the higher the inhibition percentage, the higher the antioxidant activity. The freeze-drying method is proven to scavenge the highest free radicals (42.17%), while sun drying has the lowest inhibition (35.10%). Low temperature and enzyme inactivation are the keys to freeze drying in protecting against damage to antioxidant compounds. The free radical scavenging activity of DPPH decreased with increasing time and temperature. Prolonged heat exposure causes more damage to the antioxidant compounds in sun drying. Drying using high temperatures causes a drastic decrease in bioactive compounds such as flavonoids, which are antioxidant compounds in cocoa beans [17]. The occurrence of oxidation reactions in cabinet drying and sun drying is another reason for the lower antioxidant activity compared to freeze drying. The value of the antioxidant activity of cocoa beans is significantly influenced by the drying method ($P<0.05$). Due to phenols and flavonoids being antioxidant compounds, the antioxidant activity correlates positively with both content (Pearson value 0.831 and 0.796, respectively). Cabinet drying and sun drying were not significantly different in antioxidant activity. It shows that the amount of damage to antioxidant compounds due to drying is the same. Majid and Rining also reported high antioxidant activity in freeze drying compared to sun drying and cabinet drying [18], indicated by the lower IC50 value.

D. Volatile Compounds

Clustering by PCA (Fig. 4.) showed that variations in drying methods of cocoa beans affected the bioactive compounds forming, especially its volatile compound. Volatile compound of each drying method was placed in 3 different quadrants. Freeze drying generated more volatile compounds in cocoa beans than other drying methods, followed by cabinet and sun drying. The PCA figure shows that the volatile compound of cocoa beans dried by freeze drying characterized by 1-(3-Methoxy-2- pyrazinyl)-2-methyl-1-propanone (24),which was responsible for -nutty scent [19]; 5-Hydroxymethylfurfural (33) is formed by thermal processing (roasting) [20] and methyl eicosanoate (1). Cocoa beans dried by sun drying were characterized by linoleic acid (19) and caffeine (68), and theobromine (69) were the signature of cocoa beans dried with cabinet drying.

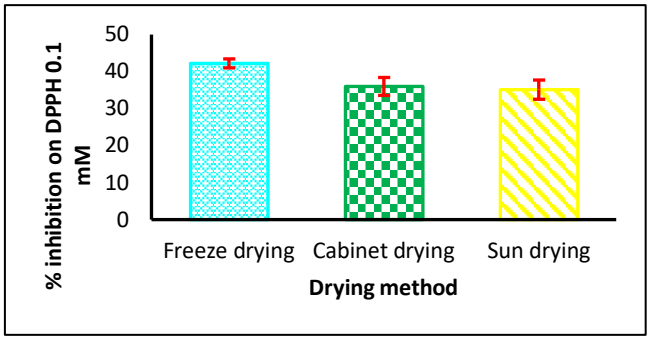

Fig. 3 Antioxidant capacity of cocoa beans in different drying methods

The freeze-drying treatment generated more volatile compounds on cocoa beans compared with other drying, especially lactones and ketones (17.87%), alcohols (4.47%), aldehydes (7.63%), and phenols (2.37%). The high polyphenol content in cocoa beans treated freeze drying (Fig. 1) aligned with the volatile compound phenol identified. The result was similar to Karyadi et al. [21], which revealed that freeze-drying maintains the nutrition content of dried plants/vegetables.

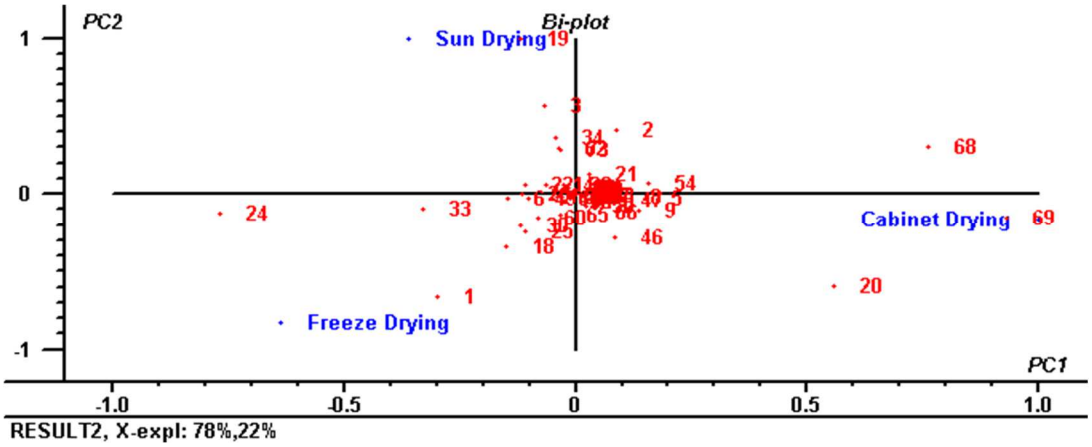

Fig. 4 Biplot graph of cocoa beans in different drying method

TABLE I
VOLATILE COMPOUNDS OF COCOA BEANS IN DIFFERENT OF DRYING METHODS

No	Ret Time	Compound	LRI	Freeze Drying		Cabinet Drying		Sun Drying	
				Area (10^4)	% Area	Area (10^4)	% Area	Area (10^4)	% Area
	Esters								
1	8.68	Methyl eicosinonate	2040	37,383	6.27%	nd	nd	nd	nd
2	8.69	Methyl palmitate	2043	30,854	5.18%	515	7.43%	58,732	8.91%
3	9.027	L-ascorbyl 2,6-dipalmitate	2188	Nd	nd	nd	nd	32,351	4.91%
4	10.295	Ethyl linoleate	2203	5,520	0.93%	nd	nd	3,744	0.57%
5	10.344	Methyl-cis-oleate	2208	Nd	nd	169	2.44%	nd	nd
6	10.38	Methyl-trans-oleate	2208	14,780	2.48%	nd	nd	13,211	2.00%

No	Ret Time	Compound	LRI	Freeze Drying		Cabinet Drying		Sun Drying	
				Area (10^4)	% Area	Area (10^4)	% Area	Area (10^4)	% Area
7	10.356	Methyl petroselinate	2210	Nd	nd	46	0.66%	nd	nd
8	10.399	10-Octadecenoic acid, methyl ester	2216	Nd	nd	117	1.69%	nd	nd
9	10.54	Methyl stearate	2244	8,286	1.39%	240	3.46%	4,622	0.70%
10	11.94	Glycidyl palmitate	3296	1,144	0.19%	nd	nd	253	0.04%
11	13.415	1,2,3-propanetriyl tris[(E)-9-octadecenoate	2805	1,086	0.18%	nd	nd	1,112	0.17%
12	13.419	Ethylene glycol monooleate	2774	258	0.04%	nd	nd	nd	nd
13	13.635	Eicosyl 2-ethylbutanoate	2602	4,194	0.70%	72	1.04%	4,489	0.68%
14	13.81	2-Monopalmitin	2644	5,589	0.94%	nd	nd	9,172	1.39%
15	15.148	Heptadecyl hexanoate	2902	1,881	0.32%	nd	nd	4,357	0.66%
16	15.365	1-Monostearin	2948	2,596	0.44%	nd	nd	4,262	0.65%
	Total Area			**113,571**	**19.06%**	**1,159**	**16.72%**	**136,305**	**20.68%**
	Acids								
17	7.055	Myristic acid	1952	663	0.11%	nd	nd	228	0.03%
18	9.01	Palmitic acid	2174	19,099	3.21%	nd	nd	nd	nd
19	10.652	Linoeic acid	2273	Nd	nd	nd	nd	56,961	8.64%
20	10.675	Oleic Acid	2281	26,365	4.43%	867	12.51%	nd	nd
21	10.85	Stearic acid	2383	10,262	1.72%	169	2.44%	19,140	2.90%
22	20.41	3β-Hydroxy-5-cholen-24-oic acid	6203	9,963	1.67%	nd	nd	13,439	2.04%
	Total Area			**66,352**	**11.14%**	**1,036**	**14.95%**	**89,768**	**13.62%**
	Lactones, Ketones								
23	2.505	2,3-dihydro-3,5-dihydroxy-6-methyl-4H-Pyran-4-one	1607	1,743	0.29%	nd	nd	5,233	0.79%
24	8.465	1-(3-Methoxy-2-pyrazinyl)-2-methyl-1-propanone	2006	77,744	13.05%	nd	nd	70,882	10.76%
25	8.627	Cyclohexanone, 3-(1,2,4-triazol-1-yl)methyl-	2030	13,698	2.30%	nd	nd	nd	nd
26	8.82	7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione	2077	11,582	1.94%	nd	nd	11,475	1.74%
27	10.785	Tetrahydro-6-nonyl-2H-Pyran-2-one 9 (delta lactone)	2337	895	0.15%	nd	nd	1,100	0.17%
28	10.785	Tetrahydro-6-octyl-2H-Pyran-2-one (delta tridecalactone)	2334	802	0.13%	nd	nd	nd	nd
29	10.8	Tetrahydro-6-tridecyl-2H-Pyran-2-one	2344	Nd	nd	nd	nd	1,154	0.18%
	Total Area			**106,464**	**17.87%**	**nd**	**nd**	**89,844**	**13.63%**
	Alcohols								
30	4.16	2-(hydroxymethyl)-2-nitro-1,3-Propanediol (nitroisobutyl glycerol)	1581	25,895	4.35%	134	1.93%	15,395	2.34%
31	16.6	Octacosanol	3143	720	0.12%	nd	nd	nd	nd
32	16.61	1-Hexacosanol	3148	Nd	nd	nd	nd	346	0.05%
	Total Area			**26,615**	**4.47%**	**134**	**1.93%**	**15,741**	**2.39%**
	Aldehydes								
33	2.888	5-Hydroxymethylfurfural	1431	45,479	7.63%	134	1.93%	41,534	6.30%
34	3.089	2,3,4,5-Tetrahydroxypentanal	3219	Nd	nd	nd	nd	20,592	**3.12%**
	Total Area			**45,479**	**7.63%**	**134**	**1.93%**	**62,126**	**9.43%**
	Alkenas								
35	7.86	Neophytadiene	1975	224	0.04%	nd	nd	nd	nd
36	9.54	p-Mentha-1,5,8-triene	2125	593	0.10%	nd	nd	639	0.10%
37	12.775	alpha-springene	3562	254	0.04%	nd	nd	nd	nd
	Total Area			**1,071**	**0.18%**	**nd**	**nd**	**639**	**0.10%**
	Alkanes								
38	11.155	Eicosane	12888	378	0.06%	nd	nd	919	0.14%
39	11.16	Heneicosane	8033	3,273	0.55%	nd	nd	493	0.07%
40	12.831	1-iodo-Triacontane	11411	196	0.03%	nd	nd	nd	nd
41	12.836	Pentacosane	22800	Nd	nd	nd	nd	838	0.13%
42	13.891	Pentatriacontane	2681	Nd	nd	nd	nd	1,725	0.26%
43	14.19	Tetracosane	4840	Nd	nd	nd	nd	409	0.06%
44	14.075	Tetrapentacontane	2918	Nd	nd	nd	nd	1,384	0.21%
45	14.385	Dotriacontane	8600	845	0.14%	nd	nd	1,392	0.21%
	Total Area			**4,692**	**0.79%**	**nd**	**nd**	**7,160**	**1.09%**
	Sterols and its derivatived								
46	16.745	Cholest-5-en-3-ol (3β)-, carbonochloridate	3807	41,337	6.94%	537	7.75%	30,161	4.58%
47	17.54	22,23-Dibromostigmasterol acetate	4020	4,696	0.79%	145	2.09%	3,753	0.57%
48	18.215	Cholesta-2,4-diene	4250	3,305	0.55%	nd	nd	2,285	0.35%
49	18.27	Stigmasta-5,22-dien-3-ol, acetate, (3β)-	4221	10,461	1.76%	nd	nd	8,748	1.33%

No	Ret Time	Compound	LRI	Freeze Drying		Cabinet Drying		Sun Drying	
				Area (10^4)	% Area	Area (10^4)	% Area	Area (10^4)	% Area
50	19.13	Cholesterol	5016	80,364	13.49%	948	13.68%	84,707	12.85%
51	19.31	9,19-Cyclochloestene-3,7-diol, 4,14-dimethyl-, 3-acetate	4936	1,352	0.23%	nd	nd	nd	nd
52	19.585	Desmosterol	5119	1,181	0.20%	nd	nd	850	0.13%
53	19.745	Lathosterol	5144	514	0.09%	nd	nd	633	0.10%
54	20.825	Stigmasterol	5404	36,686	6.16%	618	8.92%	45,993	6.98%
55	21.91	Cholest-5-en-3-ol, 24-propylidene-, (3β)-	5600	1,859	0.31%	nd	nd	2,873	0.44%
56	23.985	Cholest-4-en-3-one	6120	1,456	0.24%	nd	nd	3,269	0.50%
	Total Area			**183,211**	**30.75%**	**2,248**	**32.43%**	**183,272**	**27.81%**
	Phenols and methylated phenols								
57	4.81	2,4-Di-tert-butylphenol	1628	4,115	0.69%	nd	nd	2,942	0.45%
58	15.856	5-Octadecenal	3003	Nd	nd	nd	nd	389	0.06%
59	17.178	δ-Tocopherol	3950	Nd	nd	nd	nd	696	0.11%
60	18.572	γ-Tocopherol	4454	10,034	1.68%	nd	nd	1,257	0.19%
61	18.975	dl-α-Tocopherol	4750	Nd	nd	nd	nd	1,268	0.19%
	Total Area			**14,149**	**2.37%**	**nd**	**nd**	**6,552**	**0.99%**
	Sugars								
62	4.157	Sucrose	1580	Nd	nd	nd	nd	16,540	2.51%
63	7.951	Trehalose	2013	263	0.04%	nd	nd	819	0.12%
	Total Area			**263**	**0.04%**	**nd**	**nd**	**17,359**	**2.63%**
	Amide								
64	15.848	cis-11-Eicosenamide	3001	Nd	nd	nd	nd	1,090	0.17%
	Total Area			**Nd**	**nd**	nd	nd	**1,090**	**0.17%**
	Others								
65	4.74	1,6-Anhydro-β-D-Glucopyranose (Levoglucosan)	1618	15,730	2.64%	127	1.83%	9,099	1.38%
66	5.55	1,6-Anhydro-.β-D-Glucofuranose	1702	9,168	1.54%	125	1.80%	3,627	0.55%
67	6.595	Trimethylsilyl 3-methyl-4-[(trimethylsilyl)oxy] benzoate	1815	5,123	0.86%	nd	nd	3,731	0.57%
68	8.252	Caffeine	2411	Nd	nd	920	13.27%	25,033	3.80%
69	8.476	Theobromine	2008	Nd	nd	1,049	15.13%	nd	nd
70	13.422	1,54-dibromo-tetrapentacontane	2780	Nd	nd	nd	nd	635	0.10%
71	13.638	d-Ribose, 2-deoxy-bis(thioheptyl)-dithioacetal	2601	2,852	0.48%	nd	nd	nd	nd
72	16.152	Squalene	3091	Nd	nd	nd	nd	148	0.02%
73	18.136	(R)-2,7,8-Trimethyl-2-((3E,7E)-4,8,12-trimethyltrideca-3,7,11-trien-1-yl) chroman-6-ol (γ-Tocotrienol)	5606	Nd	nd	nd	nd	16,287	2.47%
74	22.915	9,19-Cyclolanostan-3-ol, 24-methylene-, (3β)-	5740	1,066	0.18%	nd	nd	3,315	0.50%
	Total Area			**33,939**	**5.70%**	**2,221**	**32.04%**	**49,149**	**7.46%**
	Overall Total Area			**595,806**	**100.00%**	**6,932**	**100.00%**	**659,005**	**100.00%**

Remarks: Ret Time = Retention Time; LRI = Linear Retention Index; nd = not detected

Freeze drying also maintains the content of antioxidant compounds in cacao beans. In this research, volatile compounds indicated as an antioxidant on cocoa beans treated freeze drying were γ-Tocopherol [22], 2,4-Di-tert-butylphenol [23], 5-Hydroxymethylfurfural [24], and palmitic acid. Palmitic acid exhibits anti-inflammatory and metabolic regulatory effects and antitumor activities in several tumors [25]. Freeze drying also maintains other bioactive compounds which was biological activity, such as Neophytadiene and ethyl linoleate as anti-inflammatory [26], [27], Myristic acid as an anti-diabetic [28] and anti-inflammatory [29], Octacosanol as anti-fatigue, anti-hypoxia, antioxidant, anti-inflammatory, antitumor [30], Stigmasta-5,22-dien-3-ol, acetate, (3β)- as inflammatory, antipyretic, anti-ulcer, antiarthritic [31].

IV. Conclusion

Variation drying methods on cocoa beans affected the total phenol, flavonoid, and antioxidant content. Cocoa beans treated freeze drying showed the highest phenol, flavonoid antioxidant content, and volatile bioactive compound identified.

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References

[1] A. Winiarska-Mieczan and E. Tomaszewska, "Antioxidant, anti-inflammantory, and immunomodulatory properties of tea - The positive impact of tea consumption on patients with autoimmune diabetes," *Nutrients*, vol. 13, pp. 1–25, 2021.

[2] H. Khan *et al.*, "Polyphenols in the treatment of autoimmune diseases," *Autoimmun. Rev.*, vol. 18, no. 7, pp. 647–657, 2019.

[3] M. A. El-Missiry, A. Fekri, L. A. Kesar, and A. I. Othman, "Polyphenols are potential nutritional adjuvants for targeting COVID-19," *Phyther. Res.*, vol. 35, no. 6, pp. 2879–2889, 2021.

[4] M. Maleki *et al.*, "Stabilization of telomere by the antioxidant property of polyphenols: Anti-aging potential," *Life Sci.*, vol. 259, no. August, p. 118341, 2020.

[5] D. Stagos, "Antioxidant activity of polyphenolic plant extracts," *Antioxidants*, vol. 9, no. 1, 2020.
[6] Y. Kawakami *et al.*, "Effect of cacao polyphenol-rich chocolate on postprandial glycemia, insulin, and incretin secretion in healthy participants," *Nutrition*, vol. 85, p. 111128, 2021.
[7] E. Jean-Marie, D. Bereau, and J.-C. Robinson, "Benefits of polyphenols and methylxanthines from cocoa beans on dietary metabolic disorders," *Foods*, vol. 10, no. 2049, pp. 1–20, 2021.
[8] J. G. B. Borja Fajardo, H. B. H. Tellez, G. C. P. Atuesta, A. P. S. Aldana, and J. J. M. Arteaga, "Antioxidant activity, total polyphenol content and methylxantine ratio in four materials of Theobroma cacao L. from Tolima, Colombia," *Heliyon*, vol. 8, no. 5, 2022.
[9] J. G. B. Fajardo, H. B. H. Tellez, G. C. P. Atuesta, A. P. S. Aldana, and J. J. mendez Arteaga, "Antioxidant activity, total polyphenol content and methylxantine ratio in four materials of Theobroma cacao L . from Tolima, Colombia," *Heliyon*, vol. 8, pp. 1–6, 2022.
[10] E. Lembong, M. Djali, Zaida, and G. L. Utama, "The potential of dry fermented cocoa (Theobroma cacao L.) variety Lindak bean shell treated at different degrees of roasting as a functional food," *IOP Conf. Ser. Earth Environ. Sci.*, vol. 733, no. 1, 2021.
[11] Fahrurrozi *et al.*, "Effect of Small-Scale Box-Fermentation on Catechin and Epicatechin Content of Lampung Cocoa Beans Varieties," *Int. J. Adv. Sci. Eng. Inf. Technol.*, vol. 11, no. 3, pp. 1029–1034, 2021.
[12] S. Baliyan *et al.*, "Determination of Antioxidants by DPPH Radical Scavenging Activity and Quantitative Phytochemical Analysis of Ficus religiosa," *Molecules*, vol. 27, no. 4, 2022.
[13] E. Septianti, Salengke, and J. Langkong, "Profile of bioactive compounds, antioxidant and aromatic component from several clones of cocoa beans during fermentation," *IOP Conf. Ser. Earth Environ. Sci.*, vol. 575, no. 1, 2020.
[14] L. Goya, J. E. Kongor, and S. de Pascual-Teresa, "From cocoa to chocolate: effect of processing on flavanols and methylxanthines and their mechanisms of action," *Int. J. Mol. Sci.*, vol. 23, no. 22, 2022.
[15] N. Putriani, J. Perdana, Meiliana, and P. Y. Nugrahedi, "Effect of thermal processing on key phytochemical compounds in green feafy vegetables: A review," *Food Rev. Int.*, vol. 38, no. 4, pp. 783–811, 2022.
[16] Y. Gao *et al.*, "Impact of thermal processing on dietary flavonoids," *Curr. Opin. Food Sci.*, vol. 48, no. Figure 2, p. 100915, 2022.
[17] A. S. Menon, C. L. Hii, C. L. Law, S. Shariff, and M. Djaeni, "Effects of drying on the production of polyphenol-rich cocoa beans," *Dry. Technol.*, vol. 35, no. 15, pp. 1799–1806, 2017.
[18] H. Majid and H. A. Rining, "The Effect of Drying Techniques on the Antioxidant Capacity, Flavonoids and Phenolic Content of Fermented Local Cocoa," *J. Adv. Res. Appl. Mech.*, vol. 1, no. 1, pp. 11–19, 2018.
[19] G. Strocchi *et al.*, "Potential Aroma Chemical Fingerprint of Oxidised Coffee Note by HS-SPME-GC-MS and Machine Learning," *Foods*, vol. 11, no. 24, pp. 1–14, 2022.
[20] A. Choudhary, V. Kumar, S. Kumar, I. Majid, P. Aggarwal, and S. Suri, "5-Hydroxymethylfurfural (HMF) formation, occurrence and potential health concerns: recent developments," *Toxin Rev.*, vol. 40, no. 4, pp. 545–561, 2021.
[21] J. N. W. Karyadi, I. Kamila, A. D. Saputro, and D. Ayuni, "The Effect of Blanching on the Quality of Freeze-Dried Edamame," *Int. J. Adv. Sci. Eng. Inf. Technol.*, vol. 12, no. 2, pp. 819–825, 2022.
[22] L. Huang *et al.*, "Simultaneous determination of α-tocopherol, β-tocopherol, γ-tocopherol, δ-tocopherol, sesamin, sesamolin, sesamol, and asarinin in sesame oil by normal-phase high performance liquid chromatography," *J. Food Compos. Anal.*, vol. 104, no. May, p. 104132, 2021.
[23] F. Zhao, P. Wang, R. D. Lucardi, Z. Su, and S. Li, "Natural sources and bioactivities of 2,4-di-tert-butylphenol and its analogs," *Toxins (Basel).*, vol. 12, no. 1, pp. 1–26, 2020.
[24] A. O. Ojatula, "Screening and Evaluation of Bioactive Components of Methanol Root Extract of Anthocleista nobilis G . Don . by GCMS Analysis," *Res. Sq.*, pp. 1–25, 2020.
[25] S. Zhu *et al.*, "Palmitic acid inhibits prostate cancer cell proliferation and metastasis by suppressing the PI3K/Akt pathway," *Life Sci.*, vol. 286, no. 2021, pp. 1–9, 2021.
[26] K. B. Dar *et al.*, "Immunomodulatory efficacy of Cousinia thomsonii C.B. Clarke in ameliorating inflammatory cascade expressions," *J. Ethnopharmacol.*, vol. 300, no. 2023, pp. 1–12, 2023.
[27] M. Bhardwaj, V. K. Sali, S. Mani, and H. R. Vasanthi, "Neophytadiene from Turbinaria ornata suppresses LPS-induced inflammatory response in RAW 264.7 macrophages and Sprague Dawley rats," *Inflammation*, vol. 43, no. 3, pp. 937–950, 2020.
[28] N. Shad, A. Javaid, and Q. Kanwal, "Antifungal and other bioactive constituents in roots of a halophytic weed Suaeda fruticosa," *Pak.J.Weed Sci. Res*, vol. 28, no. 3, pp. 313–320, 2022.
[29] A. S. M. Khalil, N. Giribabu, S. Yelumalai, H. Shahzad, E. K. Kilari, and N. Salleh, "Myristic acid defends against testicular oxidative stress, inflammation, apoptosis: Restoration of spermatogenesis, steroidogenesis in diabetic rats," *Life Sci.*, vol. 278, no. 2021, pp. 1–17, 2021.
[30] Y. Zhou, F. Cao, F. Luo, and Q. Lin, "Octacosanol and health benefits: Biological functions and mechanisms of action," *Food Biosci.*, vol. 47, no. 2022, pp. 1–16, 2022.
[31] S. Arora and G. Kumar, "Gas chromatography-mass spectrometry (GC-MS) determination of bioactive constituents from the methanolic and ethyl acetate extract of Cenchrus setigerus Vahl (Poaceae)," *Pharma Innov.*, vol. 6, no. 11–I, pp. 635/1-635/6, 2017.