J. Bio-Sci. 26: 49-54, 2018 ISSN 1023-8654
http://www.banglajol.info/index.php/JBS/index

OPTIMIZATION OF POLYPHENOLS EXTRACTION FROM COCOA BEAN SHELL USING FACTORIAL DESIGN

MFQ Avilés[1*], A Barragán[1], J Hernández[2], I Chóez-Guaranda[1], R Viteri[1], K Aguaguiña[1] and P Manzano[1,3]

[1]*ESPOL Polytehcnic University, Escuela Superior Politécnica del Litoral, ESPOL, Centro de Investigaciones Biotecnológicas del Ecuador (CIBE), Ecuador*

[2]*ESPOL Polytehcnic University, Escuela Superior Politécnica del Litoral, ESPOL, Facultad de Ciencias Naturales y Matemáticas (FCNM), Ecuador*

[3]*ESPOL Polytehcnic University, Escuela Superior Politécnica del Litoral, ESPOL, Facultad de Ciencias de la Vida (FCV), Guayaquil, Ecuador*

Abstract

This investigation aims to establish the optimum parameters to extract polyphenols from cocoa bean shell (CBS). Time, solvent and method of extraction were considered as factors and Total Content of Polyphenols (TCP) expressed as Gallic Acid Equivalent (GAE) was selected as the response. Results showed that using factorial design the optimum conditions were water in reflux during 5 minutes (262.76 ± 20.66 mg GAE/ 100 g). This revealed that CBS represent a high potential of use in food industry, principally in the field of functional food development.

Key words: Antioxidant activity, By product, Cocoa bean shell, Polyphenols, Reflux, Ultrasound

Introduction

Cocoa beans shell (CBS) is one of the main by-products from cocoa processing and generate a disposal problem (Martínez et al. 2012). The use of CBS as an additive in human food have been studied, principally for the fiber content (Redgwell et al. 2003). However, studies have reported that CBS contain polyphenols (Martínez et al. 2012), which are compounds that exhibit important biological activities such as antimutagenic, anticarcinogenic, antioxidant and other beneficial health properties (Bhattacharya et al. 2011, Nsor-Atind et al. 2012, Plaza et al. 2016).

Bioactive compounds, including polyphenols, have been extracted using traditional methods for instance: maceration, reflux and soxhlet (Araujo 2009, Kaneria and Chanda 2012, Aires et al. 2016, Ćujić et al. 2016). Nevertheless, these methods consume large quantities of solvents and are time consuming (Heleno et al. 2016). Novel technologies such as ultrasound-assisted extraction (UAE) has been recently employed for polyphenols extraction (Ghitescu et al. 2015) and has proved to be more efficient than conventional method (Yang and Zhang 2008). Hence, the aim of this investigation is to compare the use of method of extraction, reflux and ultrasound assisted extraction to determinate an optimum method of extraction, solvent choice and time process to obtain a high yield of polyphenols from CBS.

*Author for correspondence: mquijano@espol.edu.ec

Materials and Methods

Chemicals and reagents

Ethanol (Panreac, USA), petroleum ether and sodium carbonate (JT Baker, USA) were of analytical grade. Deionized water was obtained from a Mili-Q water purification system (Millipore, USA). Gallic acid and Folin and Ciocalteu's reagent were supplied by Sigma-Aldrich.

Sample

Cocoa bean shells were dried at 60ºC in an oven using air circulation during 16 h. The dried sample was hand milled, sieved through a 200-mesh screen and defatted with petroleum ether in a Soxhlet apparatus.

Extraction

Reflux: Dried, defatted and powdered material was refluxed using a sample: solvent ratio (water or ethanol) of 1:10. The extraction was performed in the following time intervals: 5, 15, 30 and 60 minutes.

Ultrasound assisted extraction (UAE)

An ultrasonic bath VWR of 35 KHz and 180 W was employed. The extraction of polyphenols was performed by adding 2 g of dried, defatted and powdered sample into 20 ml of solvent (water or ethanol) in a 25 ml flask. This experiment was carried out using the same time intervals of reflux extraction.

Total polyphenol content

Total polyphenol content (TPC) was determinate by spectrophotometry using the Folin and Ciocalteu's method and was expressed as gallic acid equivalents (GAE) in mg/100 g dry material (Lachman et al. 2010). A total of 250 µl of sample and 250 µl Folin and Ciocalteu's reagents 1N were mixed, 5 minutes later 20% of sodium carbonate (750 µl) was added. Absorbance was read after 90 minutes at 765 nm.

Experimental design and statistical analysis

The experiment was performed in three replicates; each replicate consisted of 16 variable combinations (run). The factors studied where method (Factor A), solvent (Factor B) and time of extraction (Factor C). Analysis of variance (ANOVA) was used to determinate the main effects of method (X_1), solvent (X_2) and time of extraction (X_3), and the interaction between the effects on total polyphenol content expressed as mg GAE/100 g of sample.

Results

The experiment was performed in three replicates; each replicate consisted of 16 variable combinations (run). The factors studied where method (Factor A), solvent (Factor B) and time of extraction (Factor C). The factors and levels considered for the experiment are given in Table 1.

Table 1. Full factorial design experimental matrix

Run	Design			Response		
	(X_1)	(X_2)	(X_3)	Trial 1	Trial 2	Trial 3
1	UAE	Water	5 min	93.41	66.44	68.48
2	UAE	Ethanol	5 min	30.42	38.54	53.10
3	Reflux	Water	5 min	285.89	256.08	246.30
4	Reflux	Ethanol	5 min	59.37	38.70	42.00
5	UAE	Water	15 min	60.02	91.43	89.37
6	UAE	Ethanol	15 min	70.82	49.17	68.14
7	Reflux	Water	15 min	17.30	176.13	185.88
8	Reflux	Ethanol	15 min	72.01	46.37	42.47
9	UAE	Water	30 min	137.82	113.30	163.26
10	UAE	Ethanol	30 min	64.69	81.09	74.98
11	Reflux	Water	30 min	179.29	214.62	183.09
12	Reflux	Ethanol	30 min	47.01	45.75	41.36
13	UAE	Water	60 min	162.73	115.85	134.85
14	UAE	Ethanol	60 min	58.67	73.29	50.61
15	Reflux	Water	60 min	208.73	182.82	190.62
16	Reflux	Ethanol	60 min	31.03	41.20	20.24

From Table 2, the *p*-value indicated the significant effect on TPC. All factors and interactions were significant at 0.05%. F-ratio indicated that solvent (583.52) had the higher effect on TPC, followed by the interaction between method and solvent of extraction (170.83). On the other hand, time had the lowest effect of TPC (3.01). The main effect plot showed in Fig. 1 revealed that the water increased the TPC during the extraction. Longer extraction time decrease the TPC and reflux method gave higher yield of TPC than UAE.

Table 2. The ANOVA of factorial design for polyphenol extraction

Analysis of variance (ANOVA)					
	Degree of freedom (f)	Sum of Sq.	Mean Sq.	F-ratio	*p*-value
Method	1	20993	20993	90.94	0.00
Solvent	1	134702	134702	583.52	0.00
Time (min)	3	2082	694	3.01	0.04
Method*Solvent	1	39436	39436	170.83	0.00
Method*Time(min)	3	13974	4658	20.18	0.00
Solvent*Time (min)	3	5263	1754	7.60	0.00
Method*Solvent*Time (min)	3	4646	1549	6.71	0.00
Other (error)	32	7387	231	-	-
Total	47	228483	-	-	-

R^2 = 96.77%, R^2 (adj) = 95.25%

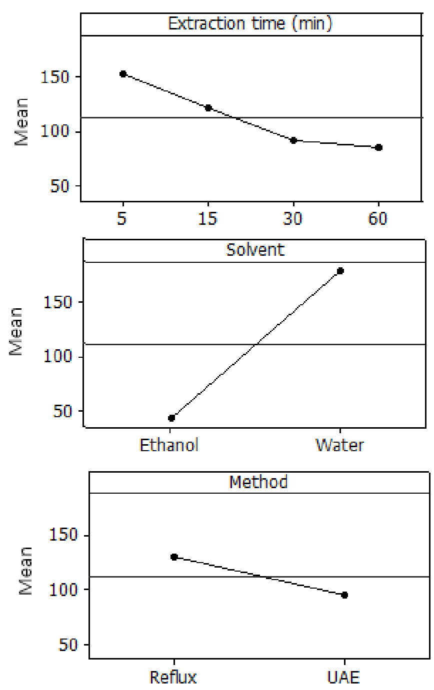

Fig. 1: Main effects plot for polyphenol extraction.

The optimum parameters for polyphenol extraction according to Fig. 2 were reflux method, water solvent and 5 minutes of extraction.

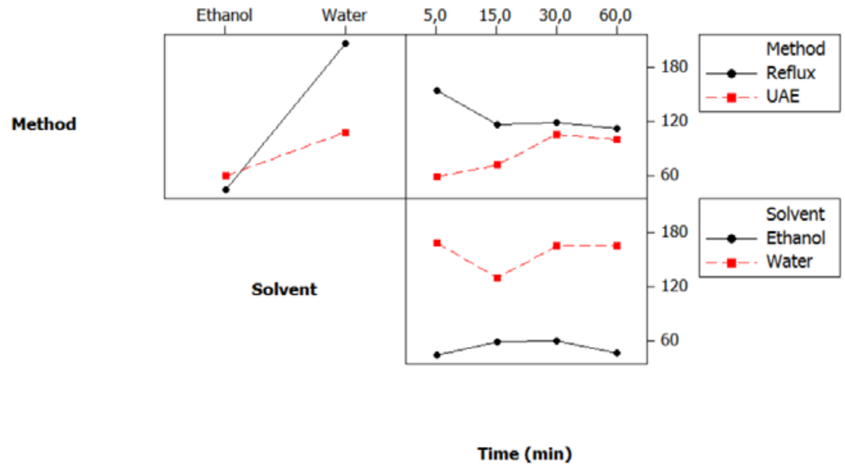

Fig. 2: Interaction effects plot for polyphenol extraction.

Discussion

Full factorial design determined that reflux used with water incremented the extraction of polyphenols. The high temperature of the process decreases water density and viscosity, which increase the mass transfer. Also, intermolecular interaction with water decreases using reflux, which allow a higher molecular motion and increment solute solubility (Wei et al. 2013). Moreover, dielectric constant of water decreases using reflux which make non polar polyphenols to solubilize into water (Filly et al. 2016).

The values of TPC of CBS (262.73 ± 20.65 mg GAE/100 g sample) extracted with reflux using water by 5 min are higher than values reported for CBS extracted with a mixture of methanol – acetone (154.43 mg GAE/100 g sample). In contrast, extraction with UAE using ethanol by 30 min (73.59 ± 8.29 mg GAE/100 g sample) was lower than TPC reported for CBS using ethanol (82.37 mg GAE/100 g sample) (Martínez et al. 2012). A comparison of TPC value with others fibers waste indicated that CBS contain more polyphenols than tomato peels (158.10 ± 7.70 mg GAE/100 g sample) (Navarro-González et al. 2011) and orange peel (49.94 mg GAE/100 g sample) (Boukroufa et al. 2015) but less TPC than parrot peel (1380 mg GAE/100 g sample) (Chantaro et al. 2008). In this context, this value added CBS extract represent a high potential of use in food industry, principally in the field of functional food development.

Conclusion

CBS represent an interesting source of polyphenols and can be used to enrich different kind of food. Extraction result feasible since water and traditional method of extraction, reflux, works well to obtain polyphenols.

Acknowledgement

We are grateful to Nardia Diez who provided expertise that greatly improved the manuscript. The financial support from Escuela Superior Politécnica del Litoral and Secretaría de Educación Superior, Ciencia, Tecnología e Innovación (SENESCYT) through project grant # SENESCYT-DMPF-2014-CO are highly acknowledged.

References

Aires A, Carvalho R and Saavedra MJ (2016). Valorization of solid wastes from chestnut industry processing: Extraction and optimization of polyphenols, tannins and ellagitannins and its potential for adhesives, cosmetic and pharmaceutical industry. Waste Management, 48: 457-464.

Araujo P (2009). Key aspects of analytical method validation and linearity evaluation. Journal of Chromatography, 877: 2224-2234.

Bhattacharya U, Mukhopadhyay S and Giri AK (2011). Comparative antimutagenic and anticancer activity of three fractions of black tea polyphenols thearubigins. Nutrition and Cancer, 63: 1122-1132.

Boukroufa M, Boutekedjiret C, Petigny L, Rakotomanomana N and Chemat F (2015). Bio-refinery of orange peels waste: A new concept based on integrated green and solvent free extraction processes using ultrasound and microwave techniques to obtain essential oil, polyphenols and pectin. Ultrasonics Sonochemistry, 24: 72-79.

Chantaro P, Devahastin S and Chiewchan N (2008). Production of antioxidant high dietary fiber powder from carrot peels. LWT-Food Science and Technology, 41: 1987-1994.

Ćujić N, Šavikin K, Janković T, Pljevljakušić D, Zdunić G and Ibrić S (2016). Optimization of polyphenols extraction from dried chokeberry using maceration as traditional technique. Food Chemistry, 194: 135-142.

Filly A, Fabiano-Tixier AS, Louis C and Fernandez X (2016). Water as a green solvent combined with different techniques for extraction of essential oil from lavender flowers. Comptes Rendus Chimie, 19: 707-717.

Ghitescu RE, Volf I, Carausu C, Bühlmann AM, Gilca I and Popa V (2015). Optimization of ultrasound-assisted extraction of polyphenols from spruce wood bark. Ultrasonics Sonochemistry, 22: 535-541.

Heleno S, Diz P, Prieto M, Barros L, Rodrigues A, Barreiro M and Ferreira I (2016). Optimization of ultrasound-assisted extraction to obtain mycosterols from *Agaricus bisporus* L. by response surface methodology and comparison with conventional soxhlet extraction. Food Chemistry, 197:1054-1063.

Kaneria M and Chanda S (2012). Evaluation of antioxidant and antimicrobial properties of *Manilkara zapota* L. (chiku) leaves by sequential soxhlet extraction method. Asian Pacific Journal of Tropical Biomedicine, 2: 1526-1533.

Lachman J, Orsák M, Hejtmánková A and Kovářová E (2010). Evaluation of antioxidant activity and total phenolics of selected czech honeys. LWT-Food Science and Technology 43: 52-58.

Martínez R, Torres P, Meneses M, Figueroa JG, Pérez-Álvarez JA and Viuda-Martos M (2012). Chemical, technological and *in vitro* antioxidant properties of cocoa (*Theobroma cacao* L.) co-products. Food Research International, 49: 39–45.

Navarro-González I, García-Valverde V, García-Alonso J and Periago M (2011). Chemical profile, functional and antioxidant properties of tomato peel fiber. Food Research International, 44: 1528-1535.

Nsor-Atind J, Zhong F, Mothibe K, Bangoura M and Lagnika C (2012). Quantification of total polyphenolic content and antimicrobial activity of cocoa (*Theobroma cacao* L.) Bean Shells. Pakistan Journal of Nutrition, 11: 672-677.

Plaza M, Batista Â, Cazarin C, Sandahl M, Turner C, Östman E and Maróstica M (2016). Characterization of antioxidant polyphenols from M*yrciaria jaboticaba* peel and their effects on glucose metabolism and antioxidant status: A pilot clinical study. Food Chemistry, 211: 185-197.

Redgwell R, Trovato V, Merinat S, Curti D, Hediger S and Manez A (2003). Dietary fibre in cocoa shell: characterisation of component polysaccharides. Food Chemistry, 81: 103-112.

Wei M, Yang Y, Chiu H and Hong S (2013). Development of a hyphenated procedure of heat-reflux and ultrasound-assisted extraction followed by RP-HPLC separation for the determination of three flavonoids content in *Scutellaria barbata* D. Don. Journal of Chromatography, 940: 126-134.

Yang Y and Zhang F (2008). Ultrasound-assisted extraction of rutin and quercetin from *Euonymus alatus* (Thunb.) Sieb. Ultrasonics Sonochemistry, 15: 308-313.