Emirates Journal of Food and Agriculture. 2016. 28(10): 741-745
doi: 10.9755/ejfa.2016-02-187
http://www.ejfa.me/

SHORT COMMUNICATION

Physicochemical characterization of *Theobroma cacao* L. sweatings in Ecuadorian coast

Carlos Balladares[1]*, Ivan Chóez-Guaranda[2], Jairo García[3], Daynet Sosa[2,3], Sebastián Pérez[4], Juan Emilio González[4], Rafael Viteri[2], Ana Barragán[2], María Quijano-Avilés[2], Patricia Manzano[2,3]

[1]Escuela Superior Politécnica del Litoral, ESPOL, Facultad de Ciencias Naturales y Matemáticas, Campus Gustavo Galindo Km 30.5 Vía Perimetral, Guayaquil, Ecuador. [2]Escuela Superior Politécnica del Litoral, ESPOL, Centro de Investigaciones Biotecnológicas del Ecuador, Campus Gustavo Galindo Km 30.5 Vía Perimetral, Guayaquil, Ecuador. [3]Escuela Superior Politécnica del Litoral, ESPOL, Facultad Ciencias de la Vida , Campus Gustavo Galindo Km 30.5 Vía Perimetral, Guayaquil, Ecuador. [4]Universidad de Las Palmas de Gran Canaria, Departamento de Ingeniería de procesos, Juan de Quesada, 3035001, Las Palmas de Gran Canaria, España

ABSTRACT

In 2014, Ecuador generated 141,000 MT of cocoa sweatings equivalent to 12,320 MT of reducing sugars that through fermentation can become Bioetanol. Cocoa sweatings was characterized to determinate its potential as biofuel. Methodologies described by AOAC 2005 standards were used. Values of 10.6 Brix; pH 3.58; density 1.10 g/ml, total sugars 12,33% reducing sugars 6.39% were observed. Chemical groups such as alkaloids, reducing sugars, and coumarins triterpenoid were identified by phytochemical screeninng. 20 chemical compounds were detected using by gas chromatography mass spectrometry (GC-MS): 3 carboxilic acids, 4 sugar acids, 3 sugar alcohols, 2 amino acids, 1 furan, 2 lactones, 1 monosaccharides, 2 disaccharides and 2 glycosides. They represent the 11.08% of total compounds separated, and Sucrose (2.15%), glucose (2.13%) and fructose (4.42%) were identified by high performance liquid chromatography (HPLC). The study showed that for each kilogram of dry cocoa produced 0.59 kg of mucilage are obtained and its sugars are an interesting source of raw material for the production of second generation bioethanol, results that contribute to reducing the environmental impact that generate these waste.

Keywords: Cocoa; Mucilage; Leachate; *Theobroma cacao*; Bioethanol

INTRODUCTION

Cocoa (*Theobroma cacao* L.) is an important cash crop around the world. There are among 5 to 6 million farmers in developing countries that produce about 90% of the cocoa production and 40 to 50 million of people depend upon cocoa for their livelihoods (Kongor et al., 2016). Ecuador is the eighth most important exporter of cocoa, a crop that is principally used for the elaboration of chocolate (Pérez -Neira, 2016). Large amounts of residue are generated during cocoa processing. Cocoa pod husk, cocoa bean shells and cocoa mucilage are the three main byproducts generated (Martínez et al., 2012). Cocoa mucilage is liquefied and removed from the bean and a large quantity of a liquid known as sweatings drain off from the bean during the fermentation step. Cocoa sweatings is the breakdown product of the mucilage and represent from 5 to 7% of fresh cocoa weight (Adams et al., 1982; Buamah et al., 1997; Nigam and Singh, 2014). Cocoa sweatings have been used as raw material for the preparation of food product such as soft drinks, wine, jams, marmalade and vinegar (Anvoh, Bi and Gnakri, 2009; Buamah et al., 1997; Oddoye et al., 2013). Nevertheless, there is still a limited information about the chemical characterization of cocoa sweating. Characterization of the physico-chemical components of cocoa sweating may add value to this byproduct and improve the utilization in other fields. This research arises to identify the main constituents of cocoa sweating by chromatographic methods (GC and LC) so novel uses will be promoted based on its characteristics as well as mitigate environmental impact.

MATERIALS AND METHODS

This research was conducted at Bioproducts and Bioprocesses Laboratory at Centro de Investigaciones Biotecnológicas del Ecuador (CIBE), in the Escuela Superior Politécnica del Litoral (ESPOL).

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

Received: 22 February 2016; **Revised:** 17 August 2016; **Accepted:** 31 August 2016; **Published Online:** 31 August 2016

Raw material

Cocoa pods were collected during morning randomly in a farm of Milagro city, Guayas province, during farmer's routine tasks. The samples have no apparent presence of pathogens such as *Moniliophthora perniciosa, M. roreri or Phytophthora palmivora*, which are major diseases in cocoa. The harvest were stored in polypropylene bags with an average temperature of 26° C. Under these conditions the samples were transferred to the laboratory.

Sample preparation

Cocoa pods were opened in a cross-section; the seeds were extracted and placed in a polypropylene bag for sweatings, simulating the conditions of storage and fermentation reported by farmers. The operation was done at room temperature and sweatings were collected in a glass container for 3 days and stored at 4° C.

Then, sweatings were filtered, frozen at -80° C and lyophilized (LABCONCO) 10^{-3} mbar x 150 to -46° C. The product obtained was pulverized in a mortar and placed in amber glass bottles of 10 mL in a desiccator for further analysis.

The analysis performed in this study correspond to a period from June to October 2015, the presence of chemical compounds and groups were evaluated. In addition, parameters such as pH, conductivity, salinity, resistivity, total solids, dissolved solids, suspended solids, density, °Brix and surface tension were done.

Physico-chemical analysis

The pH was measured with pH meter (Oakton 510), electrical conductivity (EC), salinity and resistivity were measured by electrometric methods (Mettler Toledo). Determination of total solids, dissolved solids, suspended solids and density were analyzed by the gravimetric method (AOAC, 2005). Brix were measured with a refractometer (Krüss, Germany). The equilibrium surface tension (γ st) was measured at 25° C with a manual Krüss tensiometer K6 (Krüss Optronic Hand, Hamburg, Germany) using the ring method.

The qualitative identification (phytochemical screening) of secondary metabolites in aqueous and alcoholic extracts obtained from lyophilized samples of sweatings was performed according to methodology described by (Miranda and Cuellar, 2000).

Sugars content was assessed by high performance liquid chromatography (HPLC) in a WATERS equipment using a diode array detector (DAD) and a reverse phase analytical column (Symmetry 300TM C18.5 microns - 3.9 x 150 mm).

The cocoa sweatings was derivatized with the reagent Bis (trimethylsilyl) -trifluoroacetamide Sigma-Aldrich brand mixing 2 mg of lyophilized sample with 200 uL of derivatizing agent. The procedure used for this process was adapted from the method described by (Yougen et al, 2013). After the derivatization Gas Chromatography Mass Spectrometry (GC-MS) analysis were performed in an Agilent Technologies (7890A GC system and 5975C inert XL MSD with triple axis detector). A capillary column HP-5MS (30 m × 0.25 mm) with phenyl methylpolysiloxane was used as stationary phase (0.25 micron film thickness) and helium as the carrier gas (1.5 mL/min). The injection of the sample was done at a temperature of 250°C with split mode (3:1), the interface temperature was 280°C, the detector temperature was 250°C, the oven temperature was set at 80°C for 1 minutes and then was increased to 300°C at 7°C/min. The electron ionization to 70 eV and 230°C was used as ion source and the data compounds were collect with the full scan mode (40-1000 uma) in the quadrupole mass analyzer. Finally, compounds were identified by comparison of their mass spectra and mass reference of Wiley 9th with NIST 2011 MS Library.

RESULTS AND DISCUSSION

The physical analysis of cocoa sweatings are shown in the Table 1. Studies about juice from mucilage refer 3.75 and 16.17 as pH and Brix value respectively indicating that the sweating got more grams of sucrose in 100 grams of solutions and its 0.17 less acid. The importance of the pH value found is that its concentration affects cell growth and production of secondary metabolites. The same studies show up that pH values between 3.5 and 5.0 are optimal for further development of bread yeast (Buzas et al., 1989). Given these characteristics, the cocoa sweatings have a pH favorable for the development of those microorganisms capable of splitting sugars and converting them into Bioethanol.

The results of phytochemical screening conducted to cocoa sweatings are presented in Table 2. These qualitative

Table 1: Physical characteristic of *Theobroma Cacao L.* sweattings

Parameters (Unit)	Means values (n=3) ± SD*
Density (g/ml)	1.1±0.01
°Brix	19.6±0.57
pH	3.58±0.07
Total suspends solids (mg/l)	192154
Conductivity (mS/cm)	3.29±0.06
Salinity (ppt)	3.04E+2 ± 0.02
Superficial tension (N)	52.65±1.91

*SD: Standard deviation

findings show the presence of major chemical groups evidenced with potential for conversion into other chemical species such as reducing sugars. Besides, it expose the presence of alkaloids, aminoacids, triterpenes and/or steroids. These studies are reported for the first time for this kind of waste.

The reducing sugar content was 6.39% and total sugars content was 12.33%, which the largest presence of fructose, sucrose and glucose, 4.42%, 2.15% 2.13% respectively. Cocoa sweating was partially similar to juice from mucilage of cocoa reported by (Anvoh et al., 2009) (21.4% glucose, 2.13% sucrose), Gyedu and Oppong, 2003 (total sugars 7.5%, 2.3% glucose, fructose 1.06%) and (Adams et al., 1982) (total sugars 7.38%).

The chromatogram presented in Fig. 1 shows the chromatographic profile of *Theobroma cacao L.* sweating.

Table 3 lists the components identified in cocoa sweating. 20 chemical compounds were detected using the previously describes techniques: 3 carboxylic acids, 4 sugar acids, 3 sugar alcohols, 2 amino acids, 1 furan, 2 lactones, 1 monosaccharides, 2 disaccharides and 2 glycoside. They represent the 11.08% of total compounds separated.

Table 2: Chemical groups present in sweatings of *Theobroma Cacao L.*

Chemical groups	Sweatings	Assay
Reducing sugars	+	Fehling
Triterpenes and/or steroids	+	Liebermann-Buchard
Alkaloids	++	Dragendorff
Aminoacids	+	Ninhidrina

(+): Presence of metabolite

Table 3: Chemical composition of *Theobroma cacao L.* sweating

Peak	Retention time (minutes)	Compound	Peak area (%)
1	9.49	2-Butenedioic acid	0.02
2	11.95	Butanedioic acid	0.49
3	12.29	Erythritol	0.02
4	12.49	L-Aspartic acid	0.11
5	12.87	L-Threonic acid	0.01
6	13.16	Tetronic acid	0.01
7	14.18	Glutamic acid	0.14
8	14.25	D-Arabinonic acid gamma lactone	0.01
9	14.44	L- (+)- Tartaric acid	0.13
10	15.62	Beta-D (-) Lyxopyranose	0.01
11	15.75	Xylitol	0.05
12	17.27	Rubrolide C	9.71
13	19.08	L- Ascorbic acid	0.12
14	20.023	D- Gluconic acid	0.08
15	20.10	Galacturonic acid	0.01
16	20.19	Galactofuranoside	0.04
17	21.02	Inositol	0.02
18	29.65	Maltose	0.02
19	30.11	Beta-Gentiobiose	0.05
20	35.19	Alpha D-Glucopyranoside	0.03

The compounds identified in cocoa sweating belong to some chemical groups which have relevant biological activities. Thus, there are some applications in different areas that will explain below.

2-Butenedioic acid (Fumaric acid) is used as acidifying and natural preservative in the pharmaceutical industry (Gold et al., 2012). Fumaric acid is used in the manufacture of polyester resins and polyhydric alcohols as a mordant for dyes and (Yot et al., 2016).

Other important compound to mention is succinic acid (Butanedioic Acid), for their usefulness in the chemical, cosmetic, food as a sequestrant, buffer, and a neutralizing agent (Hawley, 1993) as gastroprotector, antioxidant (Espinoza, 2007). This metabolite has not been identified in cocoa sweating, there are only references to his presence and it is related to the quality of chocolate flavor during fermentation of cocoa beans (aqueous extracts) (Fahrurrozi et al., 2015; Lucas et al, 2010).

Erythritol is a sugar alcohol (polyol) that has been approved for use as a food additive in the United States. It occurs naturally in some foods and fermented fruit (Shindou et al., 1988). In the industrial field, it is produced from glucose by fermentation with yeast, Moniliella pollinis.

This compound is sweeter than sucrose 60-70% (table sugar) but almost with no calories (Vasudevan, 2013), it do not affect blood sugar and not cause tooth decay (Noda et al., 1994; Kawanabe et al., 1992) and is partially absorbed by the body, is excreted in urine and feces.

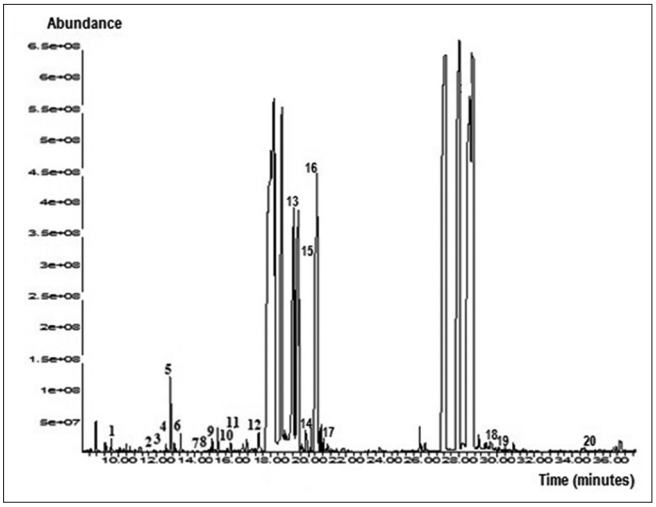

Fig 1. Analytical gas chromatogram of Theobroma cacao L. sweating.

Tetronic acid wich activity comprises of antibiotic and antiviral, cytotoxicity, mycotoxicity, as well as inhibition of the cell cycle (Athanasellis et al., 2010). Glutamic acid an aminoacid that had been identified on cocoa fermentation with a concentration of 7.4 (0 hrs) and 11.7 (72 hrs) (De Brito et al., 2001). D-(+)-Glucuronic acid γ-lactone wich has been on the synthesis of optically active glucopyranoses (Sato et al., 2008) and long-chain alkyl glucofuranosides (Raaijmakers et al., 1994). Xilitol is a sugar that is non-fermentable to alcohol and present interesting properties such as antidiabetic, antioxidant and anticarcinogenicity (Venkateswar et al., 2016). It is widely used as an artificial sweetener in food and pharmaceutical industry. As consumers prefer no calorie sweetener, xilitol demand is increasing. The main raw materials used to produce xylitol are corncob, soybean stalk, sugarcane bagasse and light woods (Dhar et al., 2016). Rubrolide C is rarely found in natural sources (Tale et al., 2012) and reports biological activity against *S. aureus*, *B. subtiliis B. megaterium*, *Escherichia coli*, *Clostridium perfringens* and *Micrococcus tetragenus* (Sikorska et al., 2012; Zhai et al., 2016).

Furthermore could identify the presence of L-ascorbic acid (vitamin C), D- gluconic acid, a compound which has been reported by (Abdul et al., 2014) from the cocoa pod, a monosaccharide Galacturonic Acid which can be polymerized used in the pharmaceutical, cosmetic and food industries as pectin (Adi-dako et al., 2016). Galacturonic Acid first identified in cocoa and could be a source for ethanol production.

Inositol (0.02%) is a natural sugar alcohol found in cell membrane of phospholipids, plama lipoproteins and in the nucleus. This compound has been employed in growth inhibition assays of human tumor cell lines (De Lima et al., 2015), as a protective agent in fishes with copper exposure (Jiang et al., 2014), as indicator during cellular apoptosis (Agarwal et al., 2010). Other compound: Maltose (0.02%) is a disaccharide that it has too many applications. It has been used as carbohydrate source in growth performance studies of gilthead sea bream (*Sparus aurata*) (Enes et al., 2010) and as stationary phase in a column of hydrophilic interaction liquid chromatography for carbohydrate separation (Fu et al., 2010). Even is a promising osmotic agent in peritoneal dialysis solution (Shu et al., 2010). The beta-Gentiobiose (0.05%) is the other disaccharide of the study. It has been used in the characterization of the glycosidic linkage of underivatized disaccharides by interaction with Pb (2+) ions through mass spectrometry (Firdoussi et al., 2007), as adjuvant of archaeal synthetic glycolipid mimetics (Sprott et al., 2008) and the extraction of crocin from saffron (*Crocus sativus*) using molecularly imprinted polymer solid-phase extraction (Mohajeri, et al., 2010).

CONCLUSIONS

The results obtained in this study allowed to establish the physical and chemical characteristics not reported in the literature for cocoa sweating. This raw material isn't used in the process of natural fermentation and the amount of ºBrix (19.6), pH (3.58) can be turn them in fermentative alcohol. Besides, the wide variety of compounds found, constitute an interesting field for future develop specific investigations. Thus, this material can be used as a feedstock for the production of bioethanol and other products for medicinal use.

AKNOWLEDGEMENTS

The authors thank the Biotechnology Research Center of Ecuador, Escuela Superior Politécnica del Litoral (ESPOL) for its financial support to the performance of this study.

Author's contributions

PM designed and conducted the work; CB and JG made the experimental work; ICG, RV, AB and MQA wrote the paper, PM, DS, SP and JEG made a major contribution to the review paper.

REFERENCES

Abdul, K. A., A. Azlan, A. Ismail, P. Hashim, S. S. A. Gani, B. H. Zainudin and N. A. Abdullah. 2014. Phenolic composition, antioxidant, anti-wrinkles and tyrosinase inhibitory activities of cocoa pod extract. BMC Complement. Altern. Med. 14: 381.

Adams, M. R., J. Dougan, E. Glossop and D. Twiddy. 1982. Cocoa sweatings - An effluent of potential value. Agric. Wastes. 4: 225-229.

Adi-Dako, O., K. Ofori-Kwakye, S. Frimpong Manso, M. E. Boakye-Gyasi, C. Sasu and M. Pobee. 2016. Physicochemical and antimicrobial properties of cocoa pod husk pectin intended as a versatile pharmaceutical excipient and nutraceutical. J. Pharm. 2016: 1-12.

Agarwal, R., S. Hassen and N. Ali. 2010. Changes in cellular levels of inositol polyphosphates during apoptosis. Mol. Cell Biochem. 345: 61-68.

Anvoh, K., A. Z. Bi and D. Gnakri. 2009. Production and characterization of juice from mucilage of cocoa beans and its transformation into marmalade. Pak. J. Nutr. 8: 129-133.

Athanasellis, G., O. Igglessi-Markopoulou, J. Markopoulos, G. Athanasellis, O. Igglessi-Markopoulou and J. Markopoulos. 2010. Tetramic and tetronic acids as scaffolds in bioinorganic and bioorganic chemistry. Bioinorg. Chem. Appl. 2010: 315056.

Buamah, R., V. P. Dzogbefia and J. H. Oldham. 1997. Pure yeast culture fermentation of cocoa (*Theobroma cacao L.*): Effect on yield of sweatings and cocoa bean quality. World J. Microbiol. Biotechnol. 13: 457-462.

Buzas, Z., K. Dallman and B. Szajani. 1989. Influenc of ph on the growth and ethanol production of free and immobilized *Saccharomyces cerevisiae* cells. Biotechnol. Bioeng. 34: 882-884.

De Brito, E. S., N. H. P. García, M. I. Gallão, A. L. Cortelazzo, P.

S. Fevereiro and M. R. Braga. 2001. Structural and chemical changes in cocoa (*Theobroma cacao L.*) during fermentation, drying and roasting. J. Sci. Food Agric. 81: 281-288.

De Lima, E. M., C. C. Kanunfre, L. F. De Andrade, D. Granato and N. D. Rosso. 2015. Cytotoxic effect of inositol hexaphosphate and its Ni(II) complex on human acute leukemia Jurkat T cells. Toxicol. In Vitro. 29: 2081-2088.

Dhar, K. S., V. F. Wendisch and K. M. Nampoothiri. 2016. Engineering of Corynebacterium glutamicum for xylitol production from lignocellulosic pentose sugars. J. Biotechnol. 230: 63-71.

Enes, P., H. Peres, A. Couto and A. Oliva-Teles. 2010. Growth performance and metabolic utilization of diets including starch, dextrin, maltose or glucose as carbohydrate source by gilthead sea bream (*Sparus aurata*) juveniles. Fish Physiol. Biochem. 36: 903-910.

Espinoza, M. M. 2007. Actividad gastroprotectora y citotoxicidad de derivados semisintéticos del diterpeno ferruginol. Memoria para optar al título de Licenciado en Tecnología Médica, Tutor: Dr. Schmeda-Hirschmann, Universidad de Talca, Chile.

Fahrurrozi, M. 2015. Microbiological and Biochemical Investigations of Cocoa Bean Fermentation. Dissertation Submitted to the Department of Chemistry Faculty of Mathematics, Informatics, and Natural Sciences, University of Hamburg for the Degree of Doctor of Natural Sciences by from Lombok, West Nusa Tenggara, Indonesia.

Firdoussi, A. E., M. Lafitte, J. Tortajada, O. Kone and J. Y. Salpin. 2007. Characterization of the glycosidic linkage of underivatized disaccharides by interaction with Pb(2+) ions. J. Mass Spectrom. 42: 999-1011.

Fu, Q., T. Liang, X. Zhang, Y. Du, Z. Guo and X. Liang. 2010. Carbohydrate separation by hydrophilic interaction liquid chromatography on a maltose column. Carbohydr. Res. 345: 2690-2697.

Gold, R., L. Kappos and D. L. Arnold. 2012. Placebo-controlled phase 3 study of oral BG-12 for relapsing multiple sclerosis. N. Engl. J. Med. 367: 1098-1107.

Gyedu, E. and H. Oppong. 2003. Cocoa pulp juice (Sweatings) and its potential for soft drink, jam and marmalade production. Paper Presented at International Workshop on Cocoa By-Products Ghana. Accra.

Hawley, G. and R. J. Lewis. 1993. Hawley's Condensed Chemical Dictionary. 12th ed. Van Nostrand Reinhold Company, New York.

Jiang, W. D., Y. Liu, K. Hu, J. Jiang, S. H. Li, L. Feng and X. Q. Zhou. 2014. Copper exposure induces oxidative injury, disturbs the antioxidant system and changes the Nrf2/ARE (CuZnSOD) signaling in the fish brain: Protective effects of myo-inositol. Aquat. Toxicol. 155: 301-313.

Kawanabe, J., M. Hirasawa, T. Takeuchi, T. Oda and T. Ikeda. 1992. Noncariogenicity of erythritol as a substrate. Caries Res. 26: 358-362.

Kongor, J., M. Hinneh, D. Van de Walle, E. Afoakwa, P. Boeckx and K. Dewettinck. 2016. Factors influencing quality variation in cocoa (*Theobroma cacao*) bean flavour profile. A review. Food Rev. Int. 82: 44-52.

Lucas, G., I. Martins, A. Figueiredo and J. Marsaioli. 2010. 1H NMR spectroscopy applied to the cocoa evaluation of *Theobroma cacao* clones. Ann. Magn. Reson. 9: 21-29.

Martínez, R., P. Torres, M. A. Meneses, J. G. 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 Rev. Int. 49: 39-45.

Mohajeri, S. A., H. Hosseinzadeh, F. Keyhanfar and J. Aghamohammadian. 2010. Extraction of crocin from saffron (*Crocus sativus*) using molecularly imprinted polymer solid-phase extraction. J. Sep. Sci. 33: 2302-2309.

Nigam, P. S. and A. Singh. 2014. Cocoa and coffee fermentations. In Encyclopedia of Food Microbiology, Elsevier, Lyngby, Denmark, pp. 485-492.

Noda, K., K. Nakayama and T. Oku. 1994. Serum glucose and insulin levels and erythritol balance after oral administration of erythritol in healthy subjects. Eur. J. Clin. Nutr. 48: 286-292.

Oddoye, E. O., C. K. Agyente-Badu and E. Gyedu-Akoto. 2013. Cocoa and its by-products: Identification and utilization. In: Chocolate in Health and Nutrition, Humana Press, Totowa, NJ, pp. 23-37.

Pérez Neira, D. 2016. Energy sustainability of Ecuadorian cacao export and its contribution to climate change. A case study through product life cycle assessment. J. Clean. Prod. 112: 2560-2568.

Raaijmakers, H. W., B. Zwanenburg and G. J. F. Chittenden. 1994. The synthesis and properties of some long-chain alkyl-D-glucofuranosidurono-6, 3-lactones, D-glucofuranosides and derivatives thereof. Recl. Trav. Chim. Pays-Bas. 113: 79-87.

Sato, K., S. Akai, H. Shoji, N. Sugita, S. Yoshida, Y. Nagai and J. Yoshimura. 2008. Stereoselective and efficient total synthesis of optically active tetrodotoxin from d-glucose. J. Organ. Chem. 73: 1234-1242.

Shindou, T., Y. Sasaki, H. Miki, T. Eguchi, K. Hagiwara and T. Ichikawa. 1988. Determination of erythritol in fermented foods by high performance liquid chromatography. Food Hyg. Saf. Sci. 29: 419-422.

Sikorska, J., S. Parker-Nance, M. T. Davies-Coleman, O. B. Vining, A. E. Sikora and K. L. McPhail. 2012. Antimicrobial rubrolides from a South African species of *Synoicum tunicate*. J. Nat. Prod. 75: 1824-1827.

Shu, Z. J., Y. M. Peng, L. Sun, L. Xiao, Y. Liu, J. Li and F. Liu, 2010. Maltose, a promising osmotic agent in peritoneal dialysis solution. Med. Hypotheses. 75: 645-647.

Sprott, G. D., C. J. Dicaire, J. P. Côté and D. M. Whitfield. 2008. Adjuvant potential of archaeal synthetic glycolipid mimetics critically depends on the glyco head group structure. Glycobiology. 18: 559-565.

Tale, N. P., A. V. Shelke, G. B. Tiwari, P. B. Thorat and N. N. Karade. 2012. New concise and efficient synthesis of rubrolides C and E via intramolecular wittig reaction. Helv. Chim. Acta. 95: 852-857.

Vasudevan, D. M. 2013. Textbook of Biochemistry for Medical Students. Jaypee Brothers Medical Publishers, New Delhi. p. 81.

Venkateswar, R. L., J. K. Goli, J. Gentela and S. Koti. 2016. Bioconversion of lignocellulosic biomass to xylitol: An overview. Bioresour. Technol. 213: 299-310.

Yot, P., L. Vanduyfhuys, E. Alvarez, J. Rodriguez, J. P. Itié, P. Fabry, N. Guillou, T. Devic, I. Beurroies, P. L. Llewellyn, V. Van Speyerbroeck, C. Serre and G. Maurin 2016. Mechanical energy storage performance of an aluminium fumarate metal-organic framework. Chem. Sci. 7: 446-450.

Yougen, W., L. Xianchao, Y. Dongmei and H. Xinwen. 2013. Isolation and identification of the water-soluble components of *Pogostemon cablin*. Chem. Eng. 3: 71-76.

Zhai, M. M., J. Li, C. X. Jiang, Y. P. Shi, D. L. Di, P. Crews and Q. X. Wu. 2016. The bioactive secondary metabolites from *Talaromyces* species. Nat. Prod. Bioprospect. 6: 1-24.