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OPTIMIZATION OF STEVIOSIDE AND REBAUDIOSIDE A EXTRACTION FROM LEAVES OF STEVIA (*Stevia rebaudiana*) BY SUPERCRITICAL FLUID EXTRACTION TECHNIQUE USING RESPONSE SURFACE METHODOLOGY

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ABSTRACT

Steviol glycosides compound in *Stevia rebaudiana* Bertoni plant act as non-caloric natural sweetener. Stevioside and rebaudioside A are the most abundant steviol glycosides in stevia leaves. It was found that extraction yield of stevioside and rebaudioside A compound using conventional method is very low with high impurities. Furthermore, there are lack of on the optimization of stevioside and rebaudioside A extraction.The aims of this study are to optimise parameters which are concentration of co-solvent, temperature and pressure, of supercritical fluid extraction (SFE) from stevia leaves using response surface methodology and to quantify the amount of stevioside and rebaudioside A using high performance liquid chromatography (HPLC) and to determine separation of rebaudioside A and stevioside using column separation. Rebaudioside A is preferred over stevioside because of its sweeter taste and with less bitter after taste and Rebaudioside A (>95%) has been approved by FDA for consumption. Extraction yield of stevioside and rebaudioside A compounds using conventional method is low. The use of SFE with CO_2 and ethanol as solvents could produce high yield of stevioside and rebaudioside A. Stevia leaves powder of variety MS012, with particle size 200 μm (diameter) were used in this study. Soxhlet extraction with distilled water and four different concentrations of ethanol in water (65%, 70%, 75% and 100%) were used. The treatment using 65% ethanol in water gave the highest percentage of stevioside and rebaudioside A compound which are 5.33% and 4.37% respectively. The fabricated SFE machine was at Faculty of Engineering and Built Environment, UKM with capacity of 5 g sample. The SFE conditions used were concentration of co-solvents within range 65% to 75% of ethanol in water, temperature within range 55°C to 75°C and pressure within range 180 bar to 220 bar. Each extraction process was conducted for 120 minutes. Due to large number of treatments required, response surface methodology (RSM) with 5-level-3-factor central composite design (CCD) was employed using MINITAB. A total of 20 out of 45 treatments were sampled. The regression equations were significantly ($p<0.05$) fitted for all responses with high R^2 (>0.87), which had no indication of lack of fit. Based on response surface study, the optimum parameters for extracting stevioside and rebaudioside A are concentration of co-solvents is 65% of ethanol in water; temperature at 75°C and pressure at 220 bar was predicted to provide the optimum response surface in terms of total extraction yield (0.243 g/g), stevioside (1.01%) and rebaudioside A (3.32%). Even though stevioside is known to be more than rebaudioside A, SFE was extracted more rebaudioside A than stevioside. Thin layer chromatography (TLC) for quick identification was used before column separation. The column separation of rebaudioside A and stevioside was eluted with ethyl acetate: ethanol: water (80: 20: 12 v/v) as mobile phase. Based on fraction analysis, most of rebaudioside A could not be separated from stevioside. Column separation using silica gel was unable to separate stevioside and rebaudioside A. From this study, SFE is the best extraction method to produce abundance of rebaudioside A.

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