

UNIVERSITI TEKNOLOGI MARA

**PROCESS PARAMETER
OPTIMISATION IN RELATION TO
SENSORY EVALUATION AND
PRODUCTION OF REBAUDIOSIDE
A AND STEVIOSIDE OF *Stevia*
rebaudiana VARIETY MS012 USING
RESPONSE SURFACE
METHODOLOGY**

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ABSTRACT

Stevia rebaudiana has been selected in this study due to its high intensity of sweetness. In this study, two accessions were chosen which were MS012a from MARDI and MS012b from UKM, Bangi. A large number of sample involvement has led to the ineffective cost of research material and time. Additionally, the use of high performance liquid chromatography (HPLC) for *S. rebaudiana* analysis involves high costs equipment, laborious technique and expensive chemicals utilisation. Finally, the lingering aftertaste and bitterness of *S. rebaudiana* extract have caused this plant unfavorable among consumer. These problems led to the approach on less expensive and effective ways of production without compromising product quality. Thus, the response surface methodology (RSM) application was the solution to a large number of sample involved through parametric optimisation. Besides, *S. rebaudiana* extraction in this study was using water which is cost-effective extraction solvent. The extraction was influenced by three variables which were temperature, particle size and duration. Refractometer was used in determining the content of stevioside and rebaudioside A as an alternative for preliminary analysis prior to HPLC quantification. The correlation values between both analyser was determined. The 9-point hedonic scale was applied in order to determine the consumer acceptance towards the optimised sample at sweetness, bitterness and aftertaste attributes. The optimisation of MS012a and MS012b extraction suggested the optimum condition at the temperature of 92°C, particle size 0.7 mm and duration of 4.7 minutes with 2.1521% and 2.0532% of stevioside and rebaudioside A content respectively. The verification value of *S. rebaudiana* obtained is less than 5% with 2.11% for MS012a and 1.99% for MS012b that shows a significant verification value. Tukey's test conducted has highlighted that optimised sample gave a significant output for both accessions. Furthermore, the correlation values for both refractometer and HPLC analysers showed Pearson coefficient, r value of 0.986 and the p-value of 0.000 (MS012a) whereas MS012b, showed r value of 0.998 with p-value 0.000. Besides, the scatter plot shows the linear correlation for both accession. This proved that refractometer was able to detect stevioside and rebaudioside A. ICP analysis was performed for MS012a and MS012b which resulted in 0.02 ppm (Hg) and 0.03 (Pb), 0.03 ppm (Hg) and 0.05 ppm (Pb) respectively. Analysis of 9-point hedonic scale for sensory evaluation for MS012a and MS012b via a Mann Whitney test revealed that there was insignificant of p-value for sweetness attributes with 0.4499 and 0.6625. Significant p-values of 0.0256 and 0.0108 for bitterness and 0.0041 and 0.0256 for aftertaste are produced for MS012a and MS012b respectively. This shows that the optimised sample extracts was accepted by the panelist with the insignificant result for sweetness attribute. The results in this study suggested that RSM can be applied for *S. rebaudiana* parametric optimisation, the refractometer can be used to detect stevioside and rebaudioside A and the taste acceptance of optimised sample by the panelist. The ICP analysis conducted was to ensure the safety of *S. rebaudiana* for the consumer. This study resulted to cost-effective research strategy for *S. rebaudiana*.

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CHAPTER ONE

INTRODUCTION

Background of Study

Stevia rebaudiana (Bertoni) is the only sweet stevia plant among 150 of stevia species. *S. rebaudiana* has been widely used in Japan, China, Russia, USA and the UK for food and beverage preparations. This plant which act as a natural sweetener is also well known as sugar, candy and sweet leaf due to its sweet taste that estimate 300 times sweeter than cane sugar (Balaswamy, Rao, Nagender, & Satyanarayana, 2014). There are eleven sweet chemical constituents of the plant such as stevioside (sv), rebaudiosides (reb) and dulcosides. Sv and reb A are the most widely studied among the sweet compounds of plant origin (Hawke, 2003).

A number of researchers have reported that *S. rebaudiana* is beneficial for humankind in the aspect of health due to its low-calorie properties (Kobus-Moryson & Gramza-Michałowska, 2015). Atteh et al. 2008 reported that regular consumption of steviol glycosides regularly leads to glucose and cholesterol level reductions. (Atteh, Onagbesan, Tona, Decuypere, Geuns, & Buyse 2008). The demand for low calorific intense sweeteners is growing not only because of sugar related health problem but also due to rising number of diabetic patients. Malaysia was reported by the International Diabetes Federation (IDF) as one of the 21 countries and territories of the IDF Western Pacific region. In the year 2015, 3.3 million cases of diabetes were recorded in Malaysia from the total population which was 31.2 million (Pacific, 2015; Statistics, 2016). Therefore, stevia is the best choice to fulfil the needs of consumers, not only combining the qualities of a sweetener, but also constituting a source of many substances with a nutritional effect on the human metabolism (Kobus-Moryson & Gramza-Michałowska, 2015). Due to the demand of low calorific sweeteners, this study was conducted with the interest to investigate *S. rebaudiana* in the aspect of its extraction process, analysis and sensory evaluation technique.

Optimisation of parameters concerning to the extraction yield which are immersion temperature, particle size and immersion duration were studied using response surface methodology (RSM). It involved the reduction in the number of