STUDY ON THE EFFECT OF PROCESSING PARAMETERS ON THE EXTRACTION OF STEVIOSIDE USING SOXHLET EXTRACTOR

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ABSTRACT

Stevioside can be obtained in *Stevia rebaudiana* leaves , which originated from Paraguay. It has been planted across Asia, Europe and Canada. It is the source of the highly sweet *ent*-kaurenoid diterpene glycosides, rebaudiana A, stevioside and other several sweet analogs. Stevioside is 300 times sweeter than sucrose and has lately gained importance as a natural non-caloric sweetener. The commercial exploration of *S. rebaudiana* has become stronger since 70's due to the Japanese researchers developed extraction and purification process of sweetener. In *S. rebaudiana,* the ratio of stevioside to rebaudiaoside-A is 2:1. For its dominance, stevioside imparts a bitter after-taste characteristic to the crude extracts. The main purpose of this to study the effect of solvent concentration, extraction time and the ratio leaves to volume of solvent on the stevioside extraction. The soxhlet extractor was used in this study. Analysis of stevioside was carried out by anthrone reaction. From the results, extraction using 70% ethanol resulted in the highest stevioside yield at 12 mg/mL. Meanwhile, the highest stevioside at 16.5 mg/mL yield can be obtained during 24 hours extraction time. For the mass leaves to volume of solvent, 0.09g/mL of the mass of the leaves gave the highest stevioside yield at 32.5 mg/mL.

ABSTRAK

Stevioside boleh diperolehi daripada daun *Stevia rebaudiana* yang berasal dari Paraguay. Pokok tumbuhan ini telah ditanam kebanyakannya di Asia, Eropah dan Kanada. Pokok tumbuhan ini mengandungi kandungan pemanis yaqng tinggi bagi ent-kaurenoid deterpene glycoside, rebaudiana A and beberapa analog pemanis yang lain. Stevioside adalah 300 kali ganda lebih manis daripada sukrosa dan kebelakangan ini telah mendapat perhatian sebagai pemanis semula jadi tanpa kalori. Penerokaan terhadap *Stevia rebaudiana* telah meningkat sejak tahun 70-an kerana para penyelidik dari Jepun telah membangunkan proses penyarian dan penulenan pemanis daun tersebut. Daun *Stevia rebaudiana,* mengandungi nisbah steviosisde terhadap A ialah 2:1 dan secara umumnya, stevioside mempunyai sedikit rasa pahit selepas rasa manis bagi ekstrak mentah. Tujuan utama projek penyelidikan ini adalah untuk mengkaji nisbah kepekatan ethanol terhadap air bagi pengektrakan stevioside, masa pengekstrakan dan nisbah jisim daun terhadap isipadu pelarut dalam pengekstrakan stevioside. Radas soxhlet digunakan dalam kajian pengekstrakan ini. Analisis stevioside pula telah dijalankan dengan menggunakan tindak balas Anthrone. Dari hasil kajian menunjukkan pengekstrakan menggunakan 70% ethanol memberikan hasil stevioside tertinggi iaitu 12 mg/mL. Manakala masa pengekstakan selama 24 jam telah memberikan hasil stevioside tertinggi sebanyak 16.5 mg/mL. Bagi nisbah jisim daun terhadap isipadu pelarut pula, 0.09 g/mL nisbah jisim daun terhadap isipadu telah menunjukkan hasil stevioside tertinggi pada 32.5 mg/mL.

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UV- Vis	Ultra- violet visible spectroscopy
HPLC	High Performance Liquid Chromatography

CHAPTER 1

INTRODUCTION

1.1. RESEARCH BACKGROUND

Stevioside can be found in *Stevia rebaudiana* leaves as shown in Figure 1.1. *S. rebaudiana* plants is from Compositae family and is originally from Paraguay. Presently, it can be found in Asia, Europe and Canada (Siddique et al., 2012). It is the source of the highly sweet *ent*-kaurenoid diterpene glycosides, rebaudian A, stevioside and other several sweet analogs. Stevioside is 300 times sweeter than sucrose at 0.4 % w/v (Geuns, 2003) and has lately gained importance as a natural noncaloric sweetener (Olabors *et al*., 1991).

Figure 1.1: *Stevia rebaudiana*

The usage of stevioside has been accepted for several years in Brazil, Argentina and Paraguay. Since 1995, United States used the stevioside as dietary supplement. In China, Korea, and Japan apply stevioside for sweetening soft drinks, soy drink, yogurt and other food (Leu *et al*., 2010).

1.2. PROBLEM STATEMENT

Extraction of stevioside compound from *S. rebaudiana* leaves are typically depending on different extraction method. The difference in polarities of extracting solvents might influence the solubility of chemical constituents in a sample and its extracting yield. One of the most relevant steps to determine stevioside content from a sample is by selection of an appropriate solvent system

1.3. RESEARCH OBJECTIVE

The main objective of this research is to study the effect of the processing parameters in the extraction of stevioside from *Stevia rebaudiana* leaves by using solid-liquid extractor (soxhlet).

1.4. SCOPE OF RESEARCH

The scopes of the research are:-

a) To evaluate the effect of ethanol-water concentrations on the stevioside yield using soxhlet apparatus. These concentrations are: ethanol 50%, ethanol 70% and ethanol 100%
b) To investigate the effect of the extraction time on the stevioside yield.
c) To examine the effect of mass over volume ratio of *Stevia rebauidiana* leaves to ethanol:water on the stevioside yield.

1.5. RATIONAL AND SIGNIFICANCE

The rationale and significance in this study is high total stevioside compounds contain in *S. Rebaudiana* act as a sweetener. Nowadays, stevia leaf extract used to sweeten soft drinks, soy sauce, soju, yogurt and other foods in Japan, Korea and some other countries in South America used In Bangladesh, they used is as anti-diabetic tea while in United State, they used it as dietary supplement (Siddique et al., 2012).

CHAPTER 2

LITERATURE REVIEW

2.1. PLANT MATERIAL

2.1.1. *Stevia rebaudiana* Leaves

Stevia rebaudiana is originated from Paraguay. The indigeneous peoples in Paraguay already used them as sweetener. In 1887, a botanist from Argentina, Antonio Bertoni, discovers the *Stevia rebaudiana* plant. Later, this plant becomes widely known outside South America. This plant also has other names including sweet leaf of Paraguay, honey leaf, sweetleaf, sweet herb, candyleaf and honey yerba (Carakostas *et al.*, 2008). This plant is an herbaceous shrub from Asteraceae family. This plant has a used in different items of food and cultivated extensively in South America, Central America, Mexico and East Asia. The benefit of *Stevia Rebaudiana* is it can be applied as an antihypertensive, antihyperglycemic, anticariogenic and anti-humanrotavirus activities.

2.1.2. Composition in *Stevia rebaudiana*

The compositions in the *Stevia rebaudiana* leaves have 2 major components which are sweet constituent and non-sweet constituent. Stevioside, rebaudiosides A, rebaudiosides B, rebaudiosides C, rebaudiosides D, rebaudiosides E,and dulcoside A are in sweet components as shown in Table 2.1. The elements for the non-sweet component are sterols, labdane diterpenes, triterpenes, flavoroids, volatile oil constituents, pigments and inorganic matters (Jahan *et al*., 2010)

According to Dacome et al (2005), the entire sweet constituent contained in the *Stevia rebaudiana* have steviol compound which exist in the scientific name of 13-hydroxykaur-16-en-18-oic acid. The structure of steviol is illustrated in Figure 2.1. Steviol consist of multiple glycosylated natural in its structure. R is represented the glycosylated. For the stevioside, the R compound is represented by β-Glc and $\beta\text{-Glc}^2 - \beta\text{-Glc}^1$. Meanwhile, the relative sweetening power post by the R compound contains in the stevioside are 250 to 300. The stevioside is consisting of 6% to 18% of the stevia leaf and the highest glycoside in the leaf.

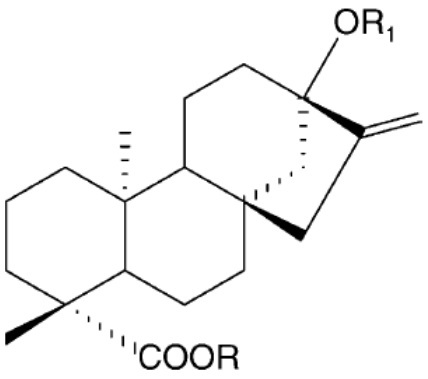

Figure 2.1: Structure of the steviol

Table 2.1: The family of steviol-derived from *Stevia rebaudiana* (Dacome *et al.*, 2005)

Diterpenic glycoside	R	R_1	Relative sweetening power (respect with sucrose =1)
Stevioside	β-Glc	β-Glc^2 – β-Glc^1	250-300
Steviosiolbioside	H	β-Glc^2 – β-Glc^1	100-125
Rebaudioside-A	β-Glc	β-Glc^2 – β-Glc^1 | ^{3} β-Glc	350-450
Rebaudioside-B	H	β-Glc^2 – β-Glc^1 | ^{3} β-Glc	300-350
Rebaudioside-C	β-Glc	ß-Glc^2 – α- Rha^1 | ^{3} β-Glc	50-120
Rebaudioside-D	β-Glc^2 – β-Glc^1	β-Glc^2 – β-Glc^1 | ^{3} β-Glc	200-300
Rebaudioside-E	β-Glc^2 – β-Glc^1	β-Glc^2 – β-Glc^1	250-300
Dulcoside	β-Glc	β-Glc^2 – β-Glc^1	50-120
Rebaudioside-F	β-Glc	β-Glc^2 – β-Xyl^1 | β-Glc	nd

From the table below, Glc represent the glucose, Rha stand for rhamnose, Xyl be a symbol of xylose and nd symbolize to not determined.

2.1.3. Advantages of Stevioside

Stevioside is a natural non-caloric as like other sweeteners. It can be used from their natural from which is directly from the dried leaves. This plant has been reported as a non-toxic compound. Only used a small quantities of stevioside needed due to the high sweetening intensity.Compared to other sweetener, this compound is secure for diabetics and Phenylketonurian (PKU) patients (Chattopadhya, 2007). It does not increase the blood sugar in the human body. So, the diabetic can use this sweetener without taking adverse glycemic response. It is also functional for those who are in overweight, obese and for individual health awareness. It can enhance the effect of other sweetener which is maple syrup and honey. By adding stevioside to the recipe, the amount of total sweetener desired can be reduced (Chattopadhya, 2007).

2.2. EXTRACTION

2.2.1. Principle of Extraction

In the extraction, its efficiency is depends on the sample matrix, analyte and the location of the analyte. Sample matrix is the sample particle that in porous and surrounded by an organic layer, while the analyte is from the sample matrix. For the process extraction, the compound is desorbed from its site in the sample matrix. This step is to remove the analyte from the extraction vessel. Then, the analyte is diffused through the organic part in order to reach the matrix-fluid interface. In this step, th analyte is distribute into extraction phase due to the convection. It is followed by the positioning and the status of the analyte within the sample matrix in the extraction process. Figure 2.2 illustrate the five different positions that have been postulated. The positions are adsorbed to the surface of the matrix; dissolved in a solvent pore and or adsorbed at the surface; dissolved or adsorbed in a matrix in micro or nano pore; chemically bond to the matrix; or dissolved in a bulk solution (Mustafa A. and Turner C., 2011).

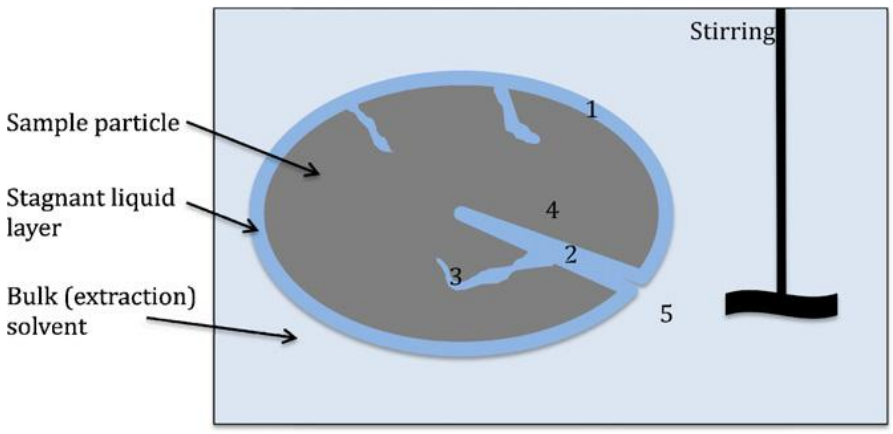

Figure 2.2: Positioning of the analyte in the sample matrix

2.3. SOXHLET EXTRACTOR

2.3.1. Principle of soxhlet

Active constituent from medical plant matrices can be extract by using classic technique based on the solvent coupled with the use of heat or agitation.There are some conventional extraction method include hyrodistillation, soxhlet, and maceration with an alcohol-water mixture or other organic solvent. Soxhlet extraction is well-established and general technique. This extraction is limited fields of application and the extraction of the thermolabile compounds (Handa *et al.,* 2008).

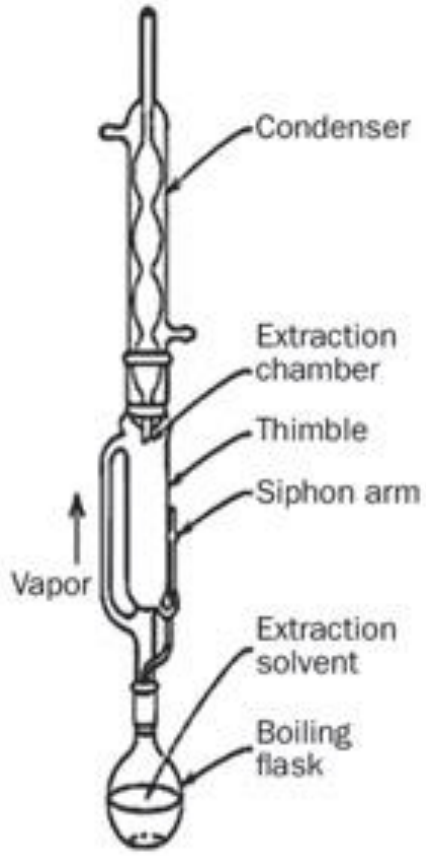

Figure 2.3: Soxhlet extractor (Handa *et al.,* 2008)

Franz von Soxhlet was the invention of the soxhlet extractor. Soxhlet extractor is a piece of laboratory apparatus that used for solid-liquid extraction. From Figure 2.3, soxhlet apparatus have condenser, extraction chamber, thimble, siphon arm and boiling flask. The plant material is put in the thimble-holder and it unloads back into the distillation flask, condensed fresh solvent from a distillation flask. The solvent inside boiling flask will evaporate in the system and become liquid again after through the condenser. The solvent carrying extracted solutes into bulk liquid. The fresh solvent passes into the plant solid bed and solute is left in the flask. The operation keeps repeating until complete extraction reached (Handa *et al.,* 2008).

2.3.2. Advantages and Disadvantage of Soxhlet Extractor

2.3.2.1. Advantages of soxhlet extractor

Separating bioactive component from natural resource by using conventional soxhlet extraction is one of the most common methods used. By using this extraction method, there have great advantages which are: from this extraction can get fresh solvent into contact with the solid matrix because of repeatedly equilibrium transfer displacement. It also can maintain in a relatively high extraction temperature with the heat from the distillation flask. After extraction, no filtration required (Handa *et al.,* 2008). Because of the basic equipment is inexpensive, the sample throughput can be increased by simultaneous extraction in parallel. By using this method, it can extract more sample mass other than other extraction methods and non-matrix dependent (Kumoro *et al*., 2006)

2.3.2.2. Disadvantage of soxhlet extractor

The most major weakness of soxhlet extractor compared to other conventional technique for solid sample preparation is this device is unable to provide agitation because it can accelerate the process (Handa *et al.,* 2008). According to Adnan et al. (2004), soxhlet extractor need a long extraction times due to slow analyte diffusion and desorption from the sample matrix to the extraction fluid. This extraction method also generates dirty extraction. Because of the large amount of solvent used, it is mandatory to undergo an evaporation or concentration step. Soxhlet technique is restricted to solvent selectivity and it is not easy to automate.

2.4. FACTOR EFFECTING EXTRACTION PROCESS

2.4.1. Solvent Polarity

By choosing the solvent in the extraction is ‘like dissolve like’ concept by the usage of polar solvent for polar analytes and also nonpolar analytes are dissolved in nonpolar solvents. The basic aspect for solvent choices is the solvent has the ability for the solubility of the desired analyte, their diffusivity in the solvent and the characteristics of the sample. Extraction with high purity can be achieved with high selectivity because the analyte should have high solubility in the solvent while other compound should not or in a minimal solubility. Using low a concentration to extract the analyte, the rate of extraction does not effect by the analyte concentration but effect the rate of mass transfer. So, choosing the right solvent chemical properties must be done in order to ensure salvation and the release of the analyte (Mustafa A. and Turner C, 2011).

2.4.2. Extraction Time

According to Handa et al., (2008), for parameter for selecting an appropriate extraction method in term of extraction time is important because incomplete extraction occurred if insufficient time used. If taking too much time for extraction time, unwanted constituents may also be extracted. For example, if tea leaves boiled for too long, tannin are extracted which can impart the astringency to the final preparation. For determination of the extraction time, shorter time can be used if the equilibrium times are excessively long.

2.4.3. Ratio Mass to Volume of Solvent

According to Abou-Arab et al., (2010) state that, the more solvent used for extraction, the more stevioside can be extracted but the concentration of the stevioside in the extract decreased. The difference purity according to Total Soluble Solid (TSS) and pigmentation could be attributed to the ability to the solvent to extract more soluble solid.

2.5. REVIEW RELATED RESEARCH FOR DIFFERENT METHOD USING *STEVIA REBAUDIANA*

Table 2.2: Total stevioside can be extracted by using different method

Method used	**Condition**					**Yield (%)**	**Reference**
	Power (W)	**Pressure (bar)**	**T (°C)**	**t (min)**	**Solvent**		
Ultrasonically Assisted Extraction	60	-	60	40	Water	43.62±1.75	Liu *et al.*,(2010)
Classical Extraction	-	-	100	120	Water	30.53±1.11	Liu *et al.*,(2010)
Supercritical CO_2 Extraction	-	211	80	60	Ethanol 17.4%	17.4	Eurkucuk *et al.*, (2009)
Supercritical fluid Extraction (200 bar)	-	200	30 10 16	720 120 250	CO_2 CO_2 + water CO_2 + water	1.6 50 50	Yoda *et al.*, (2003)
Supercritical CO_2 Extraction	-	405	50	288	Methanol 10%	5.8	Ozcan and Ozcan (2004)
Soxhlet Extraction	-	-	30	192	Methanol 10%	1.1	Ozcan and Ozcan (2004)
Hot water extraction	-	-					

Table 2.3: The extraction yield by different plant by using different method

Plant	Method used	Condition					Yield (%)	References
		T (°C)	t (min)	Pressure (bar)	Solvent	Synder's solvent polarity index		
Phyllanthus niruri	Soxhlet extraction	69	-	-	n-hexane	0.1	1.8±0.1	Markom *et al.*, (2007)
		60	-	-	Petroleum ether	0.1	2.2	Markom *et al.*, (2007)
		40	-	-	Dichloromethane	3.4	4.0	Markom *et al.*, (2007)
		61	-	-	Chloroform	4.1	9.7	Markom *et al.*, (2007)
		56	-	-	Acetone	5.4	3.9	Markom *et al.*, (2007)
		78	-	-	Ethanol	5.2	11.6	Markom *et al.*, (2007)
		65	-	-	Methanol	6.6	14.6±1.1	Markom *et al.*, (2007)
		84	-	-	Acetone 70%	6.5	18.5	Markom *et al.*, (2007)
		90	-	-	Ethanol 70%	8.2	20.8	Markom *et al.*, (2007)
		94	-	-	Ethanol 50%	7.9	22.5	Markom *et al.*, (2007)
		97	-	-	Ethanol 30%	7.1	26.4±1.6	Markom *et al.*, (2007)
		98	-	-	Ethanol 20%	6.3	27.1	Markom *et al.*, (2007)
		100	-	-	Water	9.0	26.2	Markom *et al.*, (2007)
		100	-	-	Water (sample		23.5	Markom *et al.*,