Emirates Journal of Food and Agriculture. 2017. 29(1): 45-50
doi: 10.9755/ejfa.2016-04-388
http://www.ejfa.me/

REGULAR ARTICLE

Polyphenols extracted from *Theobroma cacao* waste and its utility as antioxidant

Patricia Manzano[1,2]*, Jéssica Hernández[3], María Quijano-Avilés[2], Ana Barragán[2], Ivan Chóez-Guaranda[2], Rafael Viteri[2], Oswaldo Valle[3]

[1]Escuela Superior Politécnica del Litoral, ESPOL, Facultad de Ciencias de la Vida (FCV), Campus Gustavo Galindo, Km. 30.5 vía Perimetral, P.O. Box 09-01-5863, Guayaquil, Ecuador, [2]Escuela Superior Politécnica del Litoral, ESPOL, Centro de Investigaciones Biotecnológicas del Ecuador (CIBE), Campus Gustavo Galindo Km 30.5 Vía Perimetral, P.O. Box 09-01-5863, Guayaquil, Ecuador, [3]Escuela Superior Politécnica del Litoral, ESPOL, Facultad de Ciencias Naturales y Matemáticas (FCNM), Campus Gustavo Galindo Km 30.5 Vía Perimetral, P.O. Box 09-01-5863, Guayaquil, Ecuador

ABSTRACT

Cocoa production is economically significant in Ecuador but large quantities of waste are generated during the industrialization process. The aim of this study is to improve the quality parameters of cooking oils using polyphenols extracted from cocoa bean shell. A 32 factorial design was used in the determination of optimal conditions for the use of polyphenols as antioxidant for the oils. The polyphenol concentration added to the oil (0%, 0.02%, and 0.04%) and the times of repeated use (0, 10 and 20 times) were consider as factors and free fatty acids, peroxide index, clarity, and DPPH antioxidant activity were selected as the dependent variables. Response surface method was applied for optimization. The required concentration for stabilized cooking oil was 0.01 % with a desirability value of 97.59%. These results are novel and prove the usefulness of cocoa waste as a source of antioxidants.

Keywords: Cocoa shell; DPPH; Folin-ciocalteu; Free fatty acids; Peroxide value

INTRODUCTION

Currently, polyphenols have won interest due to their capacity to act as antioxidants. This capacity allows to stop the oxidation of fats and their subsequent oxidative alteration of the food (Zamora, 2007). Cocoa (*Theobroma cacao)* production in Ecuador is approximately 230,000 metric tons per year (PROECUADOR, 2013). The industrial process generates about 1,632 metric tons of waste per year (Murillo, 2008), including the cocoa bean shell (CBS). Studies have reported the presence of polyphenols in cocoa bean shell (Nsor-Antidana et al., 2012) using different extraction methods and kinds of solvents (acetone, ethanol, methanol and water). Boskou et al. (2006) reports the utility of the cocoa waste as oil preserver in pharmaceutical products or to increase the phenolic content in oils.

On the other hand, vegetable oils have a wide consumption in food preparation, mainly in the elaboration of fritters. Synthetic antioxidants are commonly used as additives, to maintain the characteristics of elaborated products and avoid their oxidative degradation, in spite of the harmful effects on health described in the literature (Carocho et al., 2015). Misnawi et al. (2014) have previously reported the application of natural antioxidant in cooking oil from polyphenol extracted of unfermented cocoa bean with excellent result. However, the application of polyphenol extracted of cocoa waste has not been published.

The presented study aimed to improve the quality parameters of cooking oils using polyphenols extracted from cocoa bean shell.

MATERIALS AND METHODS

Cocoa Bean Shells and chemicals: CBS were provided by Ecuacocoa C.A., located in the city of Guayaquil, Ecuador. Samples were dried at 60 ºC, size reduction was take part at a hand milled, sieved trough a 200-mesh screen and defatted with hexane in a Soxhlet apparatus.

***Corresponding author:**
Patricia Manzano, Escuela Superior Politécnica del Litoral, ESPOL, Facultad de Ciencias de la Vida (FCV), Campus Gustavo Galindo, Km. 30.5 vía Perimetral, P.O. Box 09-01-5863, Guayaquil, Ecuador. E-mail: pmanzano@espol.edu.ec

Received: 11 April 2016; **Revised:** 14 December 2016; **Accepted:** 18 December 2016; **Published Online:** 02 January 2017

Ethanol, Hexane, sodium carbonate (Na_2CO_3) and chloroform were of analytical grade and supplied by Panreac and J.T. Baker. Gallic acid, Folin-Denis reagent and 2,2-Diphenyl-1-picrylhydrazyl (DPPH) were supplied by Sigma-Aldrich. Deionized water was obtained from a Mili-Q water purification system (Millipore, Bedford, Ma, USA). Refined soy oil free of synthetic antioxidants was provided by Industrias Ales C.A., located in Manta city, province of Manabí. Sodium hydroxide, starch, Acetic acid were analytical grade and supplied by Merck and Mallinckrodt.

Sample extraction: The pulverized, defatted CBS (2 g.) was extracted in 50 mL of deionized water with a reflux extraction (5 min.) (Nsor-Atindana et al., 2012). Solutions obtained were filtered (Whatman No.1) and clarified by centrifugation at 4000 rpm, and 4ºC by 15 min. (Thermo Scientific). The supernatant obtained was subjected to a microfiltration process (0.45 μM). Sample was lyophilizate (Labconco 4.5) for further application.

Experiment condition: The fritter process was performed in a deep aluminum container, to allow the complete immersion of the sample of plantain. Two liters of soy oil were used to fry 200 g of plantain, maintaining constant the temperature of the oil and frying time at 180^{O}C for 3 minutes (Quiles et al., 2002). Samples of the soy oil, taken at different times of frying were used to evaluate potential chemical changes.

Total polyphenol content (TPC): TPC was determinate by spectrophotometry (Synergy HT, Biotec) using the Folin-Denis method and was expressed as gallic acid equivalents (GAE) in mg.g^{-1} dry material (Lachman et al., 2003).

Free Fatty Acid (FFA): FFA was determined using the AOAC Official Method 940.28 (AOAC,1995).

Peroxide Index (PI): PI was determined using the AOAC Official Method 965.33 (AOAC, 1995).

Oil clarity and DPPH radical scavenging activity: Assays were determinate by spectrophotometry (Synergy HT, Biotec), measured at 520 nm (Oomah et al., 2005) and 517 nm (Minioti et al., 2010).

Statistical analysis and optimization

In order to improve the quality parameters of cooking oils using polyphenols extracted from cocoa bean shell a 3^2 factorial design was performed. Frequency of use of the frying oil (0, 10 and 20 times) and the added quantity of the powdered polyphenol (0%, 0.02%, 0.04% (% m/m)) were consider as factors and free fatty acids, peroxide index, clarity, and DPPH antioxidant activity were selected as response variables. Response surface method was applied to achieve optimal quality parameters.

RESULTS AND DISCUSSION

The total polyphenol content was 6.04 ± 0.12 mg GAE/g of defatted sample using reflux extraction with water, this value is lower than the one described by Tapia (2015) who used water/methanol mix in polyphenols extraction (9.83 mg GAE/g). Similar studies were reported for this kind of waste using methanol and ethanol as extractions solvents (Arlorio et al, 2005; Martínez et al., 2012; Yapo et al., 2013).The response of the factorial design and the properties evaluated are shown at Table 1.

The analysis of variance (ANOVA) of the quadratic model is given in Table 2. A good fitting model is represented by a R^2 value higher than 80% (Karazhiyan et al., 2011). Furthermore, R^2 values are close to the adjusted R^2, which demonstrate that the model fits the experimental and predicted values well. The associated p-value for the model is lower than 0.05 indicating that all responses are statistically significant (Montgomery et al., 2003).

Effect of factors on free fatty acids

Free fatty acid parameter in fats and oils can be used to show the extent of its deterioration due to hydrolysis of TAG and/or cleavage and oxidation of unsaturated fatty acid (Abdulkarim et al., 2007). Response data for FFA is shown on Table 1 where none of the values obtained exceeded the permissible maximum of 0.2% FFA (as oleic acid). Additionally, the relation between the factors are exposed at Table 2 and Fig. 1, threfore, the adittion of polyphenol decrease the value of free fatty acid meanwhile the frecuency of oil use affects produce an increase on the FFA. This phenomenon is explained by the chemical reaction between the moisture of food and compounds of oil. Water food released during oil frying attacks the ester linkage of triacylglycerols and generates some compounds as diacylglycerols, monoacylglycerols, glycerol, and free fatty acids (Choe et al.,2007). Thus, free

Table 1: Oxidation values in the oil formulations

F[a]	C[b] (%)	FFA (%)	PI (meqO_2/kg)	Color (%T)	DPPH[c] (%AA)
0	0	0.055	2.254	89.847	45.026
0	0.02	0.047	2.254	88.360	73.767
0	0.04	0.046	2.187	86.192	83.975
10	0.00	0.086	3.898	86.502	42.944
10	0.02	0.064	3.732	85.155	70.835
10	0.04	0.057	3.398	82.406	80.108
20	0.00	0.126	12.843	83.704	34.934
20	0.02	0.083	8.356	82.630	65.682
20	0.04	0.073	6.887	80.345	75.124

[a]Frequency of use, [b]Polyphenol concentration, [c]DPPH antioxidant activity

Table 2: Summarized ANOVA of the variables analysis from response data

	FFA	IP	Clarity	DPPH
Model	Quadratic	Quadratic	Quadratic	Quadratic
F value (model)	40,64*	712.24*	12,90*	158,89*
R^2	93.92	96.64	85.77	99.25
Adjusted R^2	92.48	95.85	82.38	99.07
Equation	Y=0.06+0.02A-0.02B+0.01B2-0.01AB	Y=3.4+3.6A-1.1B+2.1A2-1.5AB	Y=85.1-2.9A-1.9B	Y=71.1-4.5A+19.4B-1.6A2–9.7B2
Significant factor	A, B, AB, B^2	A, B, AB, A^2	A, B	A, B, A^2, B^2

A: Frequency, B: Concentration, R^2: Coefficient of determination, level of significance *p<0.05

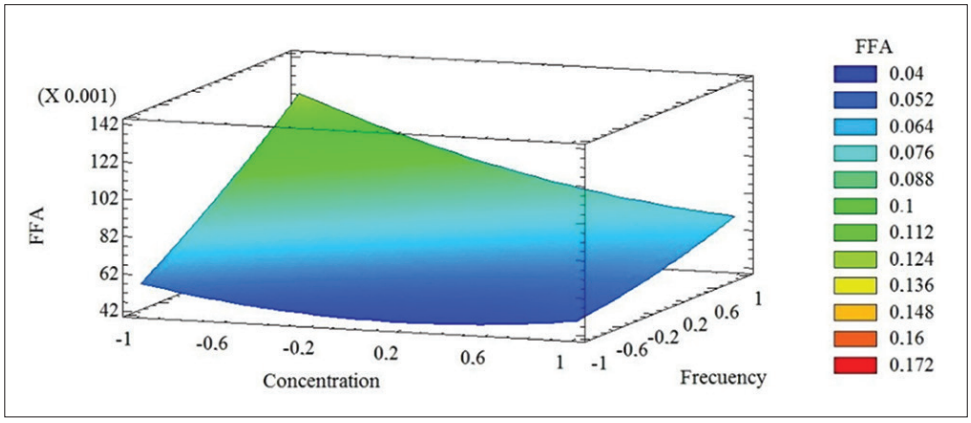

Fig 1. Surface graph showing polyphenol concentration versus frequency of oil usage and the combined effect over free fatty acids (FFA).

fatty acids content in frying oil increase with the number of fryings (Chung et al., 2004). Nonetheless, the level of free fatty acids in oils depends of other reasons as time, temperature and moisture content because the oils usually are exposed to various environments such as storage and processing (Mahesar et al., 2014). Regarding the relation with polyphenols,the reduction of fatty acids in frying oils are explained by the presence of polyphenols in the samples. Phenolic compounds slow down the oxidation of oil compounds by hydrolysis. However, some studies have reported that this fact happen at room temperature and phenolic compounds become less effective at frying temperature due to volatilization or decomposition (Choe et al., 2007; Choe et al., 1998). Factors as frequency of use (A), Polyphenol concentration (B), interaction of them (AB) and the square effect of concentration (B2) shows significant effect on FFA (Table 2).

Effect of factors on peroxide index

Peroxide index (PI) is an indicator of primary oxidation in oils and allows evaluating the quality control. The test detects the concentration of peroxides, which consists primarily of unstable hydroperoxides, which readily degrade generating products of secondary oxidations such as ketones and aldehydes, resulting in development of odd flavors. Higher PI values reflect lower oxidative stability, with detrimental effect on the oil quality (Pizarro et al., 2013). According to Table 1, PI value exceeded the permissible limit of 10 meq O2/Kg when the polyphenol concentration in the oil was 0% and was used 20 times (Table 1). Those values are established by the norms CODEX STAN 210-1999, (FAO, 2015). Furthermore, as shown in Fig. 2, there is a positive relationship between frequency and PI value. The increment in frequency of use of oil, increase the PI value. However, the addition of polyphenols, decrease PI value. Antioxidant prevent hydroperoxide and aldehyde formation in oils (Wills, 1980) and the protection increase using high concentration of polyphenols (Fig. 2). Factors as frequency of use (A), Polyphenol concentration (B), interaction of them (AB) and the square effect of frecuency (A2) shows significant effect on PI (Table 2).

Effect of factors on oil clarity

Changes on oil color intensity is affected by many factors such as products from thermo-oxidative deterioration and nonenzymatic (Blumenthal, 1991), pigments and Maillard reaction products, (Gutierrez et al., 1988; Lalas et al., 2006; Delgado-Andrade et al., 2010), particles of food left in the oil after food frying (Vijayan et al., 1996) and other aspects. Clarity response surface graph (Fig. 3) and the equation system (Table 2) shows a negative influence of the frequency and polyphenol concentration at the clarity parameter. The oil darkening is consequence of the presence of tocotrienols and phenolic that carry out the oligomerization and other chemical changes (Min et al., 1975). On the other hand, the heat cause a deteriorative effect of oxidation and polymerization of oil that affects the color (Tyagi et al., 1996). Frequency of use (A) and

concentration of polyphenols (B) on oil has demonstrate a significant effect (Table 2).

Effect of factors on DPPH antioxidant activity

Antioxidants naturally present or added to oils and foods can influence oil quality during frying (Choe E. and Min D., 2007). The Fig. 4 demonstrate that when the usage of oil increase the antioxidant activity (DPPH) decrease. Furthermore, the concentration of added polyphenol decrease such as the DPPH activity (Table 2). The presence of antioxidants improves the stability of frying oil and prevents the oxidation of fatty acids (Liu and White, 1992; Chung J., 2004). Antioxidants present phenolic structure or the phenolic configuration within their molecular structure giving them the function to inhibiting or interrupting the free-radical mechanism of glyceride autoxidation

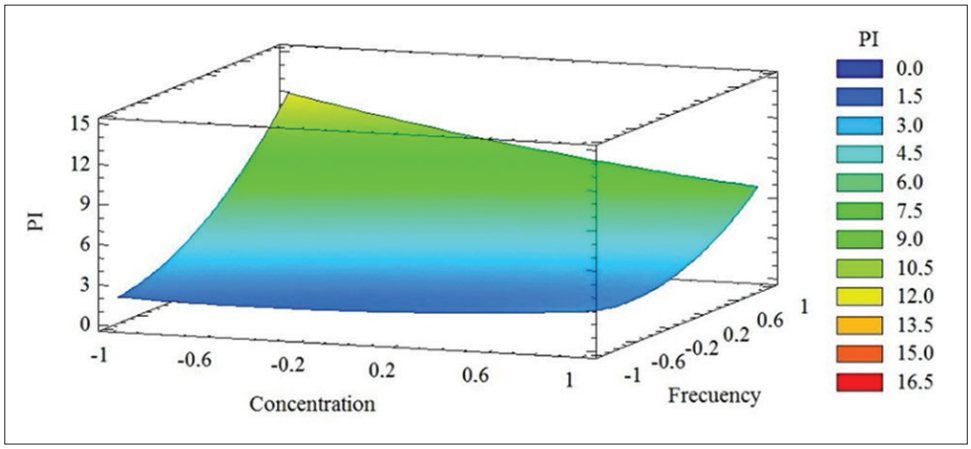

Fig 2. Surface graph showing polyphenol concentration versus frequency of oil usage and the combined effect over peroxide index (PI).

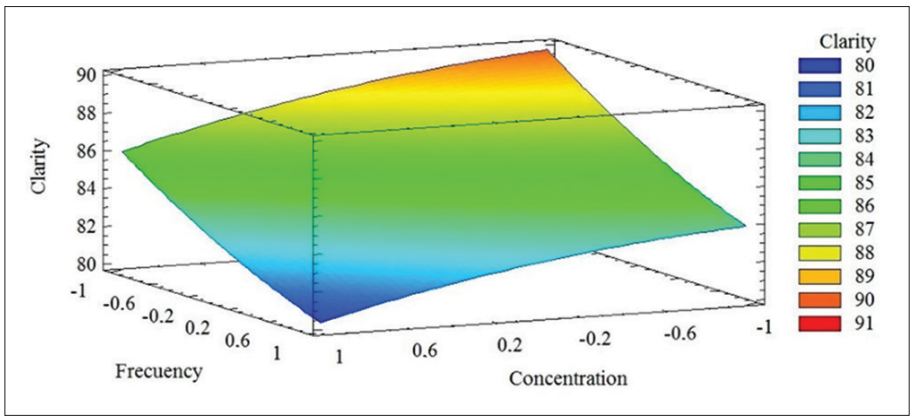

Fig 3. Surface graph showing polyphenol concentration versus frequency of oil usage and the combined effect over coloration.

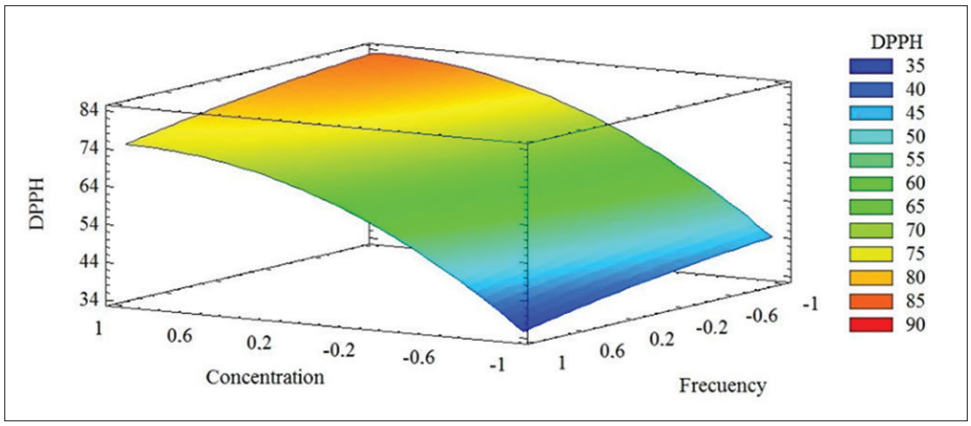

Fig 4. Surface graph showing polyphenol concentration versus frequency of oil usage and the combined effect over DPPH antioxidant activity.

Table 3: Optimization values

Variable	Target	Allowed limit	Conditions
FFA (%)	0.1	0.2	0.01% polyphenol
PI (meq)	9.99	10	20 times use of oil

(O'Brien, 2003). Factors that show significant effect on the antioxidant activity are: Frequency of use (A), Polyphenol concentration (B), the square effect of concentration (B2) and frequency (A2) shows significant effect on PI (Table 2). A, B, A2, B2.

Multiple response optimization

The desirability function approach (Derringer and Suich, 1980) was used to simultaneously optimize the multiple responses. The individual desirability values of FFA and PI responses were used to obtain a composite desirability value. This value has a range from zero to one in which the last represents the ideal case. Based on composite desirability value of 0.97, the optimum levels of frequency and polyphenol concentration are 20 times of use of oil and 0.01% of concentration. Those parameters will not exceed the maximum permissible values established in the norm CODEX STAN 210-1999, Rev. 2015 for PI (< 10 meq O2/Kg) and FFA (< 0.2%), as shown in Table 3.

CONCLUSIONS

Addition of cacao-shell derived polyphenols improved the stability of the oils, with a clearer effect in samples used 20 times. Higher concentrations of polyphenols used as anti-oxidant in frying oils retard the generation of FFA and PI, and increase the DPPH antioxidant activity. Addition of polyphenols did not affect the coloration of the oil samples.

ACKNOWLEDGMENTS

The authors thank the Escuela Superior Politécnica del Litoral (ESPOL) for its financial support to the performance of this study, and Dr. Maria C. Villacres, PROMETEO-SENESCYT, for reviewing the manuscript.

Author's contributions

P.M., M. Q. and A.B. were responsible of the conception and design of the experiment. J.H., I.C. and R.V. were in charge of the acquisition of data. P.M. and M.Q. analyzed, interpreted the data and prepared the manuscript. P. M. and O.V. did the critical revision.

REFERENCES

Abdulkarim, S. M., K. Long, O. M. Lai, S. K. S. Muhammad and H. M. Ghazali. 2007. Frying quality and stability of high-oleic *Moringa oleifera* seed oil in comparison with other vegetable oils. Food Chem. 93: 253-263.

AOAC. 1995. Official Methods of Analysis, 16th ed. Association of Official Analytical Chemists, Arlington, VA.

Arlorio, M., J. D. Coïsson, F. Travaglia, F. Varsaldi, G. Miglio, G. Lombardi and A. Martelli. 2005. Antioxidant and biological activity of phenolic pigments from *Theobroma cacao* hulls extracted with supercritical CO2. Can. Inst. Food Sci. Technol. J. 38: 1009-1014.

Blumenthal, M. M. 1991. A new look at the chemistry and physics of deep-fat frying. Food Technol. 45: 68-94.

Boskou, D., M. Tsimidou and G. Blekas. 2006. Polar phenolic compounds. Chemistry and Technology, AOCS Press, Champaign, p73-92.

Carocho, M., P. Morales and I. Ferreira. 2015. Natural food additives: Quo vadis. Trends. Food Sci. Technol. 45: 284-295.

Choe, E. and D. B. Min. 2007. Chemistry of deep-fat frying oils. J. Food Sci. 72: 77-86.

Choe, E. and J. Lee. 1998. Thermo oxidative stability of soybean oil, beef tallow, and palm oil during frying of steamed noodles. Korean J. Food Sci. Technol. 30: 288-292.

Chung, J., J. Lee and E. Choe. 2004. Oxidative stability of soybean and sesame oil mixture during frying of flour dough. J. Food Sci. 69: 574-578.

Delgado-Andrade, C., I. Seiquer, A. Haro, R. Castellano and M. P. Navarro. 2010. Development of the maillard reaction in foods cooked by different techniques. intake of maillard-derived compounds. Food Chem. 122: 145-153.

Derringer, G. and R. Suich. 1980. Simultaneous optimization of several response variables. J. Qual Technol. 12: 214-219.

FAO. 2015. Codex Alimentarius: Standard for Named Vegetable Oils. Codex Standard 210-1999, Rev 2009, Food and Agriculture Organization of United Nations.

Gutierrez, R., F. Gonzales-Quijano and M. C. Dobarganes. 1988. Analytical procedures for the evaluation of used frying fats. In: Varela, G., A. E. Bender and I. D. Morton (Eds.), Frying of Food: Principles, Changes, New Approaches, Ellis Horwood Ltd., Chicester, UK, pp. 141-154.

Karazhiyan, H., S. M. Razavi and G. O. Phillips. 2011. Extraction optimization of a hydrocolloid extract from cress seed (*Lepidium sativum*) using response surface methodology. Food Hydrocoll. 25: 915-920.

Lachman, J., D. Pronek, A. Hejtmankova, J. Dudjak, V. Pivec and K. Faitová. 2003. Total polyphenol and main flavonoid antioxidants in different onion (*Allium cepa* L.) varieties. Sci. Hortic. 30(4): 142-147.

Lalas, S., O. Gortzi and J. Tsaknis. 2006. Frying stability of moringa stenopetala seed oil. Plant Foods Hum. Nutr. 61: 99-108.

Liu, H. and P. White. 1992. High temperature stability of soybean oils with altered fatty acid compositions. J Am Oil Chem Soc. 69: 533-537.

Mahesar, S. A., S. T. H. Sherazi, A. R. Khaskheli and A. A. Kandhro. 2014. Analytical approaches for the assessment of free fatty acids in oils and fats. Anal. Methods. 6: 4956-4963.

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

Min, D. B., S. S. Lins and S. S. Chang. 1975. Study of color stability of tall oil fatty acids through isolation and characterization of minor constituents. J. Am. Oil Chem. Soc. 52: 28-33.

Minioti, K. and C. Georgiou. 2010. Comparison of different tests

used in mapping the greek virgin olive oil production for the determination of its total antioxidant activity. Grasas Aceites. 61: 45.

Misnawi, W. T., N. D. E. Susijahadi and A. Noor. 2014. The utilization of cocoa polyphenol extract to improve the shelf life of bulk frying oil used in open and deep frying. Int. Food Res. J. l: 111-118.

Montgomery, D. C., E. A. Peck and G. G. Vining. 2003. Introduction to Linear Regression Analysis, 3th ed. Wiley, New York.

Murillo, I. 2008. Evaluación de 2 Dietas Experimentales con Diferentes Niveles de Cascarilla de Cacao (*Theobroma cacao* L.) en las Fases de Crecimiento y Acabado de Cuyes (*Cavia porcellus* L.) de Raza Andina. B.Sc. Dissertation, Escuela Superior Politécnica del Litoral, Guayaquil.

Nsor-Atindana, J., F. Zhong, K. J. Mothibe, M. L. Bangoura and C. Lagnika. 2012. Quantification of total poly phenolic content and antimicrobial activity of cocoa (*Theobroma cacao* L.) Bean shells. PJN. 11: 574-579.

O'Brien, R. D., editor. 2003. Fats and oils formulation. 2th ed. Fats and oils: Formulating and Processing for Applications, CRC Press, Taylor and Francis Group, Florida, USA, pp. 263-339.

Oomah, O., A. Cardador-Martínez and G. Loarca-Piña. 2005. Phenolics and antioxidative activities in common beans (*Phaseolus vulgaris* L). J. Sci. Food Agric. 85: 935-942.

Pizarro, C., I. Esteban-Díez, S. Rodríguez and J. González. 2013. Determination of the peroxide value in extra virgin olive oils through the application of the stepwise orthogonalisation of predictors to mid-infrared spectra. Food Control. 34: 158-167.

PROECUADOR. 2013. Análisis del sector cacao y elaborados. Technical Bulletin. Instituto de Promoción de Exportaciones e Inversiones. Quito. Ecuador.

Quiles, J., M. Ramírez, J. Gómez, J. Huertas and J. Mataix. 2002. Role of vitamin E and Phenolic compounds in the antioxidant capacity, measured by ESR, of virgin olive, olive and sunflower oils after frying. Food Chem. 76: 461-468.

Tapia, C. 2015. Aprovechamiento de residuos agroindustriales, cascarilla de cacao (*Theobroma cacao* L.) variedad arriba y CCN51 para la elaboración de una infusión. B.Sc. Dissertation, Universidad Técnica de Ambato, Ambato.

Tyagi, V. K. and A. K. Vasishtha. 1996. Changes in the characteristics and composition of oils during deep-fat frying. J. Am. Oil Chem. Soc. 73: 499-506.

Vijayan, J., D. C. Slaughter and R. P. Singh. 1996. Optical properties of corn oil during frying. Int. J. Food Sci. Technol. 31: 353-358.

Wills, E. D. 1980. Effects of antioxidants on lipid peroxide formation in irradiated synthetic diets. Int. J. Radiat. Biol. Related Stud. Phys. Chem. Med. 37: 403-414.

Yapo, B. M., V. Besson, B. Koubala and K. L. Koffi. 2013. Adding value to cacao pod

husks as a potential antioxidant dietary fiber source. Am. J. Food Nutr. 1: 38-46.

Zamora, J. D. 2007. Antioxidantes: Micronutrientes en la lucha por la Salud. Rev. Chil. Nutr. 34(1): 17-26.