The effect of methods and drying temperature on glycoside content (Stevioside and Rebaudioside A) in Stevia (Stevia rebaudiana): A systematic review

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Abstract. The utilization of Stevia rebaudiana as an alternative sweetener in Indonesia is still low, this is caused by a lack of information about its processing from raw material until the extraction process to obtain the glycosides content. Glycosides are the sweetener compound in Stevia rebaudiana with a sweet taste up to 300 times of sucrose, which are stevioside and rebaudioside A. To obtain the glycosides content in stevia, stevia needs to be dried after harvest. Then, dried Stevia will be extracted to obtain the glycosides content. The research method used in this study was a systematic review of selected journals on a specific topic, then were analysed in both the theory and content. The purpose of this research is to analyse the effect of drying temperature on different drying methods on the glycoside compound of Stevia rebaudiana, observing drying temperature that is useful to obtain the optimal glycoside compound in Stevia rebaudiana, and to recommend the utilization of Stevia rebaudiana as an alternative sweetener in Indonesia. The result is the high temperature and low temperature can decrease the glycoside compound in Stevia rebaudiana. The dried temperature that is effective for drying stevia leaves to obtain the maximal glycosides is in the range of 40-60°C. The drying method that is effectively used is by an oven at 60°C as the suggested temperature. Stevia’s sweetener is potentially applied and developed in Indonesia and can be served in powder form because it is more efficient and decrease defect in dried condition and a much longer lifetime.

1. Introduction
Stevia rebaudiana is a shrub that belongs to the sunflower family (Asteraceae). Paraguay and Brazil use stevia as a natural sweetener. The use of stevia as a sweetener continuously develops in other countries, such as Japan, Korea, Malaysia, Singapore, and China [27]. According to Mondaca et. al. 2012 [16], stevia is used as a sweetener since 1909. Stevia rebaudiana is one of the sweeteners that are low-calorie, and it may be used in long-term consumption for diabetics [14].

In Indonesia, the cultivation of Stevia rebaudiana has been done since 1984 at Indonesian Plantation Biotechnology Research Institute. Stevia rebaudiana as a sweetener in Indonesia is still underused, this is because lack of information about its cultivation until extraction to get the glycoside compound. According to Djajadi, 2014 [9], one hectare of land can provide 1000-2000 Kgs dried leaves of stevia, that is means 60-70 Kgs stevioside can be generated.
Glycoside is a compound in stevia that produces a sweet taste. There is 8 di-terpene of glycoside in stevia that is 300 times sweeter than sucrose, one of the sweetest di-terpene is stevioside and rebaudioside A. Thus, glycoside is potential to use as a natural sweetener [6]. Glycoside compound is mostly found in the stevia leaves. According to Singh et. al. 2014 [25], a glycoside is the main component of stevia that is not fermented, therefore the use of stevia is possible in the diet as a food additive.

To produce the glycoside compound, stevia leaves need to be dried before extraction. According to Amrani et. al. 2018 [2], drying is the primary treatment to decrease water content in the plant and to guard against damage of microorganism activity. During the drying process, the water content in the plant decreased, so that can inhibit the growth of microorganism before it is saved. According to Zlabur et. al. 2013 [27], the drying temperature that is used for stevia leaves can impact the production of glycoside content. The purpose of the paper is to analyse the effect of drying temperature on glycoside content (stevioside and rebaudioside A) using different drying methods because drying process. Other than that, this review shows which methods and drying temperature produce the higher glycoside contents on the stevia.

2. Material and methods

2.1 Material
The material of this research is literature from international resources, such as journals and books. The journals are founded in Google Scholar and Sci-Hub and the use of books is founded in Google Books. The journals and books are issued for at least 10 years and they have to be accountable.

2.2 Methods
The review method of this paper is a systematic review. A systematic review is one of the review methods that is used in some specific journal according to the topic. Then, the journal compared and compiled to achieve the goal [5]. The flow diagram for systematic review can be seen in Figure 1. The first step is to determine the Population, Intervention, Comparison, Outcomes, and Context (PICOC), then search the keyword to find the journals or books that are relevant (Table 1). The journals and books that were found then were selected and analysed.

| PICOC     | Information                                                                 |
|-----------|------------------------------------------------------------------------------|
| Population| Stevia rebaudiana                                                            |
| Intervention| Glycoside compound (stevioside dan rebaudioside A)                          |
| Comparison| Effect of drying temperature, drying method for leaves                       |
| Outcomes  | The effect of methods and dried temperature on glycoside content (stevioside and rebaudioside A) in Stevia rebaudiana |
| Context   | Stevia rebaudiana leaves, dried temperature between 20°C – 100°C, test of glycoside content using High Performance Liquid Chromatography (HPLC) method |
2.3 Structure analysis

Based on Figure 2, structure analysis is divided into theory analysis and content analysis. Theory analysis explains the perspective theory of the topic, while content analysis explains the content about drying process, dryer, drying temperature in qualitative or quantitative data.

2.3.1 Theory and content analysis. This paper uses the drying leaves theory for theory analysis. Natural drying leaves occur due to the fall of leaves and by the time the leaves experiencing drought because of the environment. According to Babu et al. 2018 [4], the drying temperature that is used for herbal plants is 40-60°C. At 50°C of drying temperature, the plant lost least amount of their component.

2.3.2 Content analysis. The quantitative approach is done by meta-analysis, whereas making statistical identification to link cause and effect. The qualitative approach is done by meta-synthesis, whereas summarizing the result from other research to obtain the relevant topic between each literature [26].
3. Results and discussion

3.1 Drying stevia rebaudiana

Drying is a simple pre-treatment process to decrease water content in the plant [17]. In general, leaves lose 85% water content after drying [4]. The amount of water content in the stevia leaves impacts the result of the glycoside content (stevioside and rebaudioside A) content [11].

The part of Stevia rebaudiana to be dried is the leaves because the sweetener glycoside compound was highly found in the stevia leaves [20]. The drying process in stevia was carried out on stevia leaves that are in harvest, that is before the flowering. This is because photosynthesis in stevia reduces the photosynthetic that is used for the growing of stevia and it was the glucose content to increase the glycoside compound. Natural drying of stevia can be done using sunlight (sun drying or shade drying) and modern drying methods can be done using an oven or microwave. The drying temperature that is good for the leaves drying is between 20-100°C. According to Zlabur et. al. 2013 [27], drying temperature that is used for stevia leaves can impact the production of glycoside content.

3.2 Identification of stevia rebaudiana drying temperature

![Data collection flow diagram](image)

The steps to identify the drying temperature on stevia can be seen in Figure 3. The first step is identifying the literature using some keywords based on PICOC. This paper using Science Direct, IEEE Xplore and Emerald publisher. 12 literatures were already screened in the publisher database using some keywords. There are two papers in the journal from International Agrophysics and Food Chemistry. Other literature is the International Journal of Agriculture Science, Plant Archives, Plant Foods Human Nutrition, Food Science and Technology, Journal of Food and Dairy Science, Journal of Food and Nutrition Research, Horticultural Science, and Scientia Horticulturae.
3.3 Drying methods and temperature frequency analysis

Table 2. The use of drying temperature on *stevia rebaudiana* based on literature.

| Author               | Drying temperature of stevia leaves (°C) |
|----------------------|------------------------------------------|
|                      | 20 | 30 | 40 | 45 | 50 | 55 | 60 | 65 | 70 | 80 | 90 | 100 |
| Karimi *et al.*, 2015 [12] |    |    |    |    |    |    |    |    |    |    |    |    |
| Khalil *et al.*, 2015 [13]   |    |    |    |    |    |    |    |    |    |    |    |    |
| Ordonez *et al.*, 2015 [20] |    |    |    |    |    |    |    |    |    |    |    |    |
| Mondaca *et al.*, 2015 [16]  |    |    |    |    |    |    |    |    |    |    |    |    |
| Pariche *et al.*, 2015 [21]  |    |    |    |    |    |    |    |    |    |    |    |    |
| Chranioti *et al.*, 2016 [8] |    |    |    |    |    |    |    |    |    |    |    |    |
| Mondaca *et al.*, 2016 [17]  |    |    |    |    |    |    |    |    |    |    |    |    |
| Neena *et al.*, 2016 [19]    |    |    |    |    |    |    |    |    |    |    |    |    |
| Parris *et al.*, 2016 [22]   |    |    |    |    |    |    |    |    |    |    |    |    |
| Gonzalez *et al.*, 2017 [11] |    |    |    |    |    |    |    |    |    |    |    |    |
| Amrani *et al.*, 2018 [2]    |    |    |    |    |    |    |    |    |    |    |    |    |
| Huang *et al.*, 2021 [12]    |    |    |    |    |    |    |    |    |    |    |    |    |
| **Total Frequency**         | 1  | 1  | 3  | 2  | 4  | 1  | 8  | 2  | 2  | 1  | 0  | 1  |

Based on the literature resources, 4 different drying methods and 11 different drying temperatures on stevia leaves with amount different glycoside compounds were obtained. The drying temperature and drying methods can be seen in Table 2 and Table 3. To summarize all the literature resources, analysis was carried out to investigate the effect of methods and drying temperature on the amount of glycoside compound in *Stevia rebaudiana*.

3.4 Theory analysis

According to Amrani *et al.*, 2018 [2], drying is the primary treatment to decrease water content in the plant and to guard against damage of microorganism activity. During the drying process, the water content in the plant decreased, so that can inhibit the growth of microorganism before it is saved. The water content in the plant will decrease up to 85% of the weight after the drying process [4]. According
to Babu et al. 2018 [4], the drying process can be applied by a natural or modern process with technology. The research conducted by Rocha et al. 2011 [24], give the information about the drying temperature that is used in the different herbal plant. The drying temperature in the plant is used between 30-80°C [17]. The pre-treatment process that is used in *Stevia rebaudiana* is the drying process to decrease the water content because the water content can affect the glycoside compound during the extraction.

According to Babu et al. 2018 [4], the drying was done using some technology. The natural drying process generally occurs when the leaves of some plants get fall out. The natural drying might be happening with more time process. Natural drying methods consist of open sun drying, shade drying, and wind drying. The drying process using some technology consists of oven drying, microwave drying, vacuum drying, and many more.

### 3.5 Content analysis

Content analysis is done using meta-analysis and meta-synthesis approaches. The meta-analysis approach is done with quantitative data, where is making a statistic identification to connect the cause-effect and to combine the data in the literature resources. The meta-synthesis approach is done with qualitative data, where it summarizes the result of the literature research to obtain the relation based on the literature resources [26].

#### 3.5.1 Meta-analysis as a quantitative method

Table 4 shows the use of the drying method and drying temperature in each literature. There is some similarities content that uses in each literature as method and drying temperature. Figure 4 and Figure 5, show the total frequency of the use of the drying method and drying temperature. The drying method used the most is oven drying at 60°C drying temperature.

Based on the total frequency in Figure 4. the most common drying temperature that is used for the drying process of *Stevia rebaudiana* is 60°C. By the data in Table 4. the drying method that is believed to be effectively used for *Stevia rebaudiana* is by an oven. According to Babu et al. 2018 [4], the dried temperature that is used for the drying process is 60°C. This is because the 60°C temperature affects the minimum of nutrition and compound loses. The drying process with an oven is the simple drying method because there is no need for other specific tools, other than that, the drying method using an oven can decrease the drying time compared to the sun drying method. The drying method using an oven can minimalize the damage caused by the instability of temperature and also maintain the contamination from the environment [4].

![Figure 4](image-url)  
(a) Total frequency of drying temperature; (b) Total frequency of drying methods.
Table 4. Drying methods and drying temperature analysis in *stevia rebaudiana*.

| Dried method       | Temperature (°C) | Literature                      | Similarity                                                                 |
|--------------------|------------------|---------------------------------|-----------------------------------------------------------------------------|
| Sun drying         | 20               | Pariche *et al.*, 2015 [21]     | Drying methods                                                              |
|                    | 50               | Mondaca *et al.*, 2016 [17]     |                                                                              |
| Convective drying  | 30               | Mondaca *et al.*, 2015 [16]     |                                                                              |
|                    | 40               | Mondaca *et al.*, 2015 [16]     |                                                                              |
|                    | 50               | Mondaca *et al.*, 2016 [17]     | Drying temperature, HPLC (High Performance Liquid Chromatography) method for glycoside test. |
|                    | 60               | Mondaca *et al.*, 2015 [16]     | Drying temperature, HPLC (High Performance Liquid Chromatography) method for glycoside test. |
|                    |                  | Mondaca *et al.*, 2016 [17]     |                                                                              |
|                    |                  | Ordonez *et al.*, 2015 [20]     |                                                                              |
|                    |                  | Gonzalez *et al.*, 2017 [11]    |                                                                              |
|                    | 70               | Mondaca *et al.*, 2015 [16]     | Drying methods                                                              |
|                    | 80               | Mondaca *et al.*, 2015 [16]     | Drying methods                                                              |
| Oven               | 40               | Khalil *et al.*, 2015 [13]      | Drying temperature, HPLC (High Performance Liquid Chromatography) method for glycoside test. |
|                    | 45               | Chranioti *et al.*, 2016 [8]    | Drying methods                                                              |
|                    | 50               | Amrani *et al.*, 2018 [2]       | Drying methods                                                              |
|                    | 60               | Khalil *et al.*, 2015 [13]      | Drying temperature, HPLC (High Performance Liquid Chromatography) method for glycoside test. |
|                    |                  | Neena *et al.*, 2016 [19]       |                                                                              |
|                    |                  | Amrani *et al.*, 2018 [2]       |                                                                              |
| Infrared drying    | 65               | Karimi *et al.*, 2015 [12]      | Drying methods                                                              |
| Hot air drying     | 45               | Karimi *et al.*, 2015 [12]      | Drying methods                                                              |
|                    | 50               | Karimi *et al.*, 2015 [12]      |                                                                              |
|                    | 55               | Karimi *et al.*, 2015 [12]      |                                                                              |
|                    | 60               | Karimi *et al.*, 2015 [12]      |                                                                              |
|                    | 65               | Karimi *et al.*, 2015 [12]      |                                                                              |
| Vacuum oven        | 70               | Pariche *et al.*, 2015 [21]     | N/A                                                                         |
|                    |                  | Parris *et al.*, 2016 [22]      | N/A                                                                         |

Figure 5. shows the graph of stevioside and rebaudioside A content in each drying method at different drying temperatures. The method using sun drying shows the lower stevioside and rebaudioside A content in 20°C and 50°C, while according to Khalil *et al.*, 2015 [14], drying stevia leaves using an oven at 60°C can produce 14.05% of glycoside content.

From Figure 6. the drying temperature does affect the stevioside and rebudioside A contents. This graph shows that the different drying methods also produce different contents of stevioside and rebaudioside A although they were conducted at the same temperature which is 50°C. The result is that the convective drying led to the highest content of stevioside and rebaudioside A than the other drying methods. Table 5. shows the advantages and disadvantages of each drying methods. Convective drying is more efficient and stable, however, it needs higher cost and specific technologies, thus for the less complicated methods, using oven drying is recommended. The suggested temperature by an oven drying is 60°C. The drying method by an oven can produce dried stevia with 8.46% water content in 4 hours drying process.
Figure 5. Graph of the effect of drying temperature on stevioside and rebaudioside A content using (a) Sun drying, (b) Convective drying, (c) Oven drying and (d) Infrared drying (●: Stevioside, ■: Rebaudioside A, ▲: Glycoside).

Figure 6. The yield of stevioside and rebaudioside A at 50 °C using different drying methods.
Table 5. The advantage and disadvantage of drying methods.

| Drying methods   | Advantage                                      | Disadvantage                                      | References                  |
|------------------|------------------------------------------------|---------------------------------------------------|-----------------------------|
| Sun drying       | Cheaper cost, no need for specific equipment   | Takes longer times, easily get contaminated, unstable drying temperature | Babu et. al. 2018 [4]       |
| Convective drying| Faster time process, decrease contamination, stable drying temperature | Higher cost, need specific equipment               | Babu et. al. 2018 [4]       |
| Oven drying      | Faster time process, decrease contamination, stable drying temperature, no need for specific equipment, the average cost | A common method that needs to be developed         | Mondaca et al, 2016 [18]    |
| Infrared drying  | Modern technology                              | Higher cost, prone to damage the glycoside content | Huang et. al. (2021)        |

4. Conclusions
Stevia rebaudiana is a plant that can be used as an alternative sweetener. The sweetener compound is highly founded in the stevia leaves. The main glycoside content in stevia that provides the sweetest taste is stevioside and rebaudioside A. Based on 12 literature resources, it is founded that the higher or the lower temperature can affect the glycoside content in Stevia rebaudiana. The recommended drying temperature for the drying is between 40-60°C with an oven as the drying method. The drying method using an oven is the simplest and there is no need for much time during the drying process and produce a comparable yield of stevioside and rebaudioside A content.

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