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Theobromine content in chocolate products: a review

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ABSTRACT

Chocolate is one of the foods that exist in Indonesia and one of the foods that are loved by people of all ages because of its sweet taste and it easily melts in the mouth. *Theobromine* is the main ingredient in chocolate products. This compound belongs to the canteen group which has a stimulant effect, relaxes the bronchial muscles, prevents coughs, reduces asthma symptoms, and others. But consuming more than *Theobromine* has the side effect of burning the heart. To ensure that the main content is the main content in chocolate products, it is necessary to analyze the content of *theobromine* as an identity compound in chocolate. The purpose of this review article is to determine the presence of *theobromine* content in chocolate products, the amount of *theobromine* content contained in it and to find out the methods that can be used to determine *theobromine* content in chocolate. Literature searches were conducted on google scholar, PubMed, science direct, ProQuest, and springer using the keywords "analysis" OR "measurement" AND "*theobromine*" AND "chocolate" OR "cocoa". The inclusion used were articles from the period of 2010-2020, full-text articles using Indonesian and English, and the samples tested were chocolate products. Based on the search results for articles with keywords and screening according to the inclusion criteria, 6 articles were obtained. *Theobromine* contained in chocolate products has varying levels. The spectrophotometric methods UV-VIS, HPLC, and UHPLC-HRMS can be used in determining the level of *theobromine*.
Keywords: Literature Review, *Theobromine*, Chocolate Product.

INTRODUCTION

Chocolate was a familiar food in this world, even chocolate was a food favored by all ages, from children to the elderly. Technological developments make chocolate not only enjoyed in the form of chocolate fruit but now chocolate can be processed into various forms of food, including chocolate bars, chocolate candy, chocolate biscuits, ice cream, drinks, and chocolate powder. Many people like various foods made from chocolate, but only a few people know the content of the chocolate they consume. Chocolate contains two main components, namely: *theobromine* and *caffeine*, but chocolate contains more *theobromine* than *caffeine* (Darmawan, 2012).

It is important to know the *theobromine* content in chocolate products because it has benefits and side effects for the body when consumed. The benefits of *theobromine* when consumed have the effect of relaxing the bronchial muscles which can affect the psyche depending on the size of the dose (Franco *et al*., 2013; Kasabe & Badhe, 2010; Martínez-Pinilla *et al*., 2015; Mitchell *et al*., 2011). While the side effects of consuming *theobromine* in excess can cause heartburn, *theobromine* is also a weak diuretic agent and can give the effect of feelings of dislike and dysphoria, especially when taking 1000 mg capsules orally. (Baggott *et al*., 2013; Latif, 2013). Research results from several articles regarding *theobromine* content in chocolate products will be discussed in this review article.

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MATERIALS AND METHOD

Article Search Strategy

Article reviews are based on previous studies, which are Indonesian journals, international journals, and articles related to *theobromine* content in chocolate products. Literature searches were obtained using the *Google Scholar* search engine and online journal provider sites, such as *Science Direct, PubMed, ProQuest,* and *Springer*. The keyword sentences used in the literature search were "*analysis*" OR "*measurement*" AND "*theobromine*" AND "*chocolate*" OR "*cocoa*".

Inclusion and Exclusion Criteria

The inclusion criteria in the article screening process, namely: (1) the year of publication of the article with a period of 10 years back, namely 2010-2020, (2) national and international articles that are available in full text, (3) the sample tested was chocolate products. The exclusion criteria used are (1) articles with a publication year not within the past 10 years, namely 2010-2020, (2) national and international articles that are not available in full text, (3) articles that don't use chocolate products as samples, (4) articles that don't discuss *theobromine* assay in chocolate products.

Article Selection Method

The chosen articles in this traditional review were conducted by choosing articles that matched the title, abstract, background, results, and discussion of the *theobromine* content in chocolate products.

Article information

The information that will be explored from the article can be seen in table I.

Table I. Information searched from articles

Article	Title
	Author (Year of publication)
	Article Origin
	Sample type
	Sample type
	% Chocolate Solids
Treatment	The analytical method used
Results	*Theobromine* levels

RESULT AND DISCUSSION

Article Search

The results of the search for articles that have been identified from the database as a whole are 3,035 articles. Based on each journal obtained by Google Scholar (2,380 articles), PubMed (61 articles), Science Direct (26 articles), ProQuest (152 articles), and Springer (156 articles). Titles and abstracts of the identified articles, 31 relevant articles were obtained based on the title and abstract, then 25 articles were excluded. 6 articles meet the criteria. The article search scheme can be seen in Figure 1.

The excluded articles were 25 articles obtained from Google Scholar, Science Direct, PubMed, ProQuest, and Springer. These articles were excluded because they did not discuss the determination of *theobromine* levels and did not use the chocolate product as samples.

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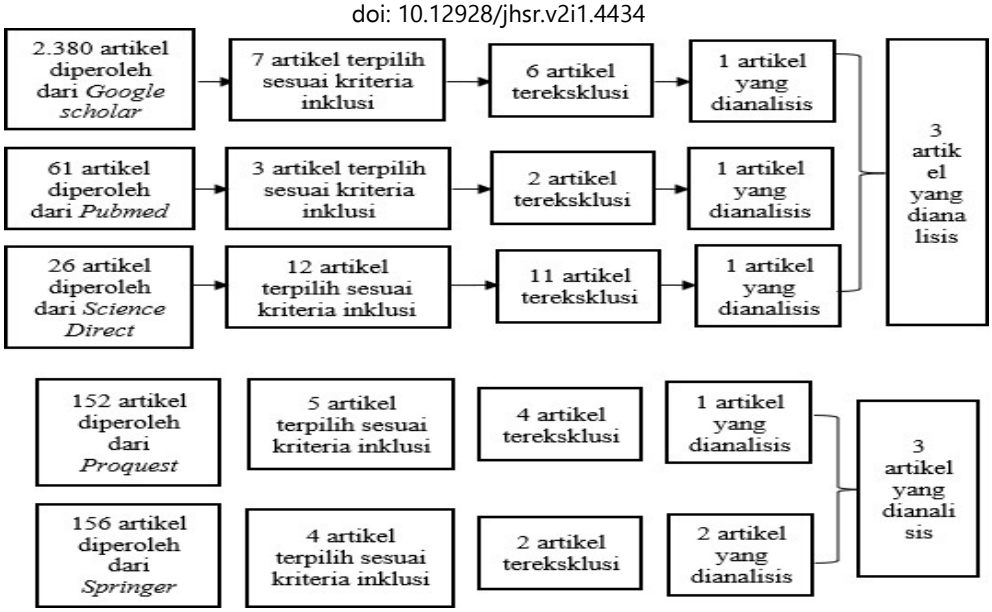

Figure 1. Article Search Scheme

Article Characteristic

The characteristics of the article include the author, year of publication, the title of the article, the origin of the article, and the sample used. The articles used in the traditional review are articles that meet the inclusion and exclusion criteria and obtained 6 articles that match. The characteristics of the 6 selected articles are described in Table II.

Table II. Article Characteristic

No.	Title, Author (Years)	Article source	Sample	Sample type	% Chocolate solids
1.	*Polyphenols, methylxanthines, fatty acids, and minerals in cocoa beans and cocoa products* Grassia *et al*., (2019) [Article 1]	ProQuest	Cocoa Beans (B)	B1, B2, B3	-
			Chocolate Products (P)	P1 = Modicana chocolare bar	70 %
				P2 = Traditional chocolate bar	75% 70%
				P3 = Traditional chocolate	85%
				P4 = Chocolate bar	100%
				P5 = Chocolate paste	
2.	UHPLC-HRMS *Analysis of Theobromine in Theobroma cacao and its Products* *Mladenovic et al.,* (2018) [Article 2]	Google Scholar	Stage of manufacture	1. Roasted Cocoa	-
				2. Dried Cocoa	-
				3. Raw Cocoa	-
				4. Chocolate bar	100%
			Branded chocolate	1. Sample A	11%
				2. Sample B	30%
				3. Sample C	31%
				4. Sample D	34%
				5. Sample E	45%
				6. Sample F	72%

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				7. Sample G	85%
				8. Sample H	90%
				9. Sample I	70%
				10. Sample J	70%
3.	*Assesment of flavanol stereoisomers and caffeine and theobromine content in commercial chocolate* Alañón et al., (2016) [Article 3]	Science Direct	Milk Chocolate (MC)	-	20 – 34%
			Dark chocolate (DC)	-	36 – 90%
4.	*Ultrasound-Assisted Method for Extraction of Theobromine and Caffeine from Cacao Seeds and Chocolate Products* Jiménez & Macías. (2013) [Article 4]	Springer	-	Biji cokelat,	-
			–	Bubuk cokelat	-
				Hershey	
			Chocolate bar	Lindt Excellence	70%
			Chocolate bar	Lindt Excellence	85%
			Chocolate bar	Lindt Excellence	90%
			Chocolate bar	Hershey	42%
			Chocolate bar	Turin Exoticas	-
			Milk chocolate	World table	-
			Free sugar chocolate bar	Chocozero	-
			Chocolate bar	Nestle-Abuelita	-
			Chocolate drink	Hershey	-
			Chocolate syrup	Great Value	-
			Milk Chocolate	Nestle-Carlos V.	-
			-	Cocoa skin	-
5.	*Colorimetry and photoacoustic spectroscopy as a suitable tool for determination of fat-free cocoa solids in a dark chocolates* Dóka et al.,(2013) [Artikel 5]	Springer	Branded chocolate	C1, C2, C3, C4, C5, C6, C7, and C8	-
6.	*Flavanols and methylxanthines in commercially available dark chocolate: a study of the correlation with nonfat cocoa solids* Langer et al., (2011) [Artikel 6]	PubMed	Black chocolate (branded)	-	-
			Milk chocolate	-	-
			White chocolate	-	-

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Analysis Method

Based on the data obtained from the articles that have been listed in Table II in the form of the characteristics of the sample whose *theobromine* content will be examined using the analytical method. The analytical methods used in each article are different and the details of the analytical methods used can be seen in Table III.

Table III. Analysis Methods of Theobromine Level Determination

Article	Methods	Stationary phase	Mobile phase	Flow rate	Wavelength	Time retention (TR)	Detector	Injection Volume	Absorbance
Article 1	HPLC	C18	(Solvent A) Water; Phosporic acid 85% 99,7 ; 0,3 v/v (Solvent B) Water; Acetonitrile; Phosporic acid 85% 57,7; 40,0; 0,3 v/v	2 mL/ *minutes*	280 nm	6,9 *minutes*	PDA	-	-
Article 2	UHPLC – HRMS	C18	Water; Acetonitrile (contains 0,1 % Phosporic acid)	300 µL/ *minutes*	-	1,8 *minutes*	LTQ Orbitra p	-	-
Article 3	HPLC	C18	(Solvent A) Water (Solvent B) 200 mM Sodium acetate; Methanol 84;16	10 mL/ *minutes*	274 nm	-	PDA	100 µL	-
Article 4	UV-Vis Spectrofotometry	-	-	-	273 nm	-	-	-	0,06
Article 5	HPLC	RP_{18}	Acetonitrile; Sodium dihydrogen *buffer* orthophosphate 8; 92 v/v	0,6 mL/*minutes*	274 nm	-	PDA	5 µL	-
Article 6	HPLC	Devosil Diol	(Solvent A) Acetonitrile; Phosphoric acid 98; 3 v/v Solvent B) Methanol; Water; Phosphoric acid 95; 3; 2 v/v	0,6 mL/ *minutes*	280 nm	-	PDA	5 µL	-

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Based on Table III, several methods can be used to determine the levels of *Theobromine* in chocolate products. Among them are High-Performance Liquid Chromatography (HPLC), Ultra High-Performance Liquid Chromatography High-Resolution Mass Spectrometry (UHPLC-HRMS), and UV-Vis spectrophotometry. Of the several methods, the most widely used of the 6 articles in Table III is the analysis method using HPLC, the HPLC method is widely used because it has good separation power and is sensitive.

***Theobromine* Contain Results**

Identification of theobromine with different methods in each article resulted in different levels of theobromine. In addition to the different methods, the samples used are also different it is the cause of the differences in the levels obtained by each article. Analysis of theobromine levels can be seen in Table IV.

Table IV. Rate of *Theobromine*

No.	Article	Sample	Sample type	% Chocolate solids	Rate±SD
1.	Article 1	Cocoa Beans (B)	B1,B2, B3	-	10,4 mg/g
		Chocolate Products (P)	P1 = Modicana Chocolate bar	70 %	-
			P2 = Traditional Chocolate bar	75%	-
			P3 = Traditional Chocolate	70%	-
			P4 = Chocolate Bar	85%	-
			P5 = Chocolate Paste	100%	14,9 mg/g
2.	Article 2	Stage of manufacture	1. Roasted Cocoa	-	(371,37 ± 25,66) ng/g
			2. Dried Cocoa	-	(401,96 ± 27,46) ng/g
			3. Raw Cocoa	-	(420,74 ± 22,15) ng/g
			4. Chocolate bar	100%	(739,93 ± 12,80) ng/g
		Branded chocolate	1. Sample A	11%	(33,70 ± 7,02) ng/g
			2. Sample B	30%	(37,21 ± 3,51) ng/g
			3. Sample C	31%	(42,56 ± 3,71) ng/g
			4. Sample D	34%	(61,29 ± 3,59) ng/g
			5. Sample E	45%	(89,47 ± 11,12) ng/g
			6. Sample F	72%	(173,19 ± 12,87) ng/g
			7. Sample G	85%	(182,52 ± 8,21) ng/g
			8. Sample H	90%	(200,99 ± 8,43) ng/g
			9. Sample I	70%	(230,17 ± 7,19) ng/g
			10. Sample J	70%	(243,21 ± 13,55) ng/g
3.	Article 3	Milk Chocolate (MC)	1. MC01	25%	(0,05 ± 0,00) %
			2. MC02	20%	(0,12 ± 0,01) %
			3. MC04	20%	(0,12 ± 0,02) %
			4. MC05	27%	(0,16 ± 0,01) %
			5. MC06	30%	(0,14 ± 0,02) %
			6. MC07	25%	(0,10 ± 0,00) %
			7. MC17	32%	(0,12 ± 0,02) %
			8. MC21	30%	(0,10 ± 0,00) %
			9. MC23	-	(0,05 ± 0,00) %
			10. MC24	30%	(0,10 ± 0,00) %
			11. MC25	31%	(0,06 ± 0,01) %
			12. MC26	27%	(0,12 ± 0,01) %
			13. MC27	-	(0,07 ± 0,01) %
			14. MC28	28%	(0,15 ± 0,03) %

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		15. MC31	32%	(0,22 ± 0,04) %
		16. MC32	34%	(0,45 ± 0,03) %
		17. MC33	30%	(0,21 ± 0,00) %
	Dark chocolate (DC)	1. DC03	74%	(0,76 ± 0,10) %
		2. DC08	50%	(0,34 ± 0,04) %
		3. DC09	55%	(0,85 ± 0,20) %
		4. DC10	72%	(0,81 ± 0,14) %
		5. DC11	70%	(0,81 ± 0,00) %
		6. DC12	48%	(0,22 ± 0,01) %
		7. DC13	90%	(0,88 ± 0,17) %
		8. DC14	70%	(0,83 ± 0,10) %
		9. DC15	47%	(0,30 ± 0,02) %
		10. DC16	49%	(0,43 ± 0,03) %
		11. DC18	70%	(1,02 ± 0,07) %
		12. DC19	85%	(1,37 ± 0,15) %
		13. DC20	72%	(1,27 ± 0,09) %
		14. DC22	47%	(0,41 ± 0,03) %
		15. DC29	39%	(0,25 ± 0,01) %
		16. DC30	70%	(0,61 ± 0,00) %
		17. DC34	60%	(0,84 ± 0,04) %
		18. DC35	40%	(0,79 ± 0,09) %
		19. DC36	72%	(0,90 ± 0,03) %
		20. DC37	74%	(0,87 ± 0,14) %
		21. DC38	70%	(0,58 ± 0,07) %
		22. DC39	85%	(0,70 ± 0,05) %
		23. DC40	36%	(0,49 ± 0,03) %
		24. DC41	50%	(0,46 ± 0,04) %
4. Article 4	-	Biji cokelat,	-	(25,91 ± 0,22) mg/g
	-	Bubuk cokelat Hershey	-	(18,63 ± 0,12) mg/g
	Chocolate bar	Lindt Excellence	70%	(16,75 ± 0,24) mg/g
	Chocolate bar	Lindt Excellence	85%	(20,01 ± 0,21) mg/g
	Chocolate bar	Lindt Excellence	90%	(23,12 ± 0,18) mg/g
	Chocolate bar	Hershey	42%	(10,75 ± 0,14) mg/g
	Chocolate bar	Turin Exoticas	-	(9,38 ± 0,21) mg/g
	Milk chocolate	World table	-	(3,80 ± 0,24) mg/g
			-	(9,80 ± 0,16) mg/g
	Free sugar chocolate bar	Chocozero	-	(16,22 ± 0,12) mg/g
	Chocolate bar	Nestle-Abuelita Hershey	-	(0,34 ± 0,15) mg/g
	Chocolate drink	Great Value	-	(0,24 ± 0,14) mg/g
	Chocolate syrup			
	Milk Chocolate	Nestle-Carlos V.	-	(2,36 ± 0,15) mg/g
	-	Cocoa skin	-	(15,10 ± 0,13) mg/g
5. Article 5	Branded chocolate	1. C1	-	(6,55 ± 0,010) mg/g
		2. C2	-	(5,05 ± 0,016) mg/g
		3. C3	-	(7,87 ± 0,032) mg/g
		4. C4	-	(6,14 ± 0,027) mg/g
		5. C5	-	(6,87 ± 0,036) mg/g
		6. C6	-	(7,94 ± 0,020) mg/g
		7. C7	-	(9,74 ± 0,008) mg/g
		8. C8	-	(11,39 ± 0,040) mg/g

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6. Article 6	Black chocolate (branded), Milk chocolate, White chocolate	1. Sample 1	0%	(0,00 ± 0,00) %
		2. Sample 2	20%	(0,15 ± 0,02) %
		3. Sample 3	39%	(0,53 ± 0,03) %
		4. Sample 4	51%	(0,85 ± 0,13) %
		5. Sample 5	63%	(1,10 ± 0,05) %
		6. Sample 6	70%	(1,12 ± 0,09) %
		7. Sample 7	70%	(1,04 ± 0,09) %
		8. Sample 8	70%	(0,90 ± 0,02) %
		9. Sample 9	70%	(0,91 ± 0,08) %
		10. Sample 10	72%	(0,97 ± 0,09) %
		11. Sample 11	72%	(0,91 ± 0,10) %
		12. Sample 12	72%	(0,97 ± 0,15) %
		13. Sample 13	100%	(1,16 ± 0,05) %
		14. Sample 14	100%	(1,64 ± 0,05) %

Based on the analysis of *theobromine* content in the article, with different samples, it can be concluded that the *theobromine* content is influenced by the percentage of chocolate solids in the composition and manufacturing process. So far, there are no levels that are allowed by the FDA regarding the *theobromine* content in chocolate products. However, according to Baggott et al. (2013) When the *theobromine* dose is increased, it will produce negative effects, such as feelings of discomfort and dysphoria, especially when taking 1000 mg *theobromine* capsules orally.

CONCLUSION

There are varying levels of *theobromine* in chocolate products based on the articles reviewed. UV-Vis, HPLC, and UHPLC-HRMS spectrophotometry can be used in the assay of *theobromine.*

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