http://dx.doi.org/10.4067/S0717-75182021000400500

# Artículo Original / Original Article

## Proximal chemical evaluation, antioxidant content and fatty acids in fermented and dried cocoa beans, roasted cocoa beans and cocoa pulp bar (*Theobroma cacao* L. Criollo cultivar)

## Evaluación química proximal, contenido de antioxidantes y ácidos grasos en el grano de cacao fermentado y seco, grano de cacao tostado y barra de pulpa de cacao (*Theobroma cacao* L. Criollo cultivar)

*José Alberto Ariza Ortega[1*]. https://orcid.org/0000-0002-2163-4593*
*Ernesto Alanís García[1]. https://orcid.org/0000-0003-1540-4908*
*Carla Taryn Rodríguez Meléndez[1]. https://orcid.org/0000-0003-4529-3639*

*1. Área Académica de Nutrición, Centro de Investigación Interdisciplinario Instituto de Ciencias de la Salud, Universidad Autónoma del Estado de Hidalgo, San Agustín Tlaxiaca, Hidalgo, México.*

*Corresponding autor: José Alberto Ariza Ortega.
Área Académica de Nutrición, Centro de Investigación Interdisciplinario Instituto de Ciencias de la Salud, Universidad Autónoma del Estado de Hidalgo, Carretera Actopan-Tilcuautla, Ex-Hacienda la Concepción s/n, 42160 San Agustín Tlaxiaca, Hidalgo, México.
Email: jose190375@hotmail.com

Este trabajo fue recibido el 09 de noviembre de 2020.
Aceptado con modificaciones: 05 de abril de 2021.
Aceptado para ser publicado: 04 de mayo de 2021.

**ABSTRACT**

*The objective of this work was to evaluate the proximal chemical composition, antioxidant activity assays with total phenol, 2,2-Diphenyl-1-Picrylhydrazyl, 2'2-azino-bis(3-ethylbenzothiazolin-6-sulfonic acid) diammonium salt, iron reducing antioxidant power, and the fatty acid composition of fermented and dried cocoa beans, roasted cocoa beans and cocoa pulp bar. The fermented and dried cocoa beans were purchased, roasted (115 °C for 5 min) and homogenized. The mixture was brought to its melting point (58 °C for 5 min). In the cocoa pulp bar there was an increase in lipids (53.0%). The roasted affected the concentration of ashes (3.38–2.98%). Total phenol (1.8, 1.5 and 1.3 mg AEG $kg^{-1}$), 2,2-Diphenyl-1-Picrylhydrazyl (65.8, 64.4 and 65.0 µmol ET $kg^{-1}$), 2'2-azino-bis(3-ethylbenzothiazolin-6-sulfonic acid) diammonium salt (3.60, 3.00 and 2.30 µmol EAC $kg^{-1}$) and iron reducing antioxidant power (0.09, 0.07 and 0.06 mg $Fe^{2+}$ $kg^{-1}$) presented significant differences ($p<0.05$) in the fermented and dried cocoa beans, roasted cocoa beans and cocoa pulp bar respectively, however, the changes were minimal. The cocoa pulp bar had a higher concentration of unsaturated fatty acid (89.2%), where oleic (48.6%) was predominated. Therefore, the cocoa pulp bar is a food with an important antioxidant content , moreover, it has a higher concentration of unsaturated fatty acid, which is desirable in the diet.*

*Key words: Antioxidants; Cocoa pulp bar; Fatty acids; Roasted; Theobroma cacao L.*

**RESUMEN**

*El objetivo de este trabajo fue evaluar la composición química proximal, el contenido de antioxidantes con los ensayos de fenoles totales, 2,2-Difenil-1-Picrilhidrazil, Ácido 2'2-azino-bis (3-etilbenzotiazolin-6-sulfónico), sal de diamonio y poder antioxidante reductor del hierro, y la composición de ácidos grasos en los granos de cacao fermentados y secos, granos de cacao tostados y barra de pulpa de cacao. Se compraron los granos de cacao fermentado y seco y se tostaron (115 °C durante 5 min), y se homogeneizaron. La mezcla se llevó a su punto de fusión (58 °C durante 5 min). En la barra de pulpa de cacao hubo un aumento de lípidos (53,0%). El tostado afectó la concentración de cenizas (3,38 - 2,98%). En los resultados de fenoles totales (1,80, 1,50 y 1,30 mg AEC $kg^{-1}$), 2,2-Difenil-1-Picrilhidrazil (65,8, 64,4 y 65,0 µmol ET $kg^{-1}$), Ácido 2'2-azino-bis (3-etilbenzotiazolin-6-sulfónico), sal de diamonio (3,6, 3,0 y 2,3 µmol EAC $kg^{-1}$) y poder antioxidante reductor del hierro (0.09, 0.07 y 0.06 mg $Fe^{2+}$ $kg^{-1}$) presentaron diferencias significativas ($p<0.05$) en los granos de cacao fermentados y secos, granos de cacao tostados y barra de pulpa de cacao respectivamente, sin embargo, los cambios fueron mínimos. La barra de pulpa de cacao presentó una mayor concentración de ácidos grasos insaturados (89%), donde predominó el oleico (48,6%). Por lo tanto, la barra de pulpa de cacao es un alimento con importante contenido en antioxidantes, además, tiene una mayor concentración de ácidos grasos insaturados, lo que es deseable en la dieta.*

*Palabras clave: Ácidos grasos; Antioxidantes; Barra de pulpa de cacao; Theobroma cacao L.; Tostado.*

## INTRODUCTION

The cocoa tree pertains to the genus *Theobroma*, each of the species is differentiated by size, the shape of the plant and its leaves, the color of the fruit and its beans, as well as its nutritional composition[1].

Cocoa beans are mainly used for chocolate production, where they are exposed to several processes (fermentation, drying, roasting, alkalization and packaging) to obtain cocoa pulp. The product is pressed to get its derivatives such as: cocoa butter, cocoa liquor and cocoa powder (residue), which are used for the diversity of chocolates, and these are qualified according to their cocoa content[2,3].

Cocoa pulp contains 100% of its nutritional composition and consists of macromolecules, micronutrients and bioactive compounds, however, this composition depends on both extrinsic and intrinsic conditions[4,5]. The main components of cocoa-based products are lipids and antioxidants, the former include unsaturated fatty acids; in which oleic acid is dominant (37,5%), and has been shown to have a positive effect on the body, reducing total blood cholesterol and low-density lipoproteins, and increasing high-density lipoproteins[6,7,8]. In the latter, polyphenols (5,00-23,9 mg of polyphenols/g of chocolate), which neutralize reactive oxygen species, these chemical components are desirable in the diet, since their consumption could prevent several diseases such as hypercholesterolemia, cardiovascular diseases, cancer and could improve insulin sensitivity, among others. However, due to its bitter taste, the industry adds sugar, milk, and seeds, among other constituents in order to reduce the bitter taste and diversify the production of chocolates. These additives change the chemical composition of the cocoa pulp, which is why some products, such as dark chocolate, are added or enriched with flavanols, to enhance their beneficial effect in health. On the other hand, the addition of sugar increases the caloric value of chocolates and decreases the percentage of cocoa, so its consumption in excess can favor the increase prevalence of conditions such as metabolic syndrome[1,4,5,6,7,8]. Therefore, the objective of this work was to evaluate the proximal chemical composition in fermented and dried cocoa beans, roasted cocoa beans and cocoa pulp bar (*Theobroma cacao* L.), as well as their antioxidants content [total phenol, 2,2-Diphenyl-1-Picrylhydrazyl, 2'2-azino-bis(3-ethylbenzothiazolin-6-sulfonic acid) diammonium salt and iron reducing antioxidant power] and fatty acid composition, in order to determine their quality.

## MATERIALS AND METHODS

The work was a controlled experimental design, where 500 g of fermented and dried cocoa beans (FDCB) (*Theobroma cacao* L. Criollo cultivar), commercialized in the Mayordomo store, located in the City of Puebla, State of Puebla, Mexico, were acquired. The quarter method was used to select 250 g of FDCB with the characteristic shape, color and aroma, while beans with impurities, defective and damaged were eliminated. FDCB were washed and roasted at 115 °C for 5 min, then the epicarp was manually removed. The roasted cocoa beans (RCB) without epicarp were ground in a manual bean mill (Tisamatic brand, Mexico). The cocoa pulp bar (CPB) obtained was melted in a water bath at 58 °C for 5 min, placed in 1 g molds, after which they were refrigerated. Once the product solidified, it was demolded and the CPB was extracted and the bar was obtained. This preparation of the CPB is because in the future it will be administered to a population to determine its effect on anthropometric indicators, blood pressure, lipid profile and glucose.

The FDCB, RCB and CPB were homogenized

separately in a blender (Oster Best02-E01; Milwaukee, Wisconsin, United States) until a particle size of 1 µm was obtained. The three samples were analyzed for moisture (method 926,08), protein (method 955,04), lipid (method 945,16), ash (method 900.02) and water activity (method 32,004 - 32,009), according to the methods reported by the Official Association of Analytical Chemistry[9]. The amount of carbohydrates was obtained by difference. Each of the treatments was performed in triplicate.

Total phenol content was evaluated using the methodology of Stintzing, et al.[10]. Sample absorbance was measured at 765 nm in a microplate reader (Power Wave XS UV-Biotek, KC Junior software, USA) and gallic acid was used as a reference standard. Values were expressed in milligrams of gallic acid equivalents per liter for each of the homogenised samples (FDCB, RCB and CPB).

For the determination of antioxidant activity by 2'2-azino-bis(3-ethylbenzothiazolin-6-sulfonic acid) diammonium salt (ABTS) and 2,2-Diphenyl-1-Picrylhydrazyl (DPPH), the samples were diluted in each of the reagents and a product was obtained and read at an absorbance of 754 nm and 540 nm in a microplate reader (Power Wave XS UV-Biotek, KC Junior software, USA), according to the methodology of Kuskowski et al.[11] and Morales et al.[12] Results were expressed as equivalent micromoles of ascorbic acid of each of the homogenized samples (FDCB, RCB and CPB).

The iron reducing antioxidant power (FRAP) assay was performed according to the methodology of Benzie et al.[13] and results expressed in milligrams of $Fe^{+2}$ per kg of the homogenised samples (FDCB, RCB and CPB).

For extractic lipid, each one of the homogenised samples (1 g) was placed in an extraction thimble-holder. The thimble-holder was placed inside the Soxhlet extractor (method 945,16) and the process was run for 4 h[9]. The solvent-oil mixture was separated using a rotary vacuum evaporator at 50 °C at medium rotational speed (Büchi, Heating Bath B-490, Mexico) attached to a vacuum pump (Büchi, Vacuum Rump V-700, Mexico).

Fatty acid content was estimated as the total percentage of fatty acid methyl esters (FAMEs) according AOAC methodology (method 963,22)[9]. To determine the fatty acid content, a Gas Chromatograph (GC) (GC HP-7890, Agilent Technologies, Santa Clara, CA, United States) fitted with a 5973N SM was used. A column with the following specifications was used: DB-5 ms, 30-m long × 0,25-mm inner diameter × 0,25-µm film (5 % phenyl) methylpolysiloxane (GC J&W Agilent J&W Ultra Inert column, Agilent Technologies, Santa Clara, CA). Additionally, the injection volume was 2 µL (with a division radius of 20:2). The oven temperature was 100 °C (with 4-min retention) and was increased to 250 °C at a rate of 3 °C/min (with 10 min holding). The injection system and the detector were maintained at 230 °C and 250 °C, respectively. The carrier gas was He with a flow rate of 1.2 mL/min. Fatty acid comparison was performed with the standard of 37 fatty acid components (Food Industry FAMEs Mix, Restek Corporation, Bellefonte, PA, USA). Each analysis was performed in duplicate.

Results are expressed as mean plus standard deviation by applying one-factor analysis of variance (ANOVA) and Duncan's mean comparison test at significance level ($p<0.05$) using IBM SPSS Statistics for Windows, Version 22.0 statistical software (IBM Corp. Released 2013. Armonk, NY: IBM Corp).

## RESULTS

Table 1 shows the results of the proximate chemical composition and water activity performed on FDCB, RCB and CPB. Table 1 shows that the moisture percentage was higher in RCB compared to FDCB and CPB. On the other hand, the percentage of protein in FDCB was higher ($p<0.05$), compared to the RCB (115 °C for 5 min) and CPB (58 °C for 5 min) ($p>0.05$).

Lipids were the chemical compounds with the highest percentage. In the FDCB, a higher concentration of lipids was quantified, compared to the result of the RCB (115 °C for 5 min). In this research, the results of the ash percentages in FDCB and CPB (58 °C for 5 min) did not present significant differences ($p>0.05$), compared to the RCB (115 °C for 5 min) ($p<0.05$). The percentage of carbohydrates in FDCB was lower if compared to the RCB and CPB.

Table 2 shows that the RCB (115 °C for 5 min) and in the CPB (58 °C for 5 min), a lower concentration of TP was quantified, compared to the FDCB ($p<0.05$).

Results of DPPH and ABTS assays for determining antioxidant capacity in FDCB and RCB (115 °C for 5 min) and CPB (58 °C for 5 min) were statistically different ($p<0.05$).

Table 2 shows that the radical scavenging activity for the DPPH assay was higher compared to the ABTS assay. On the other hand, DPPH radical scavenging activity results, a slight increase was determined in the CPB (58 °C for 5 min) compared to the RCB (115 °C for 5 min). In the CPB, a lower radical scavenging activity was quantified by ABTS assay.

In the fatty acids (FA) analysis of the FDCB and RCB (115 °C for 5 min) and CPB (58 °C for 5 min), the results noted that their concentration changed with the application of the heat treatment (Table 3). The saturated FA that showed an increase in its concentration in the RCB (115 °C for 5 min) and in the CPB (58 °C for 5 min) compared to the FDCB were butyric, tridecanoic, myristic, myristic, margaric

and heneicosanoic; and butyric, lauric and margaric respectively. In the CPB, the concentration of myristic FA was similar to the percentage quantified in the FDCB. While the unsaturated FA that were quantified in the RCB in higher concentration were myristoleic, pentadecenoic, palmitoleic, heptadecenoic and paullanic; and in CPB, in addition to the FA quantified in the RCB, oleic FA was also found.

The saturated FA that decreased in concentration in RCB were caproic, caprylic, capric, capric, undecanoic, lauric, myristic, pentadecanoic, palmitic, stearic, arachidic and behenic, and in CPB, in addition to the above FA, tridecanoic and heneicosanoic were also found. Moreover, the unsaturated FA that decreased in concentration in the RCB were oleic, linoleic, γ-linolenic, linolenic, eicosadienoic and eicosatrienoic, and in the CPB in addition to the above FA in the RCB, there were also myristoleic and non-oleic FA.

**Table 1.** Results in percentage of the proximal chemical analysis of cocoa beans.

| Parameters | FDCB | RCB | CPB |
|---|---|---|---|
| Moisture | 2.81±0.35b | 3.60±0.95a | 2.77±0.15b |
| Protein | 14.0±0.09a | 13.5±0.05b | 13.0±0.04b |
| Lipids | 42.5±1.32b | 35.6±2.29c | 53.0±0.85a |
| Ash | 3.38±0.12a | 2.98±0.34b | 3.10±0.09a |
| Carbohydrates | 37.2±1.60b | 44.1±2.74a | 28.0±0.48c |
| Water activity | 0.02±0.00b | 0.03±0.00a | 0.02±0.00b |

The results are averages of three repetitions ± SD. Different lowercase letters per row indicate statistical difference ($p<0.05$) between treatments. FDCB: Fermented and dried cocoa beans, RCB: Roasted cocoa beans and CPB: Cocoa pulp bar.

**Table 2.** Results of TP, DPPH, ABTS and FRAP of the cocoa beans.

| Parameters | FDCB | RCB | CPB |
|---|---|---|---|
| TP (mg AEG $kg^{-1}$) | $1.80\pm0.50^{a}$ | $1.50\pm0.60^{b}$ | $1.30\pm0.10^{c}$ |
| DPPH (μmol ET $kg^{-1}$) | $65.8\pm3.60^{a}$ | $64.4\pm3.20^{c}$ | $65.0\pm3.00^{b}$ |
| ABTS (μmol EAC $kg^{-1}$) | $3.60\pm0.20^{a}$ | $3.00\pm0.10^{b}$ | $2.30\pm0.20^{c}$ |
| FRAP (mg $Fe^{2+}$ $kg^{-1}$) | $0.09\pm0.00^{a}$ | $0.07\pm0.00^{b}$ | $0.06\pm0.00^{c}$ |

The results are averages of three repetitions ± SD. Different lowercase letters per row indicate statistical difference ($p<0.05$) between treatments. Total phenol (TP), 2,2-Diphenyl-1-Picrylhydrazyl (DPPH), 2'2-azino-bis(3-ethylbenzothiazolin-6-sulfonic acid) diammonium salt (ABTS), iron reducing antioxidant power (FRAP), FDCB: Fermented and dried cocoa beans, RCB: Roasted cocoa beans and CPB: Cocoa pulp bar.

**Table 3.** Percentage results of fatty acids.

| Name | Formula | Retention time (min) | FDCB | RCB | CPB |
|---|---|---|---|---|---|
| Saturated fatty acids | | | | | |
| Butyric | C4:0 | 3.58 | 0.00±0.00 | 0.07±0.00$^{a}$ | 0.07±0.00$^{a}$ |
| Caproic | C6:0 | 7.49 | 0.32±0.00$^{a}$ | 0.03±0.00$^{b}$ | 0.03±0.00$^{b}$ |
| Caprylic | C8:0 | 12.5 | 0.30±0.00$^{a}$ | 0.03±0.00$^{b}$ | 0.03±0.00$^{b}$ |
| Capric | C10:0 | 20.0 | 0.28±0.01$^{a}$ | 0.02±0.00$^{b}$ | 0.03±0.00$^{b}$ |
| Undecanoic | C11:0 | 22.8 | 0.38±0.01$^{a}$ | 0.03±0.00$^{b}$ | 0.03±0.00$^{b}$ |
| Lauric | C12:0 | 25.5 | 0.41±0.01$^{b}$ | 0.03±0.00$^{c}$ | 0.69±0.02$^{a}$ |
| Tridecanoic | C13:0 | 29.7 | 0.50±0.00$^{b}$ | 2.14±0.10$^{a}$ | 0.04±0.00$^{b}$ |
| Myristic | C14:0 | 30.1 | 0.41±0.00$^{b}$ | 1.76±0.20$^{a}$ | 0.49±0.00$^{b}$ |
| Pentadecanoic | C15:0 | 33.8 | 9.36±0.90$^{a}$ | 7.59±0.40$^{b}$ | 1.00±0.00$^{b}$ |
| Palmitic | C16:0 | 34.4 | 1.48±0.20$^{a}$ | 0.74±0.01$^{b}$ | 0.14±0.00$^{b}$ |
| Margaric | C17:0 | 35.8 | 1.03±0.01$^{c}$ | 6.22±0.20$^{a}$ | 5.62±0.30$^{b}$ |
| Stearic | C18:0 | 36.1 | 38.9±0.40$^{a}$ | 2.08±0.01$^{b}$ | 1.75±0.20$^{b}$ |
| Arachidic | C20:0 | 37.4 | 1.22±0.01$^{a}$ | 0.62±0.00$^{b}$ | 0.49±0.00$^{b}$ |
| Heneicosanoic | C21:0 | 38.2 | 0.78±0.00$^{b}$ | 0.96±0.00$^{a}$ | 0.21±0.00$^{b}$ |
| Behenic | C22:0 | 40.2 | 1.00±0.00$^{a}$ | 0.14±0.00$^{b}$ | 0.09±0.00$^{b}$ |
| | | Total | 56.4 | 22.4 | 10.7 |
| Unsaturated fatty acids | | | | | |
| Myristoleic | C14:1 (cis-9) | 35.7 | 0.64±0.00$^{b}$ | 0.85±0.00$^{a}$ | 0.13±0.00$^{c}$ |
| Pentadecenoic | C15:1 (cis-10) | 40.9 | 5.97±0.20$^{c}$ | 47.1±0.10$^{a}$ | 27.3±0.30$^{b}$ |
| Palmitoleic | C16:1 (cis-9) | 41.4 | 0.58±0.00$^{c}$ | 8.66±0.30$^{a}$ | 1.38±0.00$^{b}$ |
| Heptadecenoic | C17:1 (cis-10) | 41.5 | 7.16±0.10$^{c}$ | 10.7±0.20$^{a}$ | 9.35±0.30$^{b}$ |
| Oleic | C18:1 (cis-9) | 43.0 | 22.1±0.01$^{b}$ | 0.00±0.00 | 48.6±0.20$^{a}$ |
| Linoleic | C18:2 (cis-9,12) | 45.5 | 1.23±0.01$^{a}$ | 0.09±0.00$^{c}$ | 0.11±0.00$^{b}$ |
| γ-linolenic | C18:3 (cis-6,9,12) | 45.5 | 0.80±0.00$^{a}$ | 0.66±0.00$^{b}$ | 0.15±0.00$^{c}$ |
| Paullanic | C20:1 (cis-11) | 48.2 | 0.56±0.00$^{c}$ | 8.89±0.20$^{a}$ | 1.85±0.00$^{b}$ |
| Linoleic | C18:3 (cis-9,12,15) | 51.7 | 0.92±0.00$^{a}$ | 0.21±0.00$^{b}$ | 0.08±0.00$^{c}$ |
| Eicosadienoic | C20:2 (cis-11,14) | 52.0 | 1.07±0.01$^{a}$ | 0.08±0.00$^{b}$ | 0.09±0.00$^{b}$ |
| Eicosatrienoic | C20:3 (cis-8,11,14) | 55.1 | 2.46±0.02$^{a}$ | 0.12±0.00$^{c}$ | 0.20±0.00$^{b}$ |
| | | Total | 43.5 | 77.5 | 89.2 |

The results are averages of two repetitions ± SD. Different lowercase letters per row indicate statistical difference between treatments ($p<0.05$). FA: Fatty acids, FDCB: Fermented and dried cocoa beans, RCB: Roasted cocoa beans and CPB: Cocoa pulp bar.

## DISCUSSION

The percentage moisture content in FDCB is within the extremely desiccated seed classification (0-4 %), which affects its structure, increasing the percentage of broken, crushed beans, their viability and germination[14]. Furthermore, in the RCB (115 °C for 5 min), the moisture value increased, due to the washing of the seeds, where the water adhered to its surface was absorbed[15].

In the CPB (58 °C for 5 min), a decrease in the percentage of moisture was observed, due to the conching process, resulting in the evaporation of volatile acids, eliminating moisture and increasing sensory characteristics[16]. In a study by Abdullahi et al.[2] it was found that the percentage of moisture decreased with increasing temperature and exposure time (5.33% to 4.26%). Furthermore, in this research, water activity <0.60 was quantified in FDCB, RCB and CPB, so in these products, chemical composition is not susceptible to degradation by microorganisms[17].

The percentage of protein in FDCB is within the parameter indicated for cocoa beans 10-15 % protein5 and similar results were obtained in work by Ilesanmi[18], Djikeng et al.[3] and Abdullahi et al.[2]. These changes in the protein concentration during the different applied thermal processes are due to chemical reactions such as the Maillard reaction[17].

Lipids were the chemical compounds with the highest percentage (Table 1). However, in the RCB (115 °C for 5 min) a lower concentration of lipids was quantified. Moreno et al.[19] reported that during oven drying, denaturation, protein cross-linking, gelatinization and starch dehydration result in a physical barrier around idioblasts (oil cells), causing an increase in the resistance to pulp transfer occurring between the surface and the solvent (used for extraction), thus decreasing its yield.

In CPB, the percentage of lipids increased, because of pulp homogenization (58 °C for 5 min), as reported by Azadmard-Damirchi et al.[20]. These researchers noted that the increase in lipids during extraction is influenced by factors such as the form of homogenization (beating) and the temperature of the process (45.5 °C for 2 h), where the pores of the cell walls are produced and broken, so that changes in porosity allow lipid extraction.

The results of ash percentages were lower than the values reported by Abdullahi et al.[2] with 5.13% to 4.99% and Djikeng et al.[3] with 7.3% to 9.1%. The results obtained in this research may be due to fermentation conditions, since it has been noted that prolonged fermentation decreases the concentration of tannins due to the phenyl oxidase enzyme that produces phenols. These chemical compounds bind to minerals and their bioavailability is decreased[4].

In the RCB (115 °C for 5 min) an increase in carbohydrates was determined, due to the application of heat treatment, where the seed cells expand due to internal pressure (generated by water vapor and $CO_2$). This expansion creates porosity inside the bean and begins a rupture in the cell walls, which facilitates the crushing, exudation and extraction of water-soluble compounds. In turn, these constituents4 were increased compared with the CPB (58 °C for 5 min), where a lower percentage of carbohydrates was quantified, because these compounds have hydroxyl groups in their chemical structure, which react with tannins, reducing its concentration[5,21].

In the FDCB, RCB and CPB, it was observed that temperature affects the concentration of TP, due to oxidation chemical reactions of natural phenolic compounds (monomeric flavan-3-ols and procyanidins) to semiquinones and quinones occur[4]. Moreover, the variety, origin of the cocoa, agricultural practices and packaging conditions are factors that influence concentration[5,21].

In RCB (115 °C for 5 min), a decrease in antioxidant activity was quantified compared to FDCB, due to non-enzymatic or enzymatic oxidation of catechins to o-quinones[4].

The results of DPPH assay were superior compared to the ABTS assay, this may be due to the stereoselectivity of the radical interactions or the solubility of cocoa extracts[4,5]. The changes of the DPPH values are due to the release of phenolic compounds that bind during roasting. This is followed by a formation and accumulation of Millard reaction products, which increases melanoidin content and, thus, increases DPPH antioxidant activity[4,5,17].

In the CPB, lower activity was quantified in ABTS assay, due to the oxidative degradation of phenolic compounds during heat treatment[5], compared to the RCB where free radical scavenging activity was increased, due to the release of phenolic compounds bound to the cell matrix[4,5].

Table 2 shows the results FRAP assays. In the FDCB (115 °C for 5 min) and CPB (58 °C for 5 min) it was determined that temperature influences chelating potential. However, they have the ability to chelate ferrous ion (Fe2+)[13]. Oracz et al.[5] reported that the chelating capacity of ferrous ions depends on the cultivar and roasting conditions[13].

In the RCB (115 °C for 5 min) and CPB (58 °C for 5 min), saturated FA and unsaturated FA showed an increase in concentration due to the oxidation of some even-chain fatty acids. On the other hand, it has been observed that temperature affects the extraction of fatty acids, as reported by Moreno et al.[19] who studied the effect of different extraction methods on the cells containing the oil, the idioblasts (which are round and smooth surface), and concluded that at a temperature >100 °C these cells acquire an irregular shape and a rough surface, which affects the extraction of fatty acids.

It has also been informed that unsaturated FA, due to their chemical structure, are probably used as precursors to form dimers and polymers through intramolecular Diels-Alder reactions between an unsaturated FA (diene) with phenol (dienophile). On the other hand, the concentration of mirystic FA remained constant in the CPB, compared with the FDCB ($p>0.05$). This could be due to an ordering of this molecule between the carboxyl groups with the lipophilic groups of the hydrocarbon chains, forming Van der Waals bonds, and to fuse them, these bonds must be broken to separate them[2].

For other hand, oleic FA was not detectable in the RCB, so it may be due to the intramolecular Diels-Aldder reaction. On the other hand, an increase in the CPB was determined due to a retro Diels-Alder reaction[2], where oleic FA was released from the dienophile cycloadition, thus structural tests are needed to confirm.

## CONCLUSION

In CPB, an increase in lipids was quantified. Roasting decreased the ash concentration. In the results of TP, DPPH, ABTS and FRAP in the three samples (FDCB, RCB and CPB) the changes were minimal. The CPB had a high concentration of unsaturated fatty acids, notably oleic acid was predominate.

***Founding Source.*** *This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.*

***Acknowledgement.*** *To the Social Service student, Carla Taryn Rodríguez Meléndez, of the Bachelor's Degree in Nutrition of the Institute of Health Sciences, UAEH.*

## REFERENCES

1. *Montagna MT, Diella G, Triggiano F, Caponio GR, De Giglio O, Caggiano G, et al. Chocolate, food of the gods: History, science, and human health. Int J Environ Res Public Health. 2019; 16: 1-21.*
2. *Abdullahi G, Muhamad R, Dzolkhifli O, Rani SU. Analysis of quality retentions in cocoa beans exposed to solar heat treatment in cardboard solar heater box. Food Sci Technol. 2018; 4: 1-12.*
3. *Djikeng TF, Teyomnou TW, Tenyang N, Tiencheu B, Morfor TA, Hako TAB, et al. Effect of traditional and oven roasting on the physicochemical properties of fermented cocoa beans. Heliyon. 2018; 4: 1-17.*
4. *Oracz J, Zyzelewicz D, Nebesny E. The content of polyphenolic compounds in cocoa beans (Theobroma Cacao L.), depending on variety, growing region, and processing operations: A review. Crit Rev Food Sci Nutr. 2015; 55: 1176-1192.*
5. *Oracz J, Nebesny E. Antioxidant properties of cocoa beans (Theobroma cacao L.): Influence of cultivar and roasting conditions. Int J Food Prop. 2016; 19: 1242-1258.*
6. *Pereira AC, Martins A, Veloso MM, Reis M. Nutritional profile, bioactive compounds and antioxidant capacity of jatobá-da-mata (Hymenaea courbaril, var. stilbocarpa) by product. Rev Chil Nutr. 2020; 47: 366-371.*
7. *Valenzuela A. Chocolate, a healthy pleasure. Rev Chil Nutr. 2007; 34: 180-190.*
8. *Zamora JD. Antioxidants: Micronutrients fighting for health. Rev Chil Nutr. 2007; 34: 17-26.*
9. *Association of Official Agricultural Chemists AOAC. Official Methods of Analysis of AOAC. 25 th ed. Washington, D.C., The Association, 2019, p. 100-150.*
10. *Stintzing FC, Herbach KM, Mosshammer MR, Carle R, Yi W, Sellappan S, et al. Color, betalain pattern, and antioxidant properties of cactus pear (Opuntia spp.) clones. J Agric Food Chem. 2005; 53: 442-451.*
11. *Kuskoski EM, Asuero AG, Troncoso AM, Mancini-Filho J, Fett R. Application of various chemical methods to determine antioxidant activity in fruit pulp. Ciênc Tecnol Aliment. 2005; 25: 726-732.*
12. *Morales FJ, Jiménez-Pérez S. Free radical scavenging capacity of Maillard reaction products as related to colour and fluorescence. Food Chem. 2001; 72: 119-125.*
13. *Benzie IFF, Strain JJ. The ferric reducing ability of plasma (FRAP) as a measure of antioxidant power: the FRAP assay. Anal Biochem. 1996; 239: 70-76.*
14. *Bareke T. Biology of seed development and germination physiology. Adv Plants Agric Res. 2018; 8: 336-346.*
15. *Katsuno N, Fujimura M, Hanya A, Nishizu T. The effects of water absorption and roasting conditions on fracture properties and internal structure of sesame sedes. Biosci Biotechnol Biochem. 2017; 81: 774-778.*
16. *Aprotosoaie AC, Luca SV, Miron A. Flavor chemistry of cocoa and cocoa products-an overview. Compr Rev Food Sci Food Saf. 2015; 15: 73-91.*
17. *Damodaran S, Parkin KL. Fennema Food Science and Technology. 4 th ed. España, Pearson. 2019, p. 600.*
18. *Ilesanmi AE. Proximate, mineral and antinutrient compositions of natural cocoa cake, cocoa liquor and alkalized cocoa powders sourced in Nigeria. J Adv Pharm Sci Technol. 2016; 1: 1-17.*
19. *Moreno AO, Dorantes L, Galíndez J and Guzmán RI. Effect of different extraction methods on fatty acids, volatile compounds, and physical and chemical properties of avocado (Persea americana Mill.) oil. J Agric Food Chem. 2003; 51: 2216-2221.*
20. *Azadmard-Damirchi S, Habibi-Nodeh F, Hesari J, Nemati M, Achachlouei BF. Effect of pretreatment with microwaves on oxidative stability and nutraceuticals content of oil from rapeseed. Food Chem. 2010; 121: 1211-1215.*
21. *Coronado M, Vega y León S, Gutiérrez R, Vázquez M, Radilla C. Antioxidants: present perspective for the human health. Rev Chil Nutr. 2015; 42: 206-212.*