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Valorization of dry coffee grounds (DCG) and their oily extract: *Coffea arabica* and *Coffea canephora* and their mixture

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ABSTRACT

The coffee grounds CG are considered as a solid residue generated by consumers of ground coffee.For this purpose, three samples of dry coffee grounds DCG and its oily extract (Sample 1: Arabica, Sample 2: Robusta, Sample 3: mixture of two varieties) were subjected to a physicochemical characterization, quantification of primary and secondary metabolites and the mineral salts determination.The results show that the DCG of the three samples is a good source of carbohydrates with a max of 65.31±0.67%. In fact, the oil DCG extraction provided a yield with a maximum of 15.01±0.9%. The obtained values for the different physicochemical quality indices (refractive index RI , acidity index AI , ester index EI , saponification index SI and peroxide index PI),showed the conformity of the studied oils to the standards. Laquantification of secondary metabolites showed that the oily extract of mixture of two varieties is the richest in polyphenols with a value of 50.28±13.32 mg GAE/gr, on the other hand, the Robusta variety was found to be the richest in flavonoids (337.12±22.66 mg CE/gr).

***Keywords*:** Dry coffee grounds (DCG), dry coffee grounds oil (DCGO), physicochemical analysis, primary metabolites, quality index. secondary metabolites

INTRODUCTION

In the present day, the coffee became a major economic richness and presents the first agricultural product exchanged in volume,and the second most important commercialized resource in the world after petroleum. Its annual global production is constantly increasing and it exceeds 8 million tons per year (Stanislav *et al.,* 2014). Almost 50% of the coffee produced in the world is destined for the preparation of ground coffee (Ramalakshmi *et al.,* 2009), which generates significant quantities of a solid residue called coffee grounds (CG).The latter is usually evacuated to landfills with the environmental and economic consequences that this involves.

In recent decades, the growing awareness of the necessity to waste reduce, in order to protect the environment, has stimulated the search for valorize coffee grounds methods in direct use , in composting (Liu and price*.,* 2011) , or for energy production in the agropellets form by combustion (Jeguirim *et al.,*2014).Some studies have demonstrated the adsorptive properties of CG towards colorants (Shen and Gondal, 2017), such as methylene blue in an aqueous solution (Franca *et al.,*2009), as well as heavy metals (Kim *et al.,*2014) such as lead ions in potablewater (Tokimoto *et al.,*2005).

Other work confirmed the CG using possibility as a chelating agent to increase the iron availability in the soil for plants (Morikawa and Saigusa, 2008). Furthermore, other studies have shown that it is possible to extract up to 15% of CG oil by using an organic solvent (Kondamudi *et al.,*2008). This oil can be used for many applications; due to its richness in high value added molecules.In fact, the presence of diterpenes (Hugo *et al.,*2013), polysaccharides, galactomannans and arabinogalactans allowed the CG oil use as a source of dietary fiber (Simões *et al.,*2013). The CG is

rich in lipids (Couto *et al.,* 2009) and free fatty acids, which would make it convertible to biodiesel and bioethanol (Rocha *et al.,* 2014). It can be used to extract phenolic compounds which suggests the possibility of its use as a natural source of antioxidants for the cosmetic and pharmaceutical industry (Zuoroo *et al.,*2012).The free radical scavenging activity of CG is probably related to the presence of brown pigments (melanoids and polyphenols) formed during the coffee beans roasting process.

Moreover, it has been shown that coffee grounds also possess anti-allergenic properties and in a lesser extent of the anti-inflammatory properties (Ramalakshmi *et al.,*2009). An attractive approach for a country like Algeria of this valuable food residue, can be developed by creating apractical ideas with an innovative character to valorize the national agricultural patrimony and the development of a market or a durable agriculture as an example.

In the context of these data, our study is aimed to evaluate some physicochemical and biological properties of coffee grounds and its oily extract of the two varieties *Coffea arabica* (Arabica) and *Coffea canephora* (Robusta) as well as their mixture.

MATERIALS AND METHODS

Biological material and preparation

The CG (Arabica variety, Robusta variety, mixture of two varieties) used was collected daily after infusion using a MOKA Italian type coffee machine (coffee marker). In order to prevent the microbial degradation during the storage, the coffee grounds were air dried in the open air for a few days and then steamed at 40°C for 24 hours to obtain a dry coffee grounds (DCG). Once dried, the DCG is transferred to an opaque container and stored in the dark at room temperature, the samples were distributed as follows:

Sample 1 (DACG): dry Arabica coffee grounds

Sample 2 (DRCG): dry Robusta coffee grounds.

Sample 3 (DMCG): dry mixed Arabica and Robusta coffee grounds

Extraction of the DCG oil

This method consists of an extraction of the oil by an organic solvent (Hexane) on a solid matrix (coffee grounds) for 3 hours. The extraction was carried out in a closed chamber according to a semi-continuous process from 10 g of grindings, introduced in the soxhlet cartridge placed inside the apparatus, a flask filled with 200 ml of hexane was heated to 60°C for 3 hours. The hexane containing the dissolved lipids was then evaporated to recover the oil.

Three oil extracts of DCG are obtained: DACGO: dry Arabica coffee grounds oil; DRCGO: dry Robusta coffee grounds oil; DCGOM: dry coffee grounds oil of two varieties mixture.The oil yield is determined after extraction. It expresses the percentage of oil obtained in relation to the quantity of coffee grounds used for extraction.

The yield is calculated according to the following formula:

Yield (%) = (H/A) × 100 (I)

H: oil quantity (g) obtained after extraction; A: test sample (g) of coffee grounds used.

Analyses performed on the DCG

Physicochemical parameters

The various samples of DCG were subjected to several assays, namely moisture content (NFV 03-903), ash content (Horwitz and Latimer, 2005), pH measure (NFV 05-108, 1970), and titratable acidity measure (NFV 05-101, 1974).

Macronutrients

The protein determination was performed according to the method described by Bradford (1976), the protocol according to NF ISO 8262-3, 2006 was adopted to determine the fat content, and the total carbohydrates were calculated according to the equation:

Total carbohydrates (g/100 g) =100- (m fat+ m ash+ m proteins) (II) (Bazile *et al .,* 2016).

Mineral content

The flame spectrometry was used to determine the Na^+ and K^+cations with a CORNING 400 spectrophotometer. It is a method that depends on the fact that atoms excited by a flame can emit radiation of a characteristic wavelength whose intensity can be measured by spectrometry.The cation initial concentration to be determined is deduced from the intensity absolute value of the measured spectral emission.

Analysis performed on DCGO

Physicochemical characterization

The oil obtained from the DCG (DCGO) of the three samples cited previously had been subjected to several physicochemical analyses .

Refraction index IR (ISO 6320, 1983)

RI is determined by direct reading on a conventional Abbe refractometer (Bellingham & Stanley, BEL-44-501) . After the prism surfaces cleaning, and calibration of the instrument, a few oil drops are deposited in the prism middle.The value is indicated by the range reading.

Acid index AI (ISO N 660, 1990)

Oil (2g) is introduced into a 100 ml flask, 5 ml of ethanolic solution of neutralized potash is added, and a few drops of phenolphthalein indicator are added. Mix and titrate the liquid with the 0.1N potassium hydroxide solution contained in the burette, until the color of the solution changes and persists for 30 seconds. AI is calculated by the following formula:

AI = 56.11 x N x V / P (mg KOH / g oil) (III)

Where: P: mass (g) of the test sample; 56.11: molar mass, expressed in g/mol, of potassium hydroxide; V: volume in ml of KOH (0.1 N) required for titration; N: the potash solution normality (0.1 N).

Ester index EI (AFNOR, 2000)

In a flask, we introduce 2g of oil that we added with a burette of 25 ml, a solution of ethyl KOH (0.5 M), and pumice stone fragments.The flask is placed in a flask heater adapted to a refrigerator, with a duration of 1 hour from boiling. This time is sufficient to allow the acids release by the esters hydrolysis. We let it cool down, then we added 20 ml of distilled water with 5 drops of phenolphthalein. Finally, the excess of KOH is titrated with a HCL solution (0.5M) for the blank test, the same procedure is performed, but without the test sample. The EI determination is calculated by the following formula: EI = 28.05 /m (V0-V1) –AV .Where: V0: volume in ml of the HCl solution used to titrate the blank test; V1: volume in ml of the HCl solution used to titrate the excess KOH; m: mass in grams of the test sample; AV: the acid index determined.

Saponification index SI (NF ISO 3657)

The SI is the number in mg of potash (KOH), necessary to saponify the fatty acids contained in one gram of fat.This value is all the higher as the fatty acids are of low molecular weight. We can calculate SI from this formula: SI = EI – AV.

Peroxide Index PI (NF T 60-220)

1g of oil was dissolved in 25ml of solvent mixture, 15ml of acetic acid and 10ml of chloroform, then 1ml of potassium iodide solution (KI), mix and protect from light for 5 min. Add 25ml of distilled water, and titrate the released iodine with 0.01N sodium thiosulfate solution by vigorous mixing in the presence of starch (1g / 100ml) as a color indicator.

PI is determined by the following formula: PI = V-V0/P x10 (milliequivalents /Kg).With : V0: volume (ml) of $Na_2S_2O_3$ (0.01 N) required to titrate the blank; V: volume (ml) of $Na_2S_2O_3$ (0.01 N) required to titrate the sample; P: sample intake (g) of test.

Evaluation of secondary metabolites of phenolic extract

Preparation of phenolic extracts

For the phenolic compounds extraction, we adopted the protocol of Pirisi *et al*. (2000), 2g of oil are introduced in a tube, supplemented with 1ml of n-hexane and 2ml of 60% methanol. After agitation for 2 min, the mixture was centrifuged at 3000 rpm/5 min.The supernatant was collected, this procedure is repeated 2 to 3 times to exhaust the oil, then are combined and concentrated in dry conditions under vacuum at 40°C, then collected in 1ml of methanol 50%.

Polyphenol content

Total phenolic compounds were determined according to Vazquez Roncero *et al.*(1973). The prepared extract (100 ul) was mixed with 2ml of 2% Na_2C0_3 and then incubated for 2 minutes at room temperature. After incubation, 100µl of Folin-Ciocalteau reagent was added. The reaction mixture was left to stand for 30 minutes at room temperature in the dark.

The absorbance of all sample solutions was measured at 725 nm.The results are expressed as mg gallic acid equivalent per gr of dry plant material with reference to the gallic acid calibration curve.

Flavonoid content

Each extract (0.1ml)is added to 0.4ml of distilled water and 0.03ml of sodium nitrite (5%), all mixed with 0.02ml of aluminum chloride (10%). After incubation for 5min at room temperature, 0.2ml of sodium hydroxide (1M), and 0.25ml of distilled water were added. The absorbance was measured at 510 nm, the flavonoid content is expressed as mg catechin equivalent per g plant dry matter (Kim, 2003).

Statistical analysis

The results are expressed as means and their standard error (X±ES). The data were statistically analyzed using Microsoft Excel version 7.0 software. In all cases, a value of $p<0.05$ was considered significant.

RESULTS AND DISCUSSION

Physicochemical analysis of DCG

The different physicochemical parameters of the three DCGsamples are shown in (Figure 1). The moisture contents of DACG, DRCG and DMCG were in the range of (7.83±1.25% vs 7 ±1.5% vs 8.5±1.5%) respectively. According to Ballesteros *et al.*(2014), the moisture content of DCG of Arabica variety is 7.43% which is close to our study, while, the work of Panusa *et al.*(2013) remain higher (9.50%) than our study. On the other hand, according to Ravindranath *et al.*(1972), the moisture content of DRCG varied from 7.7 to 9.9%, while Martinez-Saez *et al.*(2017) reported a low rate of 3.6%. On the other hand, the moisture contents of the DMCG varied from 7.12% to 7.84% (Panusa *et al.,*2013), which remains lower than our results.

Several researchers have evaluated the ash content of DCG , Ravindranath *et al.,*1972 (1.8 to 2.4%), Martinez-Saez *et al.* (2017) (0.5%), Bastelleros *et al.*(2014) (1.71%), (Pujol *et al.,* 2013; Caetano *et al.,* 2012; Campos-Vega *et al.,*2015) (0.19 to 1.6%). These works synchronized with our results.

According to our results the pH of three samples is situated in the range of 5.5 to 5.85 which is in agreement with the work of several researchers who have found the values in a range of 4.2 to 5.7 (Go *et al.,*2016; Todaka *et al.,* 2013; Somnuk *et al.,* 2017).

Primary metabolites

The content of DCG in carbohydrates, lipids and proteins is represented in (Figure 2). Our results confirm that DRCG is the richest in protein 14.69±1.13% compared to DACG13.68 ±0.21% and the mixture of two varieties 13.91±0.61%.These results synchronize with those of: Ravindranath *et al.* (1972) with a rate of 14.8% for DRCG; (Ballesteros *et al.,*2014 and Mussatto *et al,.*2011b), reported a rate in the order of 17.44% and 14% protein in DMCG respectively. While the works of Lago *et al.*(2001) and Martinez-Saez *et al.*(2017) are not synchronized with our results, with values of 6.7 to 9.9% and 11.20% respectively. According to Arya and Rao (2007), the roasted coffee contains an average of 3.1% (w/w) of protein. The protein content in CG is higher than in coffee beans due to the concentration of the non-extracted components during the preparation of instant coffee. However, these estimates may be over-estimated due to the presence of other nitrogen-containing compounds such as caffeine as well as several amino acids (Delgado *et al.,* 2008). In fact, 17 amino acids are present in CG, including the nine essential amino acids. These represent almost 50% of the amino acids present (Ballesteros *et al.,*2014), mainly leucine contributing 13 or 21% of the total content. This suggests that the protein content may be over-estimated, depending on the coffee variety.

Regarding fat content, our results varied between 11.2% - 15.01%. It has been reported that CG contained 10-15% (Jenkins *et al.,* 2014), (9.3-16.2%) (Cruz *et al.,* 2012), 20%) (Lago *et al.,* 2001; Martinez-Saez *et al.,* 2017), 15.6% (Haile, 2014), 14.70% (Jorge *et al.,* 2017) of fats. The variations in content compared to our work are probably related to extraction methods and coffee varieties. In addition, since fats are not efficiently extracted in an aqueous medium, it is normal to find most of them in the CG. Also, the CG fats are composed of 84.4% of triglycerides. These are represented by linoleic acid (C18:2), palmitic acid (C18:2), oleic acid (C18:1) and stearic acid (De Melo *et al.,* 2014).The CG fats also contain sterols, including sitosterol (10-14% of coffee grounds), stigmasterol (4-5%) and campesterol (3-4%) (Campos-Vega *et al.,* 2015).

Table 1: Organoleptic characteristics and different CG oils yields

Characteristics	DACGO	DRCGO	DCGOM
Appearance and color	Yellowish brown	Chocolate brown	Dark color (brown)
Smell	Strong and agreeable coffee flavor		
Touch	Viscous, unctuous		
Taste	Lightly bitter and pungent but very sweet and persistent, as a good black coffee		
Yield (%)	**15.01±0.9**	**11.2±0.26**	**13, 06 ± 0.32**

Each value was expressed as means ± Standard deviations for triplicate experiments ,DACGO: dry Arabica coffee grounds oil; DRCGO: dry Robusta coffee grounds oil; DCGOM : dry coffee grounds oil of two varieties mixture

Table 2: Physicochemical indexes

Indexes	DACGO	DRCGO	DCGOM
Refractive Index	1.4667 ±0.004	1.4694±0.002	1.4720±0.0005
Peroxide Index meq d'O_2/Kg	7±0.25	12±0.33	8±0.15
Acid Index mg KOH/g	11.22±0.1	7.85±0.17	8.97±0.05
Saponification Index	180±0,08	190±0,07	174.6±0,31
Ester Index mg KOH/g	168.8±2,24	182.15±1.74	165.63±0,35

Each value was expressed as means ± Standard deviations for triplicate experiments ,DACGO: dry Arabica coffee grounds oil; DRCGO: dry Robusta coffee grounds oil; DCGOM : dry coffee grounds oil of two varieties mixture

Table 3: Secondary metabolite levels of different CG oils

	TPC (mg GAE/gr DM)	*TFC (mg CE/gr DM)*
DACGO	10.4 ± 3.13	110,9 ± 14,73
DRCGO	38.63 ± 1.88	337,12 ± 22,66
DCGOM	50.28 ± 13.32	169,28 ± 12 ,27

Each value was expressed as means ± Standard deviations for triplicate experiments. CE: Catechin equivalent; DM: Dry Matter; GAE: Gallic acid equivalent; TFC: Total flavonoid content; TPC: Total phenolic content; DACGO: dry Arabica coffee grounds oil; DRCGO: dry Robusta coffee grounds oil; DCGOM : dry coffee grounds oil of two varieties mixture

The carbohydrates are the most abundant elements in the CG. Indeed, it is rich in sugars of polymerized cellulose and hemicellulose structures. Specifically, the CG contains 46.8% of mannose, 30.4% of galactose, 19% of glucose and 3.8% of arabinose. This data varied from study to study, which may be due to the coffee beans variety used. In CG expresso, galactomannan is the most abundant and represents 50% of carbohydrates (Ballesteros *et al.,*2014). From our results, we can distinguish that the carbohydrate content DRCG (65.31±0.67%) is slightly higher than DACG and DMCG, which have a similar content (63.17±0.36% ,63.67±0.54%). This difference is not significant.

Mineral salts

According to our results, the Na^+ content varied between 2.22 and 3.14 mg/l for the 3 samples (Fig.3), these results are lower than the study carried out by Ballesteros *et al.*(2014) with a content of 33.70 (mg/Kg dry material). On the other hand, the different samples of CG are rich in K^+ with contents that varied from 8.85 to 9.58 mg/l (Fig .3). These results are not synchronized with those obtained by Ballesteros *et al.*(2014) and Cruz *et al.*(2012) who found K+ contents in the range of 11.7 mg/kg and 3.12 to 21.88 mg/kg respectively.

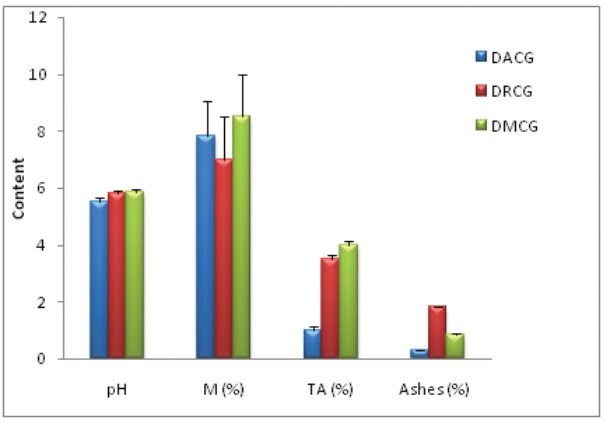

Fig. 1: Physicochemical parameters of the different DCG

Each value was expressed as means ± Standard deviations for triplicate experiments , DACG : dry Arabica coffee grounds , DRCG : dry Robusta coffee grounds , DMCG: dry mixed Arabica and Robusta coffee grounds, TA : Titratable Acidity ; M : Moisture

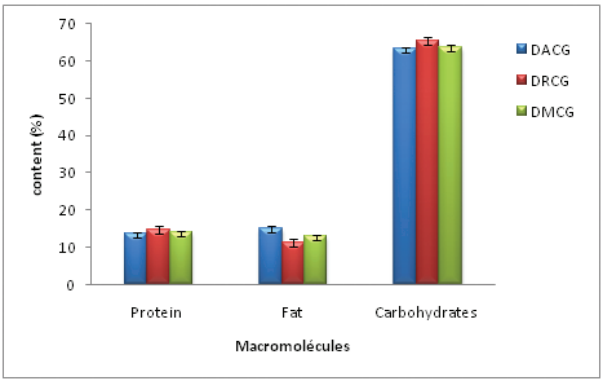

Fig. 2: Macronutrients contents

Each value was expressed as means ± Standard deviations for triplicate experiments , DACG : dry Arabica coffee grounds , DRCG : dry Robusta coffee grounds , DMCG: dry mixed Arabica and Robusta coffee grounds

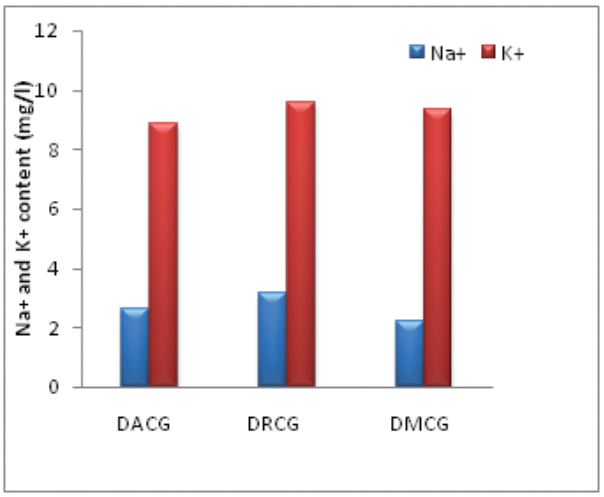

Fig. 3: Na^+ and K^+ content of the different DCG

DACG: dry Arabica coffee grounds, DRCG: dry Robusta coffee grounds, DMCG: dry mix coffee grounds of Arabica and Robusta .

Evaluation of different characteristics of DCGO

Organoleptic characterization

The organoleptic characteristics of different oils are quite similar, with a maximum yield of around (15.01±0.9%) in the Arabica variety. DACGO is in agreement with that of Haile.(2014) with a value of 15.6% and Jorge *et al.*(2017) with a value of 14.70%.While the yield of DRCGO is not in agreement with the study of Ravindranath *et al.*(1972) with a value of 7.9%. The result of DCGOM does not synchronize with the results achieved by Ballesteros *et al.*(2014) with 2.29 ±0.30% and Haddoudi *et al.*(2014) with 11% (Table.1).

Physicochemical characterization

The different physicochemical indexes of the three oil samples are shown in Table 2. The Refractive Index (RI) is a good indicator of the fat conservation state. It measures the total hydro-peroxides, which are the first oxidation products (Kiritsakis, 1998). It varied with the wavelength of the incident light and with temperature.It is proportional to the fatty acids molecular weight and their unsaturation degree, which provides a good appreciation on the oxidation possibility. The results showed that RI of the different varieties studied is comparable to that reported by Ravindranath *et al.*(1972) with a value of 1.460, and that of the codex alimentarius (1.4720-1.4750).

The results reported a high Peroxide Index (PI) for DACGO (12 meqO_2/Kg oil) compared to that of DRCGO**,** which is in the order (7 meq O_2/Kg oil) and DCGOM, which is (8.97 meq O_2/Kg). The low peroxide index of the Arabica variety may indicate that its rancidity is low and that it can be stored for a long time.

The Acid Index (AI) indicated the degree of ester alteration (mainly triglycerides) present in the fat. According to our results we noticed that the acid index is 11.22 mg KOH/g; 7.85 mg KOH/g and 8.97 mg KOH /g for DACGO, DRCGO and DCGOM respectively, our results are comparable to those reported by Ravindranath *et al.*(1972) with values in the range of (7.3-10.1 mg KOH/g).

For the Saponification Index (SI) of different varieties of DCGO ranged between174.6 to 190 (mg KOH/g), according to Ravindrarth *et al*.(1972), the SI of DRCGO varied from 180-200 mg KOH/ g. Similarly, a study performed by (Haile ,2014) revealed a value of 167.28 mg KOH/g for DACGO.

According to our results, the Ester Index (EI 0 values of three samples varied from 165.63 - 182.15 mg KOH/g, these results remained similar to the scientific literature with values of : 172.2-189.9mg KOH/g (Ravindrarth *et al.,*1972); 157.43 mg KOH/ g (Haile, 2014)

Phytonutrients

According to our results, the oil extract is relatively rich in polyphenols with a maximum of 50.28 ± 13.32 mg GAE/gr DM, this value remain higher than other works namely those of (Haddoudi *et al*., 2014) with a value of about 28 mg GAE /g; (Choi and Koh, 2017) with a level of 25.5 mg GAE/ g and (Panusa *et al*., 2013) with 12.58 -17.45 mg GAE/g using ethanol at (60%).

Similarly, the content of flavonoids of the oil extract of the three samples varies between 11.09 ± 1.47 to 33.71 ± 2.26 mg EC/gr DM, these values remain comparable with other studies, namely those Samar *et al*.(2018) with a value of 34.32 mg EC/g.

In general, several factors can influence the rate of secondary metabolites namely: the method of extraction, temperature, time of extraction (Samar *et al*., 2018), climatic conditions and area of harvest (Elkadi *et al.*, 2021).

CONCLUSION

Our results confirm the richness of DCG in macromolecules, namely carbohydrates, and the oil extracted from two varieties *viz*., Arabica and Robusta, as well as their mixture showed a conformity of the different quality indexes to the standards, with a richness in phytonutrients. On the other hand, we detected a quantitative differences in yield and in some physicochemical and biological parameters between the two varieties Arabica and Robusta and their mixture. According to our results, the rate of some primary and secondary metabolites is influenced by the origin and the variety of the coffee beans.

This study promotes the use of CG oil as an economic and ecological alternative to the use of many common cosmetic oils such as coconut or jojoba oil often imported from tropical countries.

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