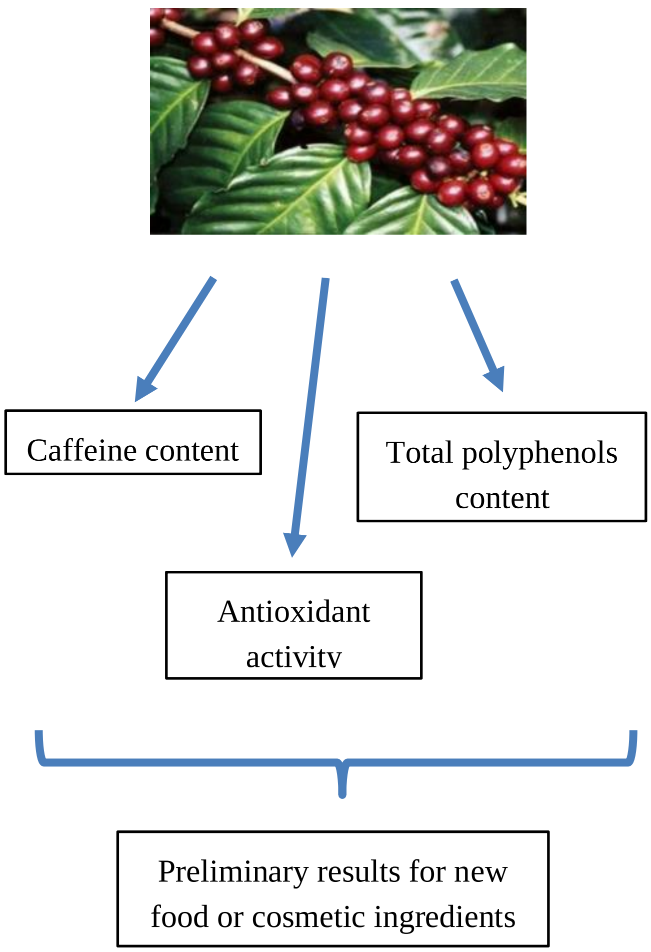
UNIVERSIDAD ESTATAL AMAZÓNICA
Educación con excelencia académica, para formar profesionales de la más alta calidad

Caffeine content, total polyphenols and antioxidant activity of the mucilage concentrate of the *Coffea arabica* L. species, variety "Catuai" and "Castillo", in the province of Pastaza (Ecuador).

Aida Salome Romero Vistin (as.romerov@uea.edu.ec)[a], José Guillermo Guaman Castillo Fabian jp.guamanc@uea.edu.ec[a], Ruperto Lucero Montaño agi2015043@uea.edu.ec[b], Matteo Radice (mradice@uea.edu.ec)[a], Jorge Luis Alba Rojas (jalba@uea.edu.ec)[a]

[a] *Universidad Estatal Amazónica*

Graphical Abstract *(mandatory)*



Abstract.
Coffee (*Coffea arabica* L.) has a gelatinous layer, called mucilage that covers the seed and must be eliminated as a waste during the transformation process. In the Pastaza province two varieties of the species have been cultivated, respectively the "catuai" and the "castle", therefore the present research aims to determine the amount of caffeine, the total content of polyphenols and the antioxidant activity of the concentrate mucilage of the two varieties. The caffeine content was evaluated using the UV-Vis spectrophotometer method, the total polyphenols through the Folin-Ciocalteu method and the antioxidant activity with the FRAP method. The coffee mucilage concentrate presented respectively a caffeine content of 4.87mg / 10g for the Catuai variety and 2.37mg / 10g for the Castillo variety. As for the polyphenols, values of 22.87 mg / g were obtained for the Catuai variety and 21.1 mg / g for the Castillo variety, while the antioxidants activity (FRAP) of the Catuai variety presented 17.78 mmol TROLOX equivalents/100 g d.w. and the Castillo variety 17.46 mmol TROLOX equivalents/100 g d.w. The Catuai variety has a higher content of caffeine and polyphenols and both varieties show a promising antioxidant activity. The results of the study allow recommending new studies of the *C. arabica* mucilage concentrates as potential ingredients of functional food or cosmetic.

Keywords: antioxidants, polyphenols, concentrate, coffee mucilage, caffeine.

Introduction.

Coffee (*Coffea arabica* L.) belongs to the *Rubiaceae* family. The seed that is found inside the cherries is also called parchment. The species cultivated worldwide and of greater economic interest are: *Coffea arabica* Linneo and *Coffea canephora*, and *Coffea arabica* (Suárez Albarracín 2018). Arabica is the most abundant variety in Ecuador is 65% and has been cultivated in Manabí, Loja and Pichincha, while the robust variety represents the 35% of the production and belongs to Santa Elena, Guayas, Orellana

MODEC 05

and Sucumbíos. The coffee industry currently only uses 5% of parchment that is used for processing, while 95% is waste that includes all by-products such as: pulp, husk, mucilage, the same as in one way or another are used as part of animal feed (Sánchez Dávila 2014). Agro-industrial coffee waste is being used as an alternative in the production of biogas, production of edible mushrooms, obtaining organic fertilizer, animal feed and obtaining wines (Días Curay 2010) The use of these alternatives depends on several factors such as the processing capacity, the availability of appropriate technology and the interest of the producers to improve the management of coffee waste, in addition to the cost involved in acquiring new technologies (Días Curay 2010). The pulp is one of the most voluminous solid by-products of coffee, representing 56% of the volume of the fruit and 40% of the weight. In fermentation, the pulp undergoes great changes thanks to its chemical composition, generating enormous amounts of organic waste. (Samayoa Toledo, Borrayo Herrera et al. 2014). The present study aims to determine the caffeine content, the total polyphenols and the antioxidant activity of the mucilage concentrate of both the *Coffea arabica* L. varieties above mentioned, in order to investigate this by-product as a new potential food or the cosmetic ingredient.

Materials and Methods

Plant material and basic chemical-physical characteristics

Mucilage from both coffee varieties have been obtained from farmers located in the Province of Pastaza, Teniente Hugo Ortiz Parish, (coordinates x; 170185, y; 9853309, z; 1141). The concentrate mucilage has been obtained by vacuum distillation using a rotary evaporator (model R-300-5482 K). For both concentrated mucilages was determinate the pH, viscosity and total solids TS (INEN 2008).

Caffeine content

10 g of the sample was exactly weighed and stirred until the sample was homogeneous. 20 drops of sodium hydroxide were placed in all the samples and then the chloroform extracts were obtained, which were deposited in a beaker with the help of a separating funnel. A centrifuge was used at 3000 rpm for 10 minutes, in order to isolate the chloroform solvent from the sample. Chloroform was evaporated in a water bath. The sample was dissolved again by adding 50 ml of hot (70 ° C) distilled water. It was allowed to cool and transferred to a 100 ml volumetric flask, made up to the mark with distilled water. It was placed in a 25 ml volumetric flask, 5 ml of the previous solution were added, 1 ml of 0.01 mol/L HCl was added and it was made up to the mark with distilled water. The absorbance of the sample was read at a wavelength of 275 nm.

Polyphenol content

Polyphenols content have been performed using the Folin-Ciocalteu method. 1 g of concentrate mucilage was exactly weighed into a 50 ml beaker, 15 ml of distilled water were added and then stirred with a rod in order to obtain an homogeneous solution. The mixture was placed in a 25 ml volumetric flask, made up to 25 ml and filtered, finally 0.3 mL of the extract was placed in a test tube with 0.5 mL of Folin's reagent. After five minutes, 0.5 mL of 20% sodium carbonate solution were added and it was completed to 3 mL with distilled water. The sample was stirred, covered with filter paper and allowed to stand for 30 minutes at room temperature. The absorbance of the three replicates was measured at 760 nm in the spectrophotometer.

FRAP assay (ferric reducing/antioxidant power)

FRAP assay was carried out using the FRAP method. For the analysis, 6.1 mg sodium acetate was prepared in 20 ml of distilled water, and 40 mM hydrochloric acid was added until the mixture reaches a pH of 3.5 and it is made up to the mark with distilled water to make up to 250 ml. The 10 mM TPTZ (tripyridyltriazine) solution was prepared by weighing 0.0352 g of TPTZ reagent and dissolved with distilled water, transferred into a 10 ml volumetric flask and made up to 40 mM hydrochloric acid. Then 0.1352 of FeCl3 • 6 H2O was dissolved in 25 mL of distilled water. Preparation of the FRAP solution consisted of mixing 2.5 ml of the TPTZ solution with 2.5 ml of iron III chloride solution and 25 ml of acetate buffer. Finally, the sample was prepared weighing 1 g of coffee mucilage, dissolving in 15 ml of distilled water, filtered in a flask and made up to 25 ml with distilled water, it was placed in a 10 mL flask adding 0.80 µL of the sample. 5 mL of FRAP solution was measured, stripped with distilled water and allowed to stand in an oven at 37 ° C for 30 minutes and finally the absorbance at a wavelength of 593 nm against white was determined.

Results and Discussion

Yield concentrate mucilage

As reported in Table 2, the final volume of the concentrate of the catuai variety was 147 mL, with a 73.5% yield, while the Castillo variety 160 mL with a 80% yield.

Table 2: Determination of the final volume and yield of the concentrate mucilage.

Variety	Initial volume	Final volume	Yield
Catuai	200 mL	147 mL	73.5 %
Castillo	200 mL	160 mL	80 %

pH determination

The pH analysis was carried out by means of three replications of the two varieties, an average and a standard deviation were also calculated. As can be seen in Table 3, the pH of the concentrate is in a range of 4.5 in the regulations (INEN 2008).

Table 2: Determination of the final volume and yield of the concentrate mucilage

Variety	pH	Reference values (Normative 2337)
Catuai	3.71± 0.02	<4.5
Castillo	3.67± 0.01	

UNIVERSIDAD ESTATAL AMAZÓNICA
Educación con excelencia académica, para formar profesionales de la más alta calidad

Total soluble solids

Brix value was performed for both varieties shoving 27.05° Brix and 20.93° Brix for Catuai and Castillo variety respectively. According to the regulations (INEN 2008), the brix value must be under 20° Brix and the Castillo variety is very close to the approved range, while the Catuai variety does not comply the normative. This difference can be partially explained due to the collected coffee beans conditions, when the beans are overripe a higher concentration of total solids is quite common. All data have been reported the table 4.

Tabla 4. Determination of total solids from coffee mucilage concentrate

Variety	Total solids	Reference values (Normative 2337)
Catuai	27.05± 0,56	<20°Brix
Castillo	20.93± 0,56	

Viscosity

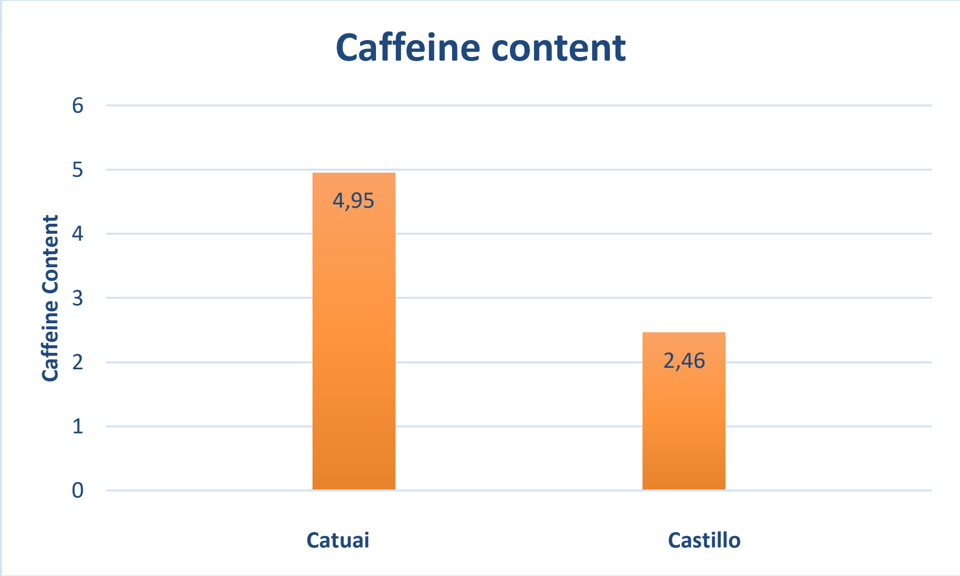
Viscosity data have been obtained for both varieties, Catuai sample showed 738.37 cP and Castillo sample 871.67 cP. The Castillo variety has a higher viscosity than the Catuai variety, probably due to concentration under the conditions already mentioned, all the values are described in table 5.

Table 5. Determination of the viscosity of coffee mucilage concentrate.

Variety	**Viscosity** (mpa*s)
Catuai	738.17± 33.02
Castillo	871.67±4.92

Caffeine content

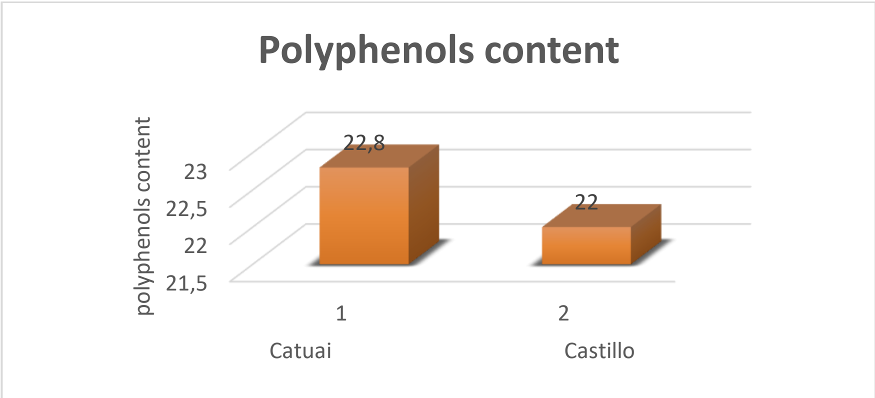
The caffeine determination showed respectively 4.87 (Catuai variety) and 2.37 mg (Castillo variety) of caffeine for each 10 g of concentrated mucilage. Data in triplicate are available in Graphic 1. Bondesson (2015) founded an amount of 13.0 mg of caffeine/10 g of concentrated mucilage, which is extremely higher than data obtained in the present research and can be explained as a physiological variation of the different varieties.



Graph 1. Caffeine content for both varieties

Total polyphenols content (TPC)

TPC have been evaluated for both varieties and data are available at Graphic 2, showing 22.87 mg/g and 21.1 mg/g for Catuai and Castillo respectively. Therefore, the values obtained by applying of the Folin method are superior in comparison to that proposed by (Adrianzén and Greyce 2018) which obtained a value of 18.77 mg / g.

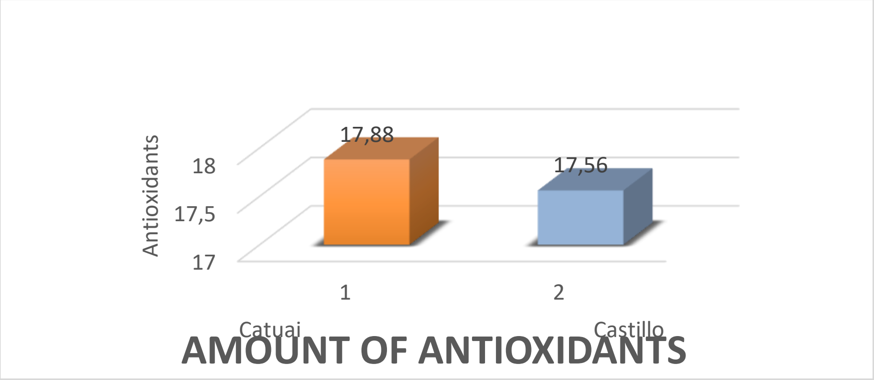


Graph 1. Polyphenols content for both varieties

Antioxidant activities (FRAP assay)

Concerning the FRAP assay, both varieties showed similar results, with 17.78 + 0.11 mmol TROLOX equivalents/100 g d.w. and 17.46 + 0.11 mmol TROLOX equivalents/100 g d.w. for Catuai and Castillo samples respectively. The results obtained are close to those reported (Fonseca Libia, Calderón Lilia et al. 2014) which applied the Wet FRAP method obtaining 10.72 mmol TROLOX equivalents/100 g d.w. with a sample of". Data concerning antioxidant activities have been detailed in graph 3.

To the date, as reported by Iriondo-DeHond et al., (2020) the information concerning the application of the mucilage is very poor, have just been reported a commercial product promoted for its health-promoting properties due the presence of vitamins and polyphenols.



Graph 3. Antioxidants activity

Conclusions

The concentrated mucilage obtained from both coffee varieties have been obtained avoiding fermentation, controlling time and temperature in the proposed technological process. Based on the analyzes carried out on the concentrated mucilage from both varieties, Catuai variety presents the highest content of polyphenols and caffeine, showing also a higher value concerning the antioxidant activity. The physical properties such as pH, Brix and Viscosity do not present a major difference between the varieties. Even if further investigations are need in order to optimized the concentrated mucilage production, these findings represent a promising approach in order to obtain new food or cosmetic ingredients from coffee by-products.

References

Adrianzén, P. and Y. Greyce (2018). Determinación de la capacidad antioxidante y polifenoles totales de la cascara y mucílago de la especie coffea arábica ly sus posibles usos, San Ignacio, Cajamarca–2018. HUACHO UNIVERSIDAD NACIONAL JOSÉ FAUSTINO SÁNCHEZ CARRIÓN.

Iriondo, DeHond. O., et al. (2020). "Applications of Compounds from Coffee Processing By-Products." Biomolecules **10**(1219): 2-20.

Días Curay, A. T. (2010). PULPA DE CAFÉ: Coffea arabica L: COMO FUENTE ALTERNATIVA DE ANTIOXIDANTES. Loja, Universidad Técnica Particular de Loja.

Fonseca Libia, G., et al. (2014). "Capacidad antioxidante y contenido de fenoles totales en café y subproductos del café producido y comercializado en Norte de Santander (Colombia)." Vitae **21**(3): 228-236.

INEN, N. T. E. (2008). "Jugos, pulpas, concentrados, néctares, bebidas de frutas y vegetales." Requisitos. NTE INEN **2337**: 1-10.

Samayoa Toledo, A. L., et al. (2014). Extracción de mucílago, azúcares, y taninos de la pulpa del café y producción de ácido acético comercial a partir de las mieles del café. Guatemala, San Carlos de Guatemala.

Sánchez Dávila, J. A. (2014). Localización y diseño de una planta productora de café tostado y molido en la Provincia de Loja bajo la normativa legal vigente. Quito, Universidad de las Américas.

Suárez Albarracín, L. D. (2018). Aprovechamiento agroindustrial de la pulpa y cascarilla del café (coffea arábiga) variedad caturra en el noroccidente de Pichincha. Quito, Universidad de las Américas.