Theobromine content in chocolate products: a review

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Submitted: 15-12-2020 Reviewed:. 14-01-2021 Accepted: 12-02-2021

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.
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.

| Article | Title |
|---------|-------|
|         | Author (Year of publication) |
|         | Article Origin |
|         | Sample type |
|         | Sample type |
| Treatment | % Chocolate Solids |
| Results   | The analytical method used |
|           | 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.
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 | ProQuest | Cocoa Beans (B) B1, B2, B3 | Chocolate Products (P) | Modicana chocolate bar 70% |
|     | Grassia et al., (2019) [Article 1] | | | | Traditional chocolate bar 75% |
|     | | | | | Traditional chocolate bar 70% |
|     | | | | | Traditional chocolate bar 85% |
|     | | | | | Traditional chocolate bar 100% |
|     | | | | | Traditional chocolate bar 72% |
| 2.  | UHPLC-HRMS Analysis of Theobromine in Theobroma cacao and its Products | Google Scholar | Stage of manufacture | Roasted Cocoa 100% | Sample A 11% |
|     | Mladenovic et al., (2018) [Article 2] | | | Dried Cocoa 30% | Sample B 11% |
|     | | | | Raw Cocoa 31% | Sample C 11% |
|     | | | | Chocolate bar 34% | Sample D 11% |
|     | | | | Branded chocolate 45% | Sample E 11% |
|     | | | | | Traditional chocolate bar 45% |
|     | | | | | Traditional chocolate bar 45% |
|     | | | | | Traditional chocolate bar 45% |
|     | | | | | Traditional chocolate bar 45% |
|   | 1. JOURNAL OF HALAL SCIENCE AND RESEARCH |
|---|---|
|   | ISSN: 2715-6214 (Print) |
|   | Journal homepage: http://journal2.uad.ac.id/index.php/jhsr/index |
|   | doi: 10.12928/jhsr.v2i1.4434 |

|   |   |   |   |   |   |   |   |   |
|---|---|---|---|---|---|---|---|---|
| 7. | Sample G | 85% |
| 8. | Sample H | 90% |
| 9. | Sample I | 70% |
| 10. | Sample J | 70% |

3. **Assessment 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 | - |
| Chocolate bar | 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 | - |
1. **JOURNAL OF HALAL SCIENCE AND RESEARCH**

ISSN: 2715-6214 (Print)
Journal homepage: http://journal2.uad.ac.id/index.php/jhsr/index
doi: 10.12928/jhsr.v2i1.4434

**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 |
|---------|------------------|------------------|--------------|-----------|------------|--------------------|----------|------------------|------------|
| 1       | HPLC C18         | (Solvent A) Water; Phosphoric acid 85% 99.7; 0.3 v/v (Solvent B) Water; Acetonitrile; Phosphoric acid 85% 57.7; 40.0; 0.3 v/v | 2 mL/ minutes | 280 nm    | 6.9 minutes | PDA                | -        | -                | -          |
| 2       | UHPLC – HRMS C18 | Water; Acetonitrile (contains 0.1 % Phosphoric acid) | 300 μL/ minutes | -         | 1.8 minutes | LTQ Orbitrap       | -        | -                | -          |
| 3       | HPLC C18         | (Solvent A) Water (Solvent B) 200 mM Sodium acetate; Methanol 84;16 | 10 mL/ minutes | 274 nm    | -          | PDA 100 μL         | -        | -                | 0.06       |
| 4       | UV-Vis Spectrofotometry | - | - | - | 273 nm | - | - | - | 0.06 |
| 5       | HPLC RP18        | Acetonitrile; Sodium dihydrogen orthophosphate buffer 8: 92 v/v | 0.6 mL/minutes | 274 nm    | -          | PDA 5 μL           | -        | -                | -          |
| 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           | -        | -                | -          |
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 Content 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 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) % |
| Dark chocolate (DC) | 1. DC03 | 32% | (0.22 ± 0.04) % |
|--------------------|---------|-----|------------------|
|                    | 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

| Milk chocolate | Biji cokelat, | 25.91 ± 0.22 mg/g |
|----------------|---------------|-------------------|
|                | Bubuk cokelat | 18.63 ± 0.12 mg/g |
| Chocolate bar  | Lindt Excellence | 16.75 ± 0.24 mg/g |
| Chocolate bar  | Lindt Excellence | 20.01 ± 0.21 mg/g |
| Chocolate bar  | Lindt Excellence | 23.12 ± 0.18 mg/g |
| Chocolate bar  | Hershey        | 10.75 ± 0.14 mg/g |
| Chocolate bar  | Turin Exoticas | 9.38 ± 0.21 mg/g  |
| Free sugar     | Chocozero      | 6.55 ± 0.010 mg/g  |
| chocolate bar  | Nestle-Abuelita Hershey | 3.04 ± 0.006 mg/g |
| Chocolate drink| Great Value    | 15.10 ± 0.013 mg/g |
| Chocolate syrup|               |                   |
| Milk Chocolate | Nestle-Carlos V. | 8.14 ± 0.017 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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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.

**ACKNOWLEDGEMENT**

Prof. Dr. is apt. Any Guntarti, M.Si. who has provided advice, support, and guidance during the preparation of this research.

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