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Determination of Lipids and Fatty Acids in Green Coffee Beans (*Coffea arabica* L.) Harvested in Different Agroclimatic Zones of the Department of Quindío, Colombia

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Abstract: The objective of this work was to quantify and characterize the lipid fraction of coffee beans grown in different agroclimatic zones of the department of Quindío, seeking to provide coffee growers with useful information about the coffee produced on their farms and the ability to determine coffee quality and sensory attributes. The lipid extraction process was carried out using two different methods: Soxhlet and Soxtec. The bias was established through concordance analysis using the Bland–Altman test to determine the correlation between the two extraction methods. The final results were compared for each agroclimatic zone, and no significant differences were found between the values for average lipid content of the coffee, which varied between 12.01% and 12.90%. When characterizing the profile of fatty acids present in the lipid fraction of coffee using the technique of gas chromatography coupled to mass spectrometry, it was evidenced that the major acids present in the green coffee bean were linoleic acid and palmitic acid, with an average content between 39.83–40.83% and 36.92–37.90%, respectively, and that these fatty acid contents presented statistically significant differences in areas with higher elevations, higher relative humidity and less sunlight (zones 3 and 4). Stearic acid presented differences in all of the agroclimatic zones.

Keywords: Colombian coffee; fatty acids; fully washed; Soxhlet; Soxtec

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1. Introduction

Coffee is one of the main agricultural products of Colombia, ranking among the top exported products [1]. In 2022, Colombia exported 11.4 million 60-kilogram bags of coffee (Colombian Coffee Growers Federation, FNC), contributing approximately 13% to the agricultural gross domestic product (GDP) of Colombia [2]. Quindío, Colombia, is one of the best coffee-producing departments nationwide, with more than 500 thousand coffee-growing families, 92% of whom use technified coffee growing and 72% of whom plant resistant varieties (FNC). Its high environmental diversity provides agroclimatic conditions necessary to generate coffee with differentiated attributes [3,4].

The climate of the Colombian coffee zone is defined as equatorial mountain tropical, i.e., tropical because of the latitudinal position with respect to the geographical equator and mountainous because of the location of crops on the slopes of Andes mountain ranges. Tropical conditions facilitate optimal growth of coffee trees because there is a good supply of light and radiation throughout the year [5].

In regions with temperatures ranging between 19 °C and 21 °C, coffee grows well. The coffee zone is located on the slopes of mountain ranges that cross the country from south to north at an elevation of 1000 to 2000 m. In the department of Quindío, the elevation variation ranges from 1250 m to 2040 m [6]. Elevation is a modifying factor of the appropriate climatic conditions for the development of the coffee crop because

the temperature decreases at higher elevations. In Colombia, in the Andean region, the temperature decreases at a rate of 0.61 °C/100 m, and in the Pacific, Orinoquía and Amazon regions, it decreases at a rate of 0.55–0.58 °C/100 m [7,8].

The quality of coffee is established throughout the coffee production chain and is the result of a series of factors, such as the species and variety, the soil, the environmental conditions, and the age of the crop [9–14]. Coffee quality also depends on harvesting and postharvest processes, which include the harvesting of the fruits as well as the process used [15].

Wet processing is the process most used by Colombian coffee growers; the coffee produced in Colombia is recognized as mildly washed Arabica. This process includes several stages, i.e., harvesting ripe fruits, hydraulic classification, pulping, classification by size, removal of mucilage, washing and drying. Although the process requires several stages to obtain dry parchment coffee, it utilizes more control tools in each stage, allowing farmers to produce high-quality coffee [15–17]. Later stages, such as storage and threshing, contribute to the preservation of the quality of the coffee.

During the stages described above, the chemical compounds of the green coffee bean form and are stored; these compounds are transformed during the roasting process and express themselves when the beverage is prepared. The lipids that constitute the lipid fraction of coffee play an important role in the quality of the beverage; they are responsible for the transport of aromas and flavors [18], add body to the drink and store fat-soluble vitamins such as vitamin E [13,19] that protect or retard oxidative processes during green bean storage.

The lipid content in green coffee beans ranges from 10.7% to 12.3% on a dry basis for improved varieties sown in Colombia [13]. The lipids present in the green coffee bean include triacylglycerols (TGs) (75%), esterified diterpenes and free fatty acids (18%), free diterpenes (0.4%), sterols (4.9%), tocopherols (0.1%), phospholipids (0.4%) and β-N-alkanoyl-5-hydroxytryptamines (1%) [20–24]. In general, after extracting lipids from green coffee, the fatty acid composition can be determined by derivatization and separation by GC–MS. The main free fatty acids in green coffee are palmitic acid and linoleic acid, with oleic, stearic and arachidic acids occurring in smaller proportions [25,26].

Fatty acid composition has been used as a parameter to determine the quality of coffee [13] and its origin [27,28] and to differentiate Arabic coffee from Robusta [29–31]. Therefore, the characterization of these compounds is decisive when evaluating the quality of coffee. The way by which lipids are extracted from coffee is also of great importance when making analytical determinations. Depending on the compound to be studied, the extraction method and the most appropriate solvent must be selected to obtain the analyte of interest [32].

One of the most commonly used methods to extract the lipid fraction from green coffee is the Soxhlet method, which consists of solid–liquid extraction, in which the fatty material of a sample (ether extract) is obtained using an organic solvent [33,34]. Another method that is based on the same principle is the automated Soxtec method, which has several advantages, such as allowing simultaneous sample extraction, requiring a lower solvent volume for extraction, and facilitating a recovery between 70% and 80%, in addition to a shorter extraction time [35,36]. Lipids in coffee samples have been determined by both the Soxhlet extraction method and the automated Soxtec method, with contents varying between 10% and 17% [33,37].

The objective of this research was to compare two lipid extraction methods to determine the composition of fatty acids present in the lipid fraction of green coffee beans and its relationship with the agroclimatic zone (Quindío Department) where the coffee crop was located and from which the samples were collected. This research sought to provide the coffee grower with useful information about their crop with a view to knowing the quality of their product and its possible behavior in the cup.

2. Materials and Methods

2.1. Agroclimatic Zones

The agroclimatic zones were determined by the CENICAFÉ's Agroclimatology Research Group; the zones were defined mainly by the regularity of their edaphic, climatic and orographic variability, where similar environmental conditions, mainly among elevation ranges with similar production system conditions, provide a similar response in agricultural potential and, therefore, in quality. The farms were grouped based on established agroclimatic zones (Figure 1); the characteristics of the zones are presented in Table 1.

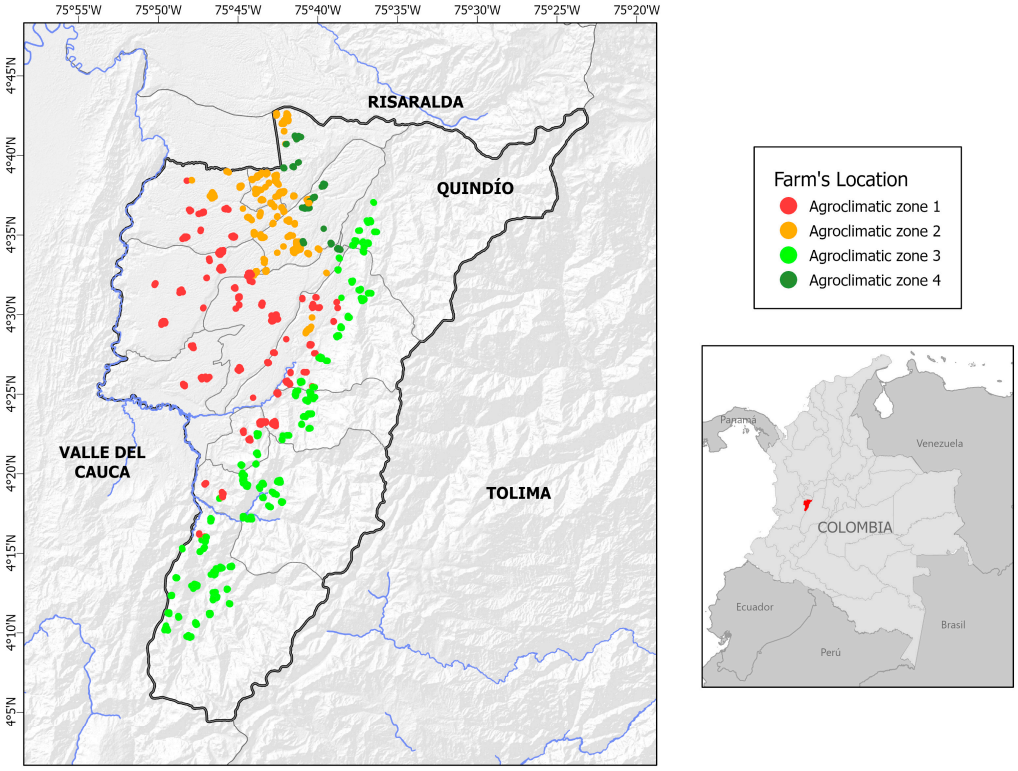

Figure 1. Geographical locations of the coffee farms in different agroclimatic zones in the department of Quindío, Colombia.

Table 1. Characteristics (average values) of the agroclimatic zones in the department of Quindío, Colombia.

Zone	1	2	3	4
Elevation (m)	1200	1400	1700	1700
Crop age (years)	4.8	5	5-jun	≤5
Density (plants/ha)	5200	4000–6000	5000–6000	4000–5000
Solar brightness (h/year)	1668	1533	1267	1332
Annual rain (mm)	2222	2798	2667	3268
Relative humidity (%)	77.0	78.3	81.9	81.3
Temperature (°C)	21.7	20.6	18.6	18.8

2.2. Coffee Samples

In total, 224 coffee-growing farms were selected randomly and proportionally to the 12 coffee-growing municipalities of the department of Quindío. The samples processed by coffee growers on their farms were evaluated during three harvests (2021–2022) for a total of 672 samples. The coffee farms are located in different agroclimatic zones of the department of Quindío (Figure 1, Table 1). The beans were harvested via standard collection and processing methods, i.e., pulping, removal of the mucilage by spontaneous fermentation or a demucilaginator, and mechanical drying and/or solar drying until reaching a humidity

percentage between 10% and 12%. The samples of dry parchment coffee (dpc) were collected by the Departmental Committee of Coffee Growers of Quindío and were sent to the National Coffee Research Center (CENICAFE) located in the municipality of Chinchiná Caldas, which is located at an elevation of 1310 m and has an average temperature of 21.2 °C, maximum temperature of 27.9 °C, minimum temperature of 17 °C, and relative humidity of 82.3%. For the chemical analyses, 2 kg of dpc was homogenized, and 500 g of dpc was threshed to later select healthy beans (without physical defects). Subsequently, the samples were divided, and 250 g was sent to the University of Quindío, located in the municipality of Armenia, which is located at an altitude of 1551 m and has an average temperature of 21 °C, maximum temperature of 26 °C, minimum temperature of 17 °C, and relative humidity of 77%, for lipid content analysis using the Soxtec method. From the same sample, 250 g of coffee was analyzed in Cenicafé laboratories to determine the lipid content by the Soxhlet method.

2.3. Determination of Lipid Content by the Soxhlet Method

Healthy beans were cryogenically ground in a Retsch ZM-200 ultracentrifuge mill and stored at −80 °C in 50 mL Falcon tubes in a Thermo Scientific Forma 900 series freezer until use. Ten grams of previously ground green coffee was used for the assessment of the lipid fraction by the Soxhlet method.

For the analysis of the lipid content, the methodology described in AOAC 945.16 was used as a reference. Ten grams of cryogenically ground green coffee was deposited on 11 × 21 cm Kimwipes (Kimtech Science Brand) and subsequently placed in a cellulose extraction cartridge with an internal diameter of 33 mm, an external diameter of 37 mm and a length of 80 mm (Advantec, Tokyo, Japan). Extraction was carried out for 16 h to ensure complete extraction. Supelco brand petroleum ether (450 mL) was used as the extraction solvent (free of peroxides) at a temperature between 165–170 °C. Then, the ether was rotary evaporated in a BÜCHI R-124 at a temperature of 50 °C and a pressure of 800 mbar. The lipid fraction was placed in an oven at 45 °C for 16 h. Subsequently, the material was weighed to determine the percentage of lipid in each sample.

2.4. Determination of Lipid Content by the Soxtec Method

Extraction was carried out using a Soxtec™ 243 (FOSS Analytical Co., Ltd., Hillerød, Germany) six-position extraction device. One gram of cryogenically ground green coffee was weighed in a cellulose thimble (26 × 60 mm; Foss, reference 15220018), and sample assembly was carried out individually in each of the six positions. Then, 40 mL of petroleum ether (RA grade; Supelco, Bellefonte, PA, USA) was added to the extract collection glasses to start the extraction at a temperature of 90 °C. The process consisted of three basic extraction steps requiring 1 h and 10 min for completion. The first phase consisted of immersing the sample in benzine for 10 min; in the second phase, extraction was carried out by reflux for 40 min; and the third phase involved the recovery of the solvent (20 min). Subsequently, the ether extract in the aluminum vessels was evaporated for 15 min at 90 °C. The samples were desiccated for 30 min and then weighed to determine the percentage of fat in each sample [38,39].

2.5. Analysis of Fatty Acids Composition

Fatty acids were determined in accordance with AOAC 969.33. One hundred microliters of lipids extracted using the Soxhlet method was saponified with 1 mL of 0.5 M sodium hydroxide (prepared in analytical grade methanol) for 10 min at 90 °C; then, the free fatty acids were esterified with 1 mL of boron trifluoride in 20% methanol for 40 min at 80 °C. Subsequently, two successive extractions were carried out with 1 mL of hexane. The samples were analyzed on an Agilent 8860 gas chromatograph with a 5977B mass detector with an autosampler. The fatty acids were separated on a DB-5MS column (Agilent; 30 µm × 0.250 mm × 0.25 µm). The injection mode was split with a constant helium flow of 1.2 mL/m and an injection volume of 0.2 µL. The injection port temperature was 300 °C.

The temperature program was as follows: 90 °C to 260 °C, with a ramp of 20 °C/min. The conditions used for the mass spectrometer were as follows: temperature, 230 °C; scan mode, 20–550 m/z; positive electron impact; and ionization energy, 70 eV. The mass spectra data were processed using MassHunter, version B.01.00 (Agilent Technologies, Santa Clara, CA, USA). The quantification of each compound was carried out by normalization, and identification by comparison with the spectra deposited in the NIST 2017 library database.

2.6. Statistical Analysis

The results for the lipids obtained by the two extraction methods, Soxhlet and Soxtec, are reported as percentages on a dry basis. To identify agreement between the extraction methods, Bland–Altman analysis was used, which allowed quantification of the mean difference between the two methods (bias or blind) at one (1σ) and two (2σ) standard deviations. Subsequently, to determine the difference between the lipid and fatty acid contents and the agroclimatic zones of origin, the t-multivariate test at 5% probability was used. The analyses were conducted using SAS version 9.4 2016 (Cary, NC, USA).

3. Results and Discussion

3.1. Comparison between Lipid Extraction Methods

Table 2 shows the operating parameters for each of the methods used, where the differences between the volume of solvent for extraction, the mass of the coffee sample, the process time and the percentage of recovery of the coffee are reported.

Table 2. Comparison of the operating parameters of the lipid fraction extraction methods (Soxhlet and Soxtec).

	Soxhlet	Soxtec
Volume of extraction solvent per sample (mL)	450	40
Green coffee sample mass (g)	10	1
Process time (h)	36	1.9
Solvent recovery percentage (%)	80	70–80

Figure 2 and Table 3 show the results of the Bland–Altman analysis, which was performed to determine the bias and the confidence interval for the two extraction techniques used.

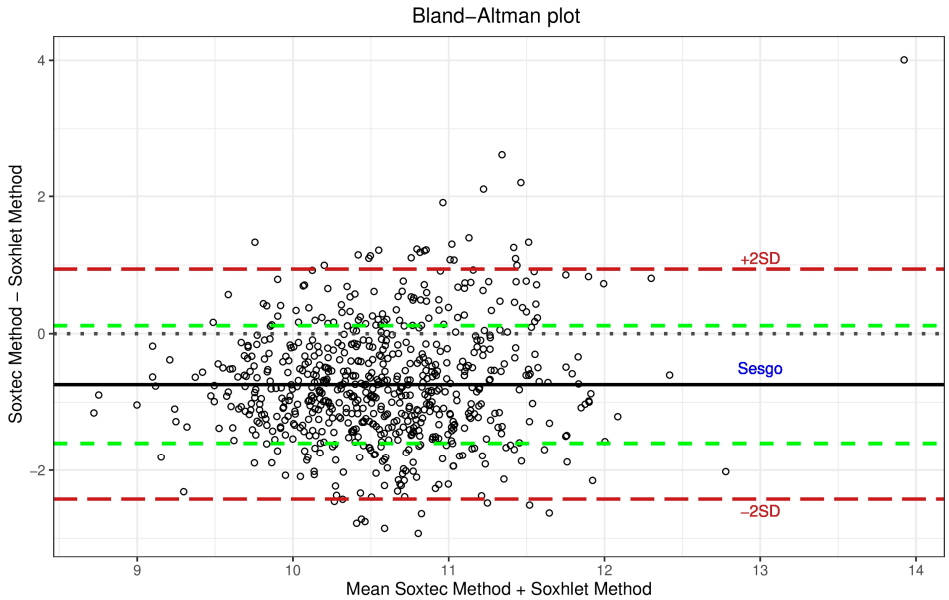

Figure 2. Bland–Altman plot for two lipid extraction methods.

Table 3. Confidence intervals at one and two deviations for lipids extracted by the Soxtec and Soxhlet methods.

Bias	Limit Greater Than 2σ	Limit Less Than 2σ	Limit Greater Than 1σ	Limit Less Than 1σ
−0.74	0.94	−2.43	0.12	−1.60

Based on the results in Table 3 and in Figure 2, on average, the difference between the lipid measurements using the Soxhlet method and the Soxtec method was 0.74 units (bias); the negative direction of the bias indicates that the Soxhlet method tended to yield higher lipid content values. Additionally, for 68% of the data, the difference between measurements was at most 0.86 units, and in general, for 95% of the data, there were no differences between measurements greater than 1.70 units.

The long extraction times with the Soxhlet method facilitated 100% extraction of the lipid fraction of the coffee, but these times exceeded 18 h, and the solvent consumption was high (450 mL per sample), making it nonenvironmentally friendly and very inefficient in terms of extraction time, in addition to requiring subsequent stages such as rotary evaporation. The Soxtec method was more efficient but yielded lower values than expected. Based on the concordance analysis, if 0.74 units on average was added, the difference between the two measurements on average was zero.

Figure 3 shows the comparison of variances in the two methods after the Bland–Altman analysis. There were no differences at the 5% significance level, which is why it is possible to extract lipids from coffee using either of the two methods; however, the Soxtec method is recommended, based on the advantages it presents.

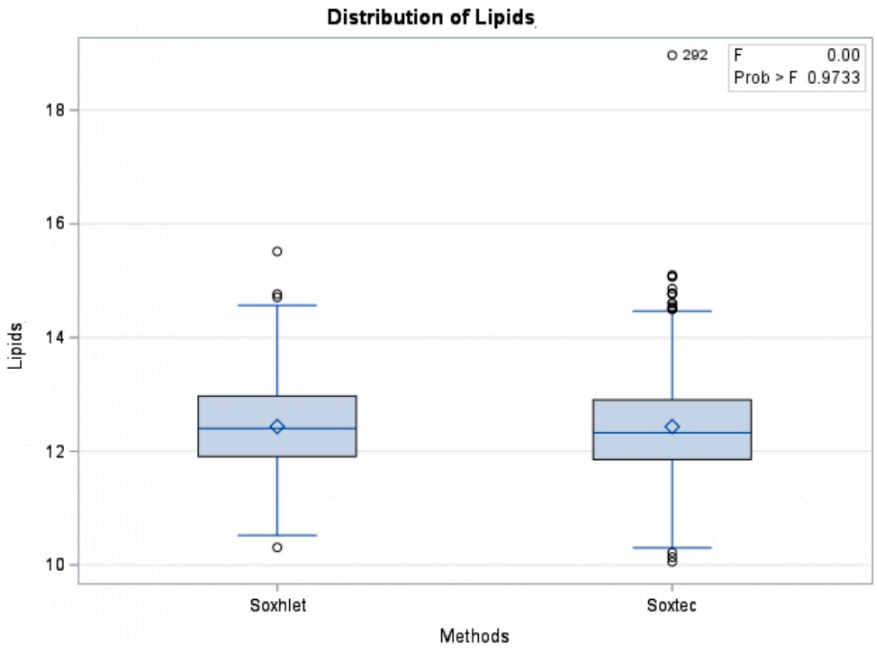

Figure 3. Lipid extraction distribution for the Soxhlet and Soxtec methods.

Table 4 presents the results for the extraction of the lipid fraction of green coffee using the two different methods. The percentage of lipids obtained by the Soxhlet method varied between 12.01 ± 0.62% and 12.81 ± 0.68%, and the percentage obtained by the Soxtec method ranged from 12.05 ± 0.60% to 12.90 ± 0.48%; both results were within the ranges reported for green coffee [37,40] and consistent with the results reported by Villarreal et al. [41]. Echeverri et al. [13] analyzed the lipid fraction content of green coffee in improved varieties of *C. arabica* grown in different locations in Colombia using the Soxhlet method and reported values between 10.7% and 12.3%. The results of this study are higher

than those reported by Osorio et al. [26], who evaluated the lipid content in three stages of maturation of green coffee in the department of Caldas, obtaining values between 10.28% and 10.45%.

Table 4. * Average lipid content in green coffee beans for three crops by zone and by extraction method (Soxhlet or Soxtec).

Method	Harvest	Zone	Mean	Stdev	Min	Max
Soxhlet	1	1	12.65	0.90	10.53	14.57
		2	12.19	0.83	10.31	15.51
		3	12.81	0.86	10.56	14.76
		4	12.53	0.72	11.75	14.02
	2	1	12.12	0.66	10.72	13.84
		2	12.14	0.64	10.52	13.44
		3	12.16	0.62	11.02	14.31
		4	12.01	0.62	10.95	13.30
	3	1	12.57	0.60	11.43	13.54
		2	12.37	0.69	11.08	13.77
		3	12.81	0.68	11.01	14.46
		4	12.23	0.70	11.46	13.67
Soxtec	1	1	12.26	0.60	10.62	14.11
		2	12.05	0.60	10.14	14.07
		3	12.42	0.28	10.92	14.60
		4	12.20	0.10	11.40	13.24
	2	1	12.73	0.80	10.99	15.01
		2	12.79	0.62	10.55	14.86
		3	12.90	0.48	10.3	15.08
		4	12.73	0.39	13.51	14.54
	3	1	12.36	0.49	10.06	14.26
		2	12.34	0.23	11.07	14.47
		3	12.17	0.38	10.85	18.97
		4	12.28	0.32	12.25	14.77

* Analysis of variance did not indicate significant differences between the two methods.

3.2. Fatty Acid Composition

The chromatographic profile obtained from the analysis of the fatty acids identified in green coffee by GC–MS is presented in Figure 4.

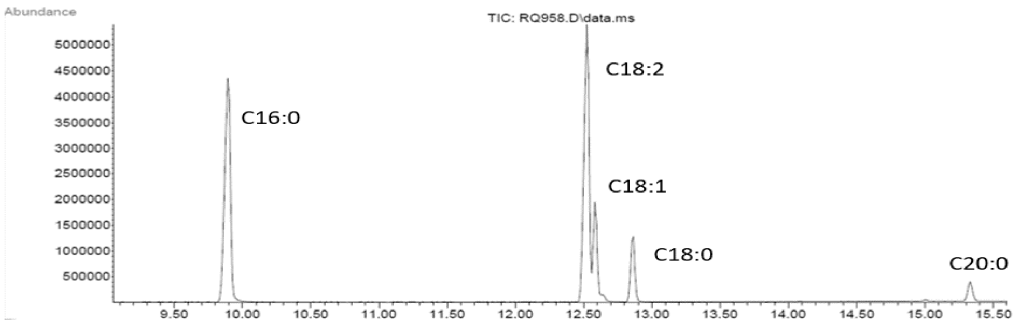

Figure 4. Chromatogram of green coffee fatty acids detected by gas chromatography–mass spectrometry. Palmitic acid (C16:0); oleic acid (C18:1); linoleic acid (C18:2); stearic acid (C18:0), arachidic acid (C20:0).

Table 5 provides the average fatty acid contents identified in the green coffee samples from the different agroclimatic zones in the department of Quindío. The average fatty acid contents are consistent with the ranges reported by other authors [25,33,40–42].

Table 5. Free fatty acid composition in green coffee (%) from each agroclimatic zone.

Total Fatty Acid (%)	Zone	Mean	Group *
Linoleic acid	1	39.83 ± 1.26	C
	2	40.37 ± 1.15	B
	3	40.83 ± 1.23	A
	4	40.54 ± 1.37	AB
Palmitic acid	1	36.92 ± 0.94	C
	2	37.27 ± 0.98	B
	3	37.90 ± 0.89	A
	4	37.77 ± 1.15	A
Oleic acid	1	12.30 ± 1.12	A
	2	11.71 ± 1.10	B
	3	11.66 ± 1.05	B
	4	11.47 ± 1.07	B
Stearic acid	1	8.55 ± 0.66	A
	2	8.28 ± 0.42	B
	3	7.62 ± 0.51	D
	4	7.94 ± 0.65	C
Arachidic acid	1	2.40 ± 0.52	A
	2	2.37 ± 0.52	A
	3	1.99 ± 0.51	B
	4	2.27 ± 0.54	A

* Different letters indicate statistically significant differences based on the multivariate test at the 5% level.

Linoleic acid (C18:2) occurred in the highest percentage in the four zones (Table 5), ranging from 39.83 ± 1.26% to 40.83 ± 1.23%; similar values were reported by Villarreal et al. [41], Dong et al. [40], and Zhu et al. [12]. Linoleic acid was highest for zone 3 (40.83 ± 1.23%), followed by zone 4 (40.54%), zone 2 (40.37%) and zone 1 (39.83 ± 1.26). The linoleic acid content differed significantly between zone 1 and all of the other zones. A multiple comparison test was performed with the objective of identifying differences among agroclimatic zones. As seen in Table 5, the oleic acid content was different in different zones, accounting for the majority in zone 1, contrary to the results for linoleic acid, for which the content was lower than that reported in the other areas. For zones 2, 3 and 4, the percentage of oleic acid ranged from 11.47 ± 1.07% to 11.71 ± 1.10%; in zone 1, the percentage was 12.30 ± 1.12%. The values obtained herein are similar to the ranges reported by Echeverri et al. [13]. The oleic acid content decreased when the elevation increased, the solar brightness decreased and the rainfall increased (Table 1). These results are also similar to those reported by Tsegay et al. [9], who obtained values between 6% and 13.6% and determined that this acid tends to increase as elevation decreases.

Among the saturated fatty acids, palmitic acid occurred in the highest proportion in the agroclimatic zones studied, ranging from 36.92 ± 0.94% to 37.90 ± 0.89%, values that are consistent with the results reported by Villarreal et al. [41], i.e., between 30.6% and 43.1%, and by Dong et al. [40], i.e., between 36.57% and 38.71%. The zones with the highest percentage of this fatty acid were zones 3 and 4, with values of 37.90% and 37.77%, respectively; there was no significant difference in the percentage of palmitic acid between these two zones. For zone 2, the percentage was 37.27%, and that in zone 1 was 36.92%. The values for both zones (1 and 2) were significantly different from those for zones 3 and 4. The content of this fatty acid tended to increase as the elevation of the crop increased, the annual rainfall increased and the solar brightness decreased. Several authors have attributed the variations in the levels of fatty acids to the altitude at which the crop is developed; as the altitude increases, the temperature decreases, which prolongs the ripening period of the fruits, favoring greater development and accumulation of these compounds in green coffee beans [14,41,43,44].

The content of stearic acid, a saturated fatty acid, significantly differed among the 4 zones. The content was highest in zone 1 (8.55%), followed by zone 2 (8.28%), zone 4 (7.94%) and zone 3 (7.62%). Puerta and Echeverri [23] and Dong et al. [40] reported contents

ranging from 6.40 to 8.30%. In this study, similarly to oleic acid (C18: 2), stearic acid (C18:0) decreased when the elevation increased, sunlight decreased and rainfall increased.

Among the saturated fatty acids, arachidic acid (C20:0) occurred at the lowest percentages in the four zones. For zones 1, 2 and 4, the values ranged from 2.27% to 2.40%; similar values were reported by Villarreal et al. [41] and Zhu et al. [12], with values of 2–4.3% and 2.45–2.72%, respectively. The percentage of this fatty acid was lowest for zone 3 (1.99%); this zone is characterized by lower solar brightness (1267 h/year, Table 1) compared to that in the other zones.

Based on the results presented in Table 5, in general, there are statistically significant differences in the composition of fatty acids in green coffee from different agroclimatic zones in the department of Quindío. In addition, the values obtained are similar to the values reported by other authors [11,22–24,37,38], who showed significant differences associated with climatic factors, confirming that environmental conditions highly influence the fatty acid content in green coffee. Linoleic acid (C18:2) was the most abundant fatty acid present in the lipid fraction, ranging from 39.8 to 40.83%, followed by palmitic acid (C16:0), ranging from 36.92% and 37.90%, oleic acid (C18:1), ranging from 11.47% to 12.30%, stearic acid (C18:0), ranging from 7.62% to 8.55%, and arachidic acid (C20:0), ranging from 1.99% to 2.40%.

Based on the results of studies carried out by Figueiredo et al. [18] and Echeverri et al. [13], a high proportion of long-chain saturated fatty acids (palmitic, stearic and arachidic) is a potential discriminator of the sensory quality of green coffee because these acids impart desirable organoleptic characteristics, providing body and flavor in coffee; those results are supported by the average saturated fatty acid values obtained in this study (48.72%). The average content of unsaturated fatty acids, i.e., oleic acid and linoleic acid, was 52.2%, indicating greater susceptibility to oxidation processes, with linoleic acid being the most prevalent in the analyzed samples (40.39% of the lipid fraction). There were significant differences in the linoleic acid and oleic acid contents among the cultivated areas. Notably, linoleic acid provides benefits for human health and nutrition because it plays a role in the synthesis of prostaglandins and other biological processes of cell regeneration [45].

4. Conclusions

Comparing the lipid content obtained using two different extraction methods, the bias was not greater than 0.74 units, and when comparing the lipid contents in green coffee beans grown in the different agroclimatic zones in the department of Quindío using the Soxhlet and Soxtec methods, the average percentage of lipids did not vary significantly among the cultivated agroclimatic zones; however, when characterizing the lipid fraction by fatty acid content, there were significant differences among the agroclimatic zones. Additionally, linoleic acid and palmitic acid were predominant in areas at higher elevations with higher relative humidity and lower sunlight (zones 3 and 4). Among the saturated fatty acids, stearic acid and arachidic acid were predominant in areas at lower elevations, with lower relative humidity and with greater sunlight (zones 1 and 2), findings similar to those for unsaturated oleic fatty acids.

The different chemical compounds present in the coffee bean have a crucial role in configuring the different sensory attributes perceived by the coffee taster. Chemical composition is the result of the interaction of the variety with the different climatic and soil conditions of the production zone, among other factors. For this reason, in this research, the characterization of the lipid composition and the fatty acid profile of the bean was approached from the perspective of the possible changes generated by the characteristics of the location in which the coffee plant grows. Differences in the contents and their possible association with zones that differ in elevation and agroclimatic variables such as precipitation, relative humidity, and solar brightness were determined. A better understanding of the chemical composition and its correlation with the particularities of production and processing will allow a better understanding of the behavior of the attributes of the beverage. Given these perspectives, it is important to improve our understanding of the conditions

of the production site and postharvest practices and how they correlate with the sensory quality of the beverage, which will allow us to continue with the differentiation of coffee from origin.

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