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Changes in triacylglycerols and free fatty acids composition during storage of roasted coffee

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ABSTRACT

Lipids are major coffee components, and changes in their composition during storage may contribute to loss of sensorial quality. In this study, changes in the triacylglycerols (TAG) and free fatty acids (FFA) composition of *Coffea arabica* seeds roasted to two color degrees (light medium and dark medium) were evaluated during storage for 6 months, under two temperature (5 °C and 25 °C) and atmosphere (air and N_2) conditions. For the first time, hydrolysis of TAG fraction was observed during storage of roasted coffee, with increases in FFA, after 1 month storage, from 0.4 to 93.5 mg/100 g in light-medium samples and from non-detected to 1.1 mg/100 g in dark-medium samples. After 3 months storage, 20% and 13% decreases in FFA from light-medium and dark-medium samples, respectively, were observed, suggesting oxidation. The N_2 atmosphere contributed to a slower loss of FFA. In the same way, at 5 °C, lower release of FFA was observed compared to 25 °C. Considering the inversion in the unsaturated FA (UFA) and saturated FA (SFA) contents observed in the dark-medium sample, the present results also show that the ratio Σ UFA/SFA, in TAG and AGL fractions might potentially be used as a tool to establish the shelf life of roasted coffee.

1. Introduction

Coffee is the most consumed food product in the world. Roasting induces severe transformation on coffee's chemical composition. Additionally, during storage, the roasted beans are susceptible to further chemical and physical changes that may greatly affect the quality and the acceptability of the brew.

Lipids are major coffee components and correspond to approximately 11–20 g/100 g of roasted *Coffea arabica* composition (Oliveira, Franca, Mendonça, & Barros-Junior, 2006; Toci, Farah, & Trugo, 2006; Trugo, 2003). Furthermore, triacylglycerols (TAG) comprise the main lipid class in coffee and account for approximately 8–17 g/100 g (75% of total coffee lipids) in freshly brewed coffee, whereas free fatty acids (FFA) account for 0.1–0.2 g/100 g (about 1% of total coffee lipids only) (Trugo, 2003). Among the most important unsaturated fatty acids for coffee freshness are oleic (18:1n-9), linoleic (18:2n-6) and linolenic (18:3n-3) acids, which account, respectively, for approximately 0.6–1.1 g/100 g, 2.9–5.4 g/100 g and 0.08–0.15 g/100 g, representing 7%, 36% and 1% of TAG fraction (Folstar, 1985; Lercker et al., 1996; Nikolova-Damyanova, Velikova, & Jham, 1998; Speer & Kolling-Speer, 2006).

Lipids may contribute to loss of sensory quality during storage. TAG can be hydrolyzed either chemically or enzymatically to produce a mixture of diacylglycerols, monoacylglycerols, FFA, and glycerols molecules (Folstar, 1985; Frankel, 2005). The rate at which these reactions occur depends mostly on factors related to environmental and technological aspects such as availability of oxygen and moisture, exposed surface area, temperature, as well as package material (Manzocco & Lagazio, 2009; Pérez-Martínez, Sopelana, Paz de Peña, & Cid, 2008; Speer & Kolling-Speer, 2006). Since during coffee roasting hydrolytic enzymes are thermally inactivated, moisture and temperature are the main factors that will rule hydrolysis reactions in roasted coffee. The presence of high moisture content in food storage systems reduces the contact between food and oxygen, which tends to cause a decrease in oxidation reactions, but promotes hydrolysis reactions. When moisture in the storage system is low, Entropy decreases in the system, which leads to a decrease in the kinetic energy of the

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molecules and thus in the rates of all types of reactions. However, when storage temperature is high, Entropy increases, accompanied by a raise in the rate of degradation reactions (Frankel, 2005, Chapter 11; Kim & Min, 2008). On the other hand, oxidation reactions are facilitated by the presence of oxygen, and unsaturated FFA are the most susceptible components to such reaction, due to the presence of double bonds (π electrons), less packed in lipid crystals than TAG (Kim & Min, 2008). The oxidation of FFA is responsible for the formation of a large number of volatile compounds, loss of positive attributes, such as "freshness", and formation of an attribute called "staleness" (Frankel, 2005).

Several studies, through evaluation of the volatile composition and sensory analysis, have focused on the shelf life of roasted coffee under various conditions of temperature, atmosphere and moisture. Data have shown that all these variables influenced the acceptability of stored roasted coffee (Manzocco & Lagazio, 2009; Ross, Pecka, & Weller, 2006; Toci, 2010). The interest on shelf life of roasted and ground coffee is especially important to consumers. However, the assessment of shelf life requires the exact definition of the criteria to determine the end of the product's life. It has been speculated that hydrolysis of TAG results in release of free fatty acids, which are oxidized to produce, as mentioned above, off-flavors in coffee (Spadone, Takeoka, & Liardon, 1990; Speer, Sehat, & Montag, 1993). Nevertheless, studies on degradation of lipids in roasted coffee are scarce.

The aim of the present study was to investigate potential changes in the content and composition of fatty acids contained in TAG and FFA fractions of roasted *C. arabica* during storage under different temperature and atmospheric conditions.

2. Material and methods

2.1. Samples

Excellent cup quality seeds of Brazilian *C. arabica* from Minas Gerais, classified as "strictly soft", were used. One hundred grams of the seeds were roasted in a spouted bed roaster (IRoast, Gurnee, IL, USA), reaching a maximum temperature of 221 °C. They were roasted for 5.5 min and 7.5 min to give light-medium and dark-medium color degrees, respectively, according to the Roast Color Classification System (AGTRON – SCAA, USA, 1995). All samples were ground to pass a 500 μm sieve.

2.2. Storage

Coffee storage was carried out by placing 2 g aliquots of each sample in 7 mL amber vials and storing them for 1–6 months, under controlled conditions of temperature (5 and 30 °C) and atmosphere (ambient air and N_2). Storage was performed in triplicate.

2.3. Analysis of total lipids content

Total lipids contents were determined according to the method number 15.028 established by AOAC (1984).

2.4. Extraction, separation and transesterification of fatty acids

Total lipids were extracted in triplicate from 2.0 g of coffee samples with 40 mL of organic solvents (isopropanol:chloform, 1:1 mL/mL), by thoroughly mixing with an Ultra Turrax mixer (IKA; Germany) for 1 min at 14,000 rpm. The extract was transferred quantitatively into an extraction tube with 14 mL chloroform:methanol (2:1 mL/mL), followed by addition of 4.6 mL of KCl (8.8 g/L) (Kaluzny, Duncan, Merritt, & Eppse, 1985). Subsequently, the tube was centrifuged for 10 min at 224× *g*. The bottom fraction containing coffee lipids was collected and stored at −20 °C until the next analytical step of lipid class separation.

The lipid fractions of interest (TAG and FFA), in half mL of sample, were separated in solid phase extraction cartridges (aminopropyl, 500 μm; Varian, USA) according to Kaluzny et al. (1985). The transesterification of both TAG and FFA fractions was performed according to the method of Lepage and Roy (1986). Samples were stored under N_2 atmosphere at −20 °C until GC analysis.

2.5. Analysis and identification of fatty acids methyl esters by gas chromatography

Gas-chromatographic peaks of FAME (Fatty Acids Methyl Esters) were identified by comparing the retention time data of certified standards with the sample retention data, expressed as relative retention times. The FAME standard mixtures used were 47 FAME Mix (ref. 47 885-U; Supelco Co.). Peaks eluting at the retention times of the FAME standards were confirmed by GC–MS. The FAME was analyzed by capillary GC according to Torres, Ney, Meneses, and Trugo (2006). Analyses were performed using a Shimadzu QP5050 GC (Kyoto, Japan). A Omegawax™ 250 (30 m × 0.25 mm × 0.25 μm film thickness) column purchased from Supelco Co. (Bellefonte, PA, USA) was used. The chromatographic conditions were: injection mode – split 1:20, injection temperature – 250 °C; column temperature setting – 160 °C (2 min) to 210 °C (15 min) at 2.5 °C/min.; detector – FID, detector temperature – 280 °C; carrier gas – helium; flow – 2.5 mL/min.

The quantifications of individual fatty acids in TAG and FFA fractions were achieved with quantitative addition of appropriate internal standards (margaric acid for FFA and trinonadecanoate for TAG; both from Sigma–Aldrich). Peak areas were used for calculating the concentration of fatty acids. After correcting the peak areas with Ackman and Sipos theoretical correction factors, as described by Wolff, Bayard, and Fabien (1995), the amount of fatty acids (mg/100 g total fatty acids) was calculated for all the samples.

2.6. Statistical analysis

Results were analyzed by factorial ANOVA (Statistica®, version 8.0, USA). Fisher LSD test was used to compare means (Statistica®, version 8.0, USA). P values < 0.05 were considered significant.

3. Results and discussion

Since previous studies have shown that the presence of defective seeds and or microorganisms contamination may alter coffee's chemical composition and cell wall structure (Dentan, 1987; Mazzafera, 1999), to prevent that changes in lipid fraction were influenced by factors other than natural changes during storage, the coffee sample used in the present experiment was of excellent quality and contained no defective seeds. Coffee seeds were roasted to reach two roasting degrees, light-medium and dark-medium, commonly used in major global consumer markets like the U.S. (in the case of light-medium roast), Brazil and Europe (in the case of dark-medium roast).

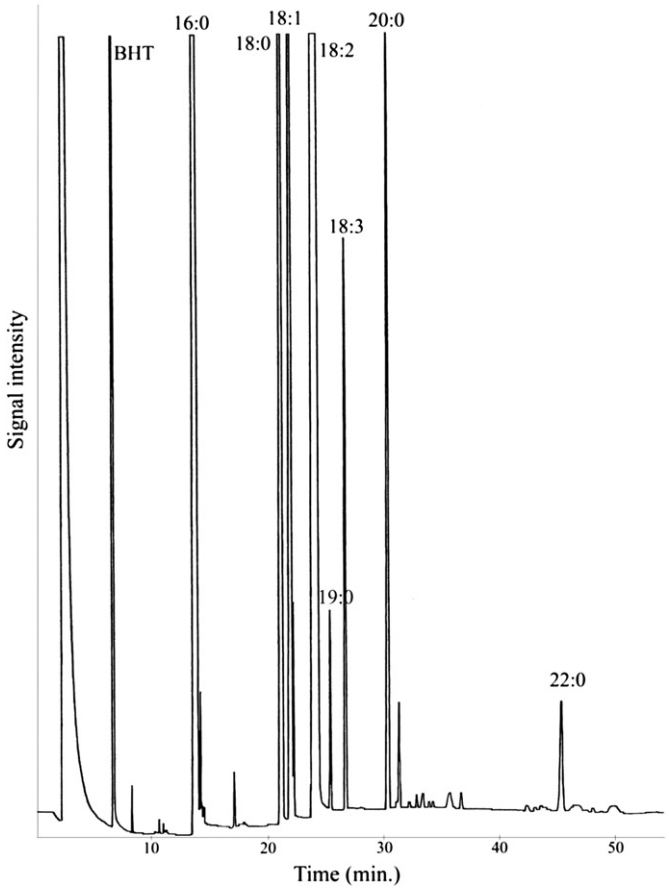
The total lipid contents observed in the samples roasted to light-medium and dark-medium roasting degrees were 10.2 g/100 g and 14.0 g/100 g (dry basis), respectively. These values agree with those from Oliveira et al. (2006) and Trugo (2003), who reported values from 11 to 20 g/100 g, for roasted *C. arabica*. Also in our previous work (Toci et al., 2006), values from 11 to 16 g/100 g were reported for light-medium and dark-medium roasting degrees, respectively. The increase in total lipid content in the darker coffee samples is actually relative and may be attributed to loss of other organic

compounds, such as carbohydrates, proteins, trigonelline and chlorogenic acids (Toci et al., 2006; Trugo, 2003).

Seven among the most important fatty acids in coffee were investigated in both TAG and FFA fractions: palmitic (16:0), stearic (18:0), oleic (18:1n-9), linoleic (18:2n-6), linolenic (18:3n-3), arachidic (20:0) and behenic (22:0) acids. The results for both fractions are presented separately bellow (Fig. 1).

3.1. TAG fraction

The TAG contents in roasted coffee samples before and after storage are presented in Tables 1 and 2. In the freshly roasted light-medium sample (control), TAG contents corresponded to 7.5 g/100 g, equivalent to about 74% of total lipids (Table 1). This is in agreement with the literature, which reports values ranging from 8 to 15 g/100 g (Folstar, 1985; Nikolova-Damyanova et al., 1998). In the dark-medium samples, TAG content decreased to 6.65 g/100 g (Table 2), a 11% decrease between the two roasting degrees. This difference might be attributed to hydrolytic and oxidative degradation of the lipid fraction during roasting, although Folstar (1985) did not report changes in fatty acids' contents and percent distribution with increasing roasting degree. In both roasting degrees (Tables 1 and 2), 16:0 and 18:2n-6 were the dominant fatty acids in TAG fraction, with contents ranging from 1.98 to 2.25 g/100 g and 3.37–3.79 g/100 g, respectively, in agreement with the study by Nikolova-Damyanova et al. (1998) and Lercker et al. (1996). The samples also presented reasonable contents of 18:0 (0.46–0.56 g/100 g) and 18:1n-9 (0.58–0.64 g/100 g), the contents of 18:3n-3 and 20:0 ranged from 0.11 to 0.14 g/100 g, and, finally, the lowest content was observed for 22:0 in both samples (25 mg/100 g), which is also in agreement with results from Nikolova-Damyanova et al. (1998) and Lercker et al. (1996) (Tables 1 and 2).



16:0 - palmitic, 18:0 - stearic, 18:1n-9 - oleic, 18:2n-6 - linoleic, 19:0 trinonadecanoate (standard), 18:3n-3 - linolenic, 20:0 – arachidic, 22:0 - behenic acids. BHT – butylated hydroxytoluene.

Fig. 1. Typical chromatogram of triacylglycerol fraction in light-medium roasted coffee prior to storage.

The total content of TAG seems to have increased during the 1st month of storage of light-medium sample, and during the 1st and 2nd months in the dark-medium sample (Tables 1 and 2). This increase might be caused by the actual loss of other coffee components such as volatile compounds (Pérez-Martinez et al., 2008; Toci, 2010). Another possibility is that the TAG fraction might be associated with other chemical structures, for example, proteins that would dissociate during storage and produce an increase in TAG content. Nevertheless, during storage, a continuous decrease in TAG content was observed in the light-medium sample, with losses of 7% after 2 months of storage, 36% after 4 months and 42% after 5 months (Fig. 2). Similar behavior was observed in the dark-medium sample, with losses of 35% after 3 months and 51% after 6 months of storage (Fig. 2). These results indicate hydrolysis of TAG.

The ratio between unsaturated and saturated fatty acids (Σ UFA/SFA) may be used as a tool to understand some responses to storage. In the light-medium sample, this ratio was nearly constant during the whole period of storage, ranging from 1.24 to 1.70 (Table 1). In the dark-medium sample, a different behavior was observed (Table 2). Until the 2nd storage month, the ratios were similar to those observed in the light-medium degree sample, ranging from 1.31 to 1.38 (Table 2). However, after the 3rd storage month, the ratio began to decrease, ranging from 1.06 to 1.38 until the 6th month, where there was a complete inversion in Σ UFA/SFA values, which ranged from 0.72 to 0.73. This phenomenon is better visualized in Fig. 2, where total contents of SFA and UFA were plotted. Based on 1.3-random-2-random distribution, Folstar (1985) studied the positional distribution of fatty acids in the triglyceride molecule of roasted coffee. It was shown that the UFA, specially linoleic acid (18:2), are preferably esterified with the secondary hydroxyl position of glycerol, resulting in two abundant species, PLP and PLL (P = 16:0 and L = 18:2). The 2-position of glycerol is more protected than 1- and 3-positions, implying that the 16:0 would be released in a faster speed than the 18:2. Additionally, it was observed that increased FA unsaturated on carbon 2 increased TAG stability (Neff & EI-Agaimy, 1996; Wada & Koizumi, 1983). Considering these studies, that 16:0 and 18:2 were major components in both TAG and FFA classes, and that the hydrolysis reaction also produces diacylglycerols and monoacylglycerols, we can suppose that the inversion phenomenon of the unsaturated and saturated contents observed after 6 months of storage , was an effect related with TAG species. It is possible that after the 6th month, for the dark-medium roasting degree, the hydrolysis of 18:2 in position 2 has been initiated, which might have resulted in an abrupt decrease of its content in TAG fraction and in an expected increase in the FFA fraction (Fig. 2). The present results agree with previous studies that showed the loss of aromatic viability after 5 or 6 months of storage (Banggenstoss, Poisson, Luethi, Perren, & Escher, 2007; Marin, Pozrl, Zlatic, & Plestenjak, 2008). Therefore, Σ UFA/SFA measurement appears to be a promising potential tool to evaluate the shelf life of roasted coffee. However, for light-medium roasted sample, due to a higher TAG content, the inverse phenomenon should occur later, because the concentrations of UFA and SFA were becoming similar in both TAG and FFA fractions (Fig. 2), requiring further investigation.

Temperature and atmosphere alone did not influence significantly the concentration of TAG in stored coffee samples (Table 3). Time alone had a significant effect in stored light-medium and dark-medium samples and the interaction between time and

Table 1
Changes in triacylglicerols content (mg/100 g) of light-medium roasted Arabica coffee stored during 6 months at different temperature and atmosphere conditions.

				Fatty acids								Σ SFA	Σ UFA	Σ UFA/SFA
				(16:0)	(18:0)	(18:1)	(18:2)	(18:3)	(20:0)	(22:0)	Total			
Freshly roasted coffee (control)				2253 ± 26	557 ± 9	640 ± 7	3778 ± 18	108 ± 0	139 ± 13	25 ± 1	7496 ± 30	2970	4527	1.52
Storage period, temperature and atmosphere	1 month	30 °C	N_2	3112 ± 349	818 ± 103	846 ± 12	4465 ± 39	119 ± 1	216 ± 33	42 ± 4	9616 ± 540	4187	5429	1.30
			Air	2901 ± 165	776 ± 49	855 ± 16	4446 ± 62	120 ± 8	229 ± 12	63 ± 19	9388 ± 277	3968	5420	1.37
		5 °C	N_2	2645 ± 170	677 ± 65	717 ± 70	3990 ± 368	106 ± 1	218 ± 37	54 ± 13	8406 ± 875	3594	4812	1.34
			Air	3286 ± 477	855 ± 132	891 ± 141	4513 ± 296	115 ± 7	247 ± 46	61 ± 13	9968 ± 873	4449	5519	1.24
	2 months	30 °C	N_2	2123 ± 86	545 ± 66	622 ± 21	3668 ± 46	116 ± 6	134 ± 13	27 ± 5	7232 ± 139	2827	4405	1.56
			Air	2168 ± 408	524 ± 158	555 ± 83	3439 ± 229	114 ± 11	149 ± 56	32 ± 10	6980 ± 862	2873	4107	1.43
		5 °C	N_2	2169 ± 17	525 ± 4	583 ± 3	3476 ± 30	104 ± 1	158 ± 2	32 ± 0	7047 ± 51	2884	4163	1.44
			Air	1977 ± 86	503 ± 9	573 ± 23	3253 ± 300	97 ± 10	145 ± 33	26 ± 1	6572 ± 501	2650	3922	1.48
	3 months	30 °C	N_2	2077 ± 26	525 ± 6	620 ± 13	3639 ± 57	106 ± 1	133 ± 0	27 ± 0	7127 ± 102	2762	4365	1.58
			Air	2313 ± 116	421 ± 203	692 ± 17	3768 ± 45	110 ± 4	145 ± 1	28 ± 0	7477 ± 20	2907	4569	1.57
		5 °C	N_2	2204 ± 58	474 ± 101	659 ± 11	3735 ± 6	108 ± 2	140 ± 1	28 ± 0	7348 ± 24	2846	4501	1.58
			Air	2479 ± 202	626 ± 58	710 ± 55	3973 ± 222	106 ± 2	157 ± 17	31 ± 3	8082 ± 395	3293	4789	1.45
	4 months	30 °C	N_2	1409 ± 1	347 ± 4	417 ± 4	2545 ± 82	79 ± 4	87 ± 1	19 ± 0	4904 ± 86	1862	3041	1.63
			Air	1401 ± 6	345 ± 3	420 ± 2	2535 ± 9	83 ± 4	88 ± 2	17 ± 0	4889 ± 15	1851	3038	1.64
		5 °C	N_2	1463 ± 122	314 ± 49	385 ± 27	2120 ± 367	101 ± 36	92 ± 5	21 ± 6	4497 ± 438	1891	2606	1.38
			Air	1405 ± 19	329 ± 23	421 ± 13	2618 ± 147	84 ± 4	88 ± 2	19 ± 3	4963 ± 156	1841	3122	1.70
	5 months	30 °C	N_2	1451 ± 11	365 ± 13	394 ± 11	2070 ± 90	50 ± 6	91 ± 4	19 ± 2	4440 ± 78	1927	2514	1.30
			Air	1269 ± 208	353 ± 48	349 ± 89	1754 ± 354	38 ± 24	90 ± 9	20 ± 1	3872 ± 369	1731	2141	1.24
		5 °C	N_2	1437 ± 4	363 ± 10	396 ± 16	2106 ± 24	58 ± 10	93 ± 1	20 ± 2	4472 ± 45	1913	2559	1.34
			Air	1419 ± 1	338 ± 2	369 ± 5	2211 ± 27	65 ± 3	90 ± 4	18 ± 1	4512 ± 21	1866	2646	1.42
	6 months	30 °C	N_2	1413 ± 289	366 ± 10	419 ± 12	2352 ± 109	65 ± 18	96 ± 26	19 ± 5	4730 ± 319	1524	2286	1.50
			Air	1449 ± 2	372 ± 3	420 ± 18	2300 ± 14	62 ± 3	95 ± 2	20 ± 0	4718 ± 41	1936	2782	1.44
		5 °C	N_2	1374 ± 36	368 ± 11	433 ± 18	2451 ± 142	69 ± 3	98 ± 5	19 ± 0	4813 ± 144	1860	2953	1.59
			Air	1415 ± 15	358 ± 13	405 ± 30	2305 ± 36	64 ± 2	94 ± 1	18 ± 1	4660 ± 98	1886	2774	1.47

(±) Standard deviation from triplicate values; saturated fatty acids (SFA); unsaturated fatty acids (UFA).

Table 2
Changes in triacylglicerols content (mg/100 g) of dark-medium roasted Arabica coffee stored during 6 months at different temperature and atmosphere conditions.

				Fatty acids								Σ SFA	Σ UFA	Σ UFA/SFA
				(16:0)	(18:0)	(18:1)	(18:2)	(18:3)	(20:0)	(22:0)	Total			
Freshly roasted coffee (control)				1976 ± 219	459 ± 42	584 ± 37	3372 ± 132	114 ± 3	117 ± 13	26 ± 4	6648 ± 314	2578	4070	1.58
Storage period, temperature and atmosphere	1 month	30 °C	N_2	2395 ± 313	608 ± 122	743 ± 98	3842 ± 286	106 ± 15	154 ± 33	49 ± 19	7897 ± 775	3205	4691	1.46
			Air	2546 ± 360	442 ± 70	413 ± 79	3810 ± 637	111 ± 2	162 ± 44	30 ± 14	7514 ± 934	3180	4334	1.36
		5 °C	N_2	2209 ± 203	517 ± 82	602 ± 149	3469 ± 392	107 ± 0	164 ± 31	32 ± 6	7101 ± 672	2922	4179	1.43
			Air	2309 ± 272	575 ± 82	682 ± 106	3820 ± 300	105 ± 2	143 ± 21	33 ± 10	7666 ± 794	3060	4606	1.51
	2 months	30 °C	N_2	2315 ± 185	552 ± 53	611 ± 43	3653 ± 112	103 ± 3	204 ± 88	35 ± 15	7508 ± 548	3107	4367	1.41
			Air	3001 ± 281	605 ± 24	702 ± 20	4373 ± 160	124 ± 6	136 ± 28	28 ± 9	9194 ± 372	3770	5199	1.38
		5 °C	N_2	2311 ± 189	554 ± 52	614 ± 53	3673 ± 228	102 ± 2	158 ± 48	34 ± 3	7545 ± 708	3056	4388	1.44
			Air	2627 ± 98	644 ± 5	689 ± 5	3921 ± 39	105 ± 4	211 ± 76	34 ± 2	8231 ± 225	3515	4716	1.34
	3 months	30 °C	N_2	1429 ± 11	341 ± 16	362 ± 3	2143 ± 138	60 ± 10	82 ± 7	18 ± 1	4434 ± 132	1869	2565	1.37
			Air	1428 ± 19	358 ± 9	362 ± 7	1912 ± 122	46 ± 5	77 ± 13	19 ± 1	4202 ± 120	1882	2320	1.23
		5 °C	N_2	1420 ± 6	363 ± 9	373 ± 16	1966 ± 179	46 ± 7	96 ± 3	20 ± 0	4282 ± 189	1897	2384	1.26
			Air	1406 ± 12	345 ± 10	368 ± 2	2128 ± 183	59 ± 14	88 ± 2	19 ± 0	4414 ± 179	1858	2555	1.37
	4 months	30 °C	N_2	1469 ± 12	370 ± 21	381 ± 2	1959 ± 197	49 ± 19	101 ± 3	22 ± 1	4350 ± 209	1962	2389	1.22
			Air	1469 ± 3	360 ± 15	381 ± 28	1960 ± 11	47 ± 2	90 ± 5	19 ± 4	4326 ± 58	1938	2388	1.23
		5 °C	N_2	1438 ± 27	349 ± 7	377 ± 11	2170 ± 208	62 ± 15	89 ± 4	19 ± 1	4504 ± 206	1896	2608	1.38
			Air	1478 ± 8	348 ± 3	353 ± 1	1945 ± 90	58 ± 17	104 ± 25	20 ± 9	4307 ± 135	1951	2355	1.21
	5 months	30 °C	N_2	1531 ± 159	385 ± 40	392 ± 22	2071 ± 119	49 ± 6	98 ± 14	20 ± 4	4546 ± 364	2033	2512	1.24
			Air	1501 ± 109	365 ± 80	343 ± 53	1721 ± 82	46 ± 24	107 ± 1	23 ± 2	4105 ± 139	1996	2109	1.06
		5 °C	N_2	1416 ± 7	346 ± 18	360 ± 28	1961 ± 111	52 ± 3	94 ± 5	19 ± 0	4248 ± 142	1875	2373	1.27
			Air	1685 ± 361	408 ± 69	421 ± 62	2363 ± 568	63 ± 27	107 ± 27	21 ± 5	5068 ± 791	2221	2847	1.28
	6 months	30 °C	N_2	1341 ± 22	350 ± 28	278 ± 3	1068 ± 32	20 ± 1	132 ± 4	45 ± 7	3233 ± 40	1868	1365	0.73
			Air	1359 ± 44	337 ± 16	271 ± 4	1072 ± 0	18 ± 0	130 ± 1	54 ± 22	3241 ± 42	1879	1362	0.72
		5 °C	N_2	1326 ± 4	327 ± 0	262 ± 3	1042 ± 17	17 ± 0	126 ± 8	24 ± 19	3123 ± 12	1802	1321	0.73
			Air	1392 ± 91	347 ± 31	281 ± 12	1103 ± 17	19 ± 0	135 ± 9	83 ± 64	3359 ± 97	1957	1403	0.72

(±) Standard deviation from triplicate values; saturated fatty acids (SFA); unsaturated fatty acids (UFA).

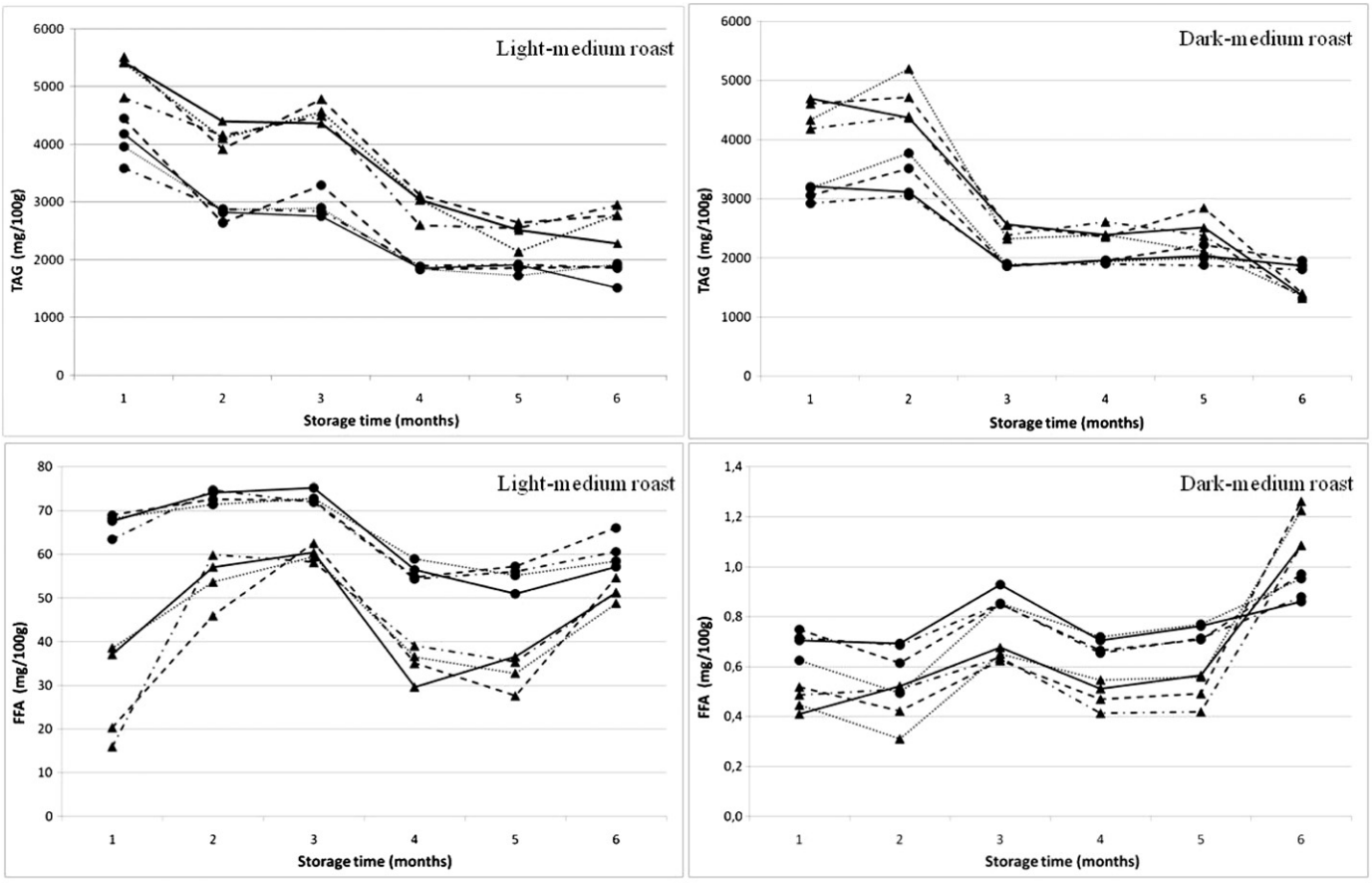


Fig. 2. Total saturated and unsaturated fatty acids contents in light-medium and dark-medium roasted coffee samples stored for 6 months, under different conditions (5 and 30 °C; N_2 and Air).

atmosphere had a significant effect in stored light-medium samples (Table 3). Ortalá, Gutiérrez, Chiralt, and Fito (1998) observed that the formation of hydroperoxides in green coffee seeds was dependent on storage temperature in samples with low moisture content, but it was almost temperature-independent in hydrated samples. Given the fact that coffee is highly hygroscopic (Ortalá et al., 1998), it is probable that the water adsorbed in the samples was the major cause for TAG hydrolysis during storage (Fig. 2), and therefore could have blunted the effects of temperature and atmosphere on TAG reduction during storage. On the other hand, the roasting process promotes free radical formation and is associated with pyrolysis reactions (Morrice, Deighton, Glidewell, & Goodman, 1993) that can accelerate degradation. Possibly, free radicals initially present in all the fresh coffee samples might explain the absence of significant differences between inert and oxidizing atmospheres. The interaction between storage time and atmosphere influenced the total TAG content in the 1st, 3rd, and 4th months of storage of light-medium samples (Fig. 2). During these months, the highest contents of TAG were observed in samples under oxidant atmosphere (Fig. 2 and Table 1). It is possible that losses of more thermolabile compounds in oxidant atmosphere, as previously mentioned (Pérez-Martínez et al., 2008; Toci, 2010), have caused this apparent increment in TAG contents.

Sigmoidal kinetic curves were obtained for TAG degradation in both roasting degrees (Fig. 2). This indicates a two-step hydrolysis process. In Fig. 2, two periods of stability may be observed in total contents of TAG during storage, from 2 to 3 months and from 4 to 6 months of storage for the light-medium sample, and from 1 to 2 months and from 3 to 5 months of storage for the dark-medium sample. These results suggest a decrease in hydrolysis in these periods. Ortalá et al. (1998) also observed a slow kinetic of lipid degradation during the first 100 days (≈3 months) of storage, followed by 100 days of stability. The classical molecular model for lipid oxidation (Frankel, 2005) establishes that reactions occur through a chain mechanism controlled by free radical formation, with three typical steps: initiation, propagation, and termination. The main factor affecting the reaction rate was the initiation reaction. On the basis of the model of Koelsch, Downes, and Labuza (1991), as well as on the basis of the present data, it appears that

Table 3
P-levels from ANOVA results for triacylglycerols (TAG) and free fatty acids (FFA) contents in roasted Arabica coffee as affected by storage time, temperature and atmosphere, and two- and three-level interactions.

Factors	Light-medium coffee		Dark-medium coffee	
	TAG	FFA	TAG	FFA
Storage time (t; months)	0.000	0.000	0.000	0.000
Storage temperature (T; °C)	0.444	0.257	0.712	0.611
Storage atmosphere (Atm)	0.748	0.462	0.143	0.366
$t \times T$	0.528	0.003	0.730	0.010
$t \times$ Atm	0.003	0.178	0.215	0.000
Atm $\times T$	0.572	0.910	0.396	0.539
$t \times T \times$ Atm	0.732	0.540	0.386	0.552

P values <0.05 were considered significant.

Table 4
Changes in free fatty acids content (mg/100 g) of light-medium roasted Arabica coffee stored during 6 months at different temperature and atmosphere conditions.

				Fatty acids								Σ SFA	Σ UFA	Σ UFA/SFA
				(16:0)	(18:0)	(18:1)	(18:2)	(18:3)	(20:0)	(22:0)	Total			
Freshly roasted coffee (control)				0.29 ± 0	0.07 ± 0	nd	nd	nd	0.03 ± 0	nd	0.39 ± 0	0.4	0.0	–
Storage period, temperature and atmosphere	1 month	30 °C	N_2	47.70 ± 1.87	13.30 ± 2.56	7.29 ± 2.84	29.54 ± 12.86	0.30 ± 0	4.80 ± 0.05	1.84 ± 0.18	104.76 ± 8.06	67.6	37.1	0.55
			Air	49.53 ± 4.49	12.64 ± 1.68	7.52 ± 1.87	30.78 ± 8.63	0.27 ± 0.21	4.97 ± 0.37	1.15 ± 0.24	106.84 ± 2.82	68.3	38.6	0.56
		5 °C	N_2	44.83 ± 3.73	11.21 ± 0.97	3.16 ± 0.22	12.57 ± 0.14	0.24 ± 0.05	6.13 ± 1.95	1.31 ± 0.15	79.43 ± 1.54	63.5	16.0	0.25
			Air	49.22 ± 1.03	12.07 ± 0.40	5.14 ± 1.22	15.00 ± 3.27	0.23 ± 0.04	5.90 ± 1.79	1.84 ± 0.23	89.38 ± 1.06	69.0	20.4	0.30
	2 months	30 °C	N_2	54.48 ± 0.05	13.39 ± 0.05	10.02 ± 2.50	41.46 ± 4.04	0.62 ± 0.30	5.05 ± 0.05	1.25 ± 0.07	131.25 ± 4.86	74.2	57.1	0.77
			Air	52.28 ± 1.03	12.79 ± 0.30	10.50 ± 0.71	42.52 ± 2.10	0.63 ± 0.04	4.92 ± 0.12	1.47 ± 0.05	125.08 ± 3.02	71.4	53.6	0.75
		5 °C	N_2	54.56 ± 0.01	13.28 ± 0.07	11.44 ± 0.03	47.69 ± 0.04	0.78 ± 0.03	5.43 ± 0.75	1.50 ± 0.14	134.68 ± 0.54	74.8	59.9	0.80
			Air	52.95 ± 0.34	13.11 ± 0.01	8.90 ± 1.88	36.42 ± 8.48	0.68 ± 0.05	5.01 ± 0.01	1.52 ± 0.02	118.57 ± 7.60	72.6	46.0	0.63
	3 months	30 °C	N_2	55.24 ± 1.39	13.53 ± 0.33	11.54 ± 0.01	48.04 ± 0.02	0.86 ± 0.06	5.23 ± 0.27	1.25 ± 0.35	135.69 ± 1.21	75.3	60.4	0.80
			Air	53.38 ± 1.88	13.28 ± 0.47	11.44 ± 0.10	47.21 ± 0.18	0.80 ± 0.02	4.74 ± 0.54	1.47 ± 0.05	132.32 ± 2.05	72.9	59.5	0.82
		5 °C	N_2	52.63 ± 0.52	12.95 ± 0.08	10.94 ± 0.09	46.43 ± 2.21	0.83 ± 0.10	5.00 ± 0.28	1.35 ± 0.07	130.13 ± 2.27	71.9	58.2	0.81
			Air	54.13 ± 0.24	13.47 ± 0.11	11.94 ± 0.35	50.09 ± 2.50	0.57 ± 0.33	3.60 ± 1.88	1.08 ± 0.56	134.88 ± 0.31	72.3	62.6	0.87
	4 months	30 °C	N_2	39.46 ± 4.31	10.10 ± 0.18	5.99 ± 0.94	23.49 ± 5.13	0.18 ± 0.14	5.61 ± 2.28	1.30 ± 0.14	86.12 ± 5.65	56.5	29.7	0.53
			Air	41.70 ± 3.23	10.57 ± 0.72	7.12 ± 0.64	28.96 ± 2.06	0.51 ± 0.09	4.79 ± 1.15	1.87 ± 0.70	95.50 ± 6.08	58.9	36.6	0.62
		5 °C	N_2	43.16 ± 3.28	9.86 ± 1.39	7.49 ± 0.73	31.18 ± 3.43	0.42 ± 0.12	4.78 ± 1.56	1.53 ± 0.28	93.43 ± 5.02	54.3	39.1	0.72
			Air	39.39 ± 0.17	9.92 ± 0.09	6.87 ± 0.08	27.72 ± 0.34	0.49 ± 0.01	4.11 ± 0.53	1.37 ± 0.01	89.85 ± 0.84	54.8	35.1	0.64
	5 months	30 °C	N_2	36.18 ± 0.42	8.83 ± 0.11	7.06 ± 0.03	29.05 ± 0.02	0.48 ± 0.01	3.27 ± 0.03	2.64 ± 0.92	87.51 ± 1.01	50.9	36.6	0.72
			Air	40.48 ± 6.00	9.81 ± 1.34	6.21 ± 2.16	26.10 ± 9.05	0.44 ± 0.17	3.68 ± 0.36	1.15 ± 0.02	87.87 ± 13.47	55.1	32.8	0.59
		5 °C	N_2	40.11 ± 4.39	11.03 ± 1.21	7.42 ± 0.60	27.34 ± 7.55	0.62 ± 0.12	3.61 ± 0.01	1.30 ± 0.30	91.43 ± 7.71	56.1	35.4	0.63
			Air	40.28 ± 4.64	12.13 ± 1.58	6.98 ± 3.71	20.18 ± 2.57	0.47 ± 0.24	3.82 ± 0.59	1.03 ± 0.32	84.89 ± 1.80	57.3	27.6	0.48
	6 months	30 °C	N_2	41.00 ± 1.41	11.05 ± 1.34	9.35 ± 0.21	41.25 ± 1.06	0.65 ± 0.07	4.05 ± 0.07	1.05 ± 0.07	108.40 ± 3.00	57.2	51.3	0.90
			Air	42.09 ± 1.29	11.07 ± 1.32	9.56 ± 0.62	38.56 ± 2.03	0.67 ± 0.04	3.95 ± 0.07	1.32 ± 0.26	107.22 ± 3.98	58.4	48.8	0.84
		5 °C	N_2	44.00 ± 1.41	11.18 ± 0.45	9.33 ± 0.47	41.40 ± 0.84	0.63 ± 0.04	4.16 ± 0.05	1.26 ± 0.05	111.96 ± 0.36	60.6	51.4	0.85
			Air	48.56 ± 0.50	11.68 ± 0.05	10.36 ± 0.08	43.63 ± 0.34	0.71 ± 0.01	4.34 ± 0.04	1.53 ± 0.41	120.82 ± 0.40	66.1	54.7	0.83

(±) Standard deviation from triplicate values; saturated fatty acids (SFA); unsaturated fatty acids (UFA).

Table 5
Changes in free fatty acids content (mg/100 g) of dark-medium roasted Arabica coffee stored during 6 months at different temperature and atmosphere conditions.

				Fatty acids								Σ SFA	Σ UFA	Σ UFA/SFA
				(16:0)	(18:0)	(18:1)	(18:2)	(18:3)	(20:0)	(22:0)	Total			
Freshly roasted coffee (control)				nd	nd	nd	nd	nd	nd	nd	nd	0	0	–
Storage period, temperature and atmosphere	1 month	30 °C	N_2	0.51 ± 0.06	0.13 ± 0	0.10 ± 0.05	0.30 ± 0.06	0.01 ± 0.01	0.06 ± 0.01	0.02 ± 0	0.97 ± 0.09	0.70	0.41	0.58
			Air	0.46 ± 0.07	0.11 ± 0.02	0.09 ± 0.02	0.35 ± 0.08	0.01 ± 0	0.04 ± 0	0.01 ± 0	1.07 ± 0.19	0.62	0.45	0.71
		5 °C	N_2	0.53 ± 0	0.13 ± 0	0.10 ± 0	0.39 ± 0.01	0.01 ± 0	0.05 ± 0	0.01 ± 0	1.20 ± 0.02	0.72	0.49	0.68
			Air	0.55 ± 0.01	0.13 ± 0	0.10 ± 0.01	0.41 ± 0.03	0.01 ± 0	0.05 ± 0	0.01 ± 0	1.27 ± 0.02	0.75	0.52	0.69
	2 months	30 °C	N_2	0.51 ± 0.01	0.12 ± 0	0.10 ± 0	0.41 ± 0.01	0.01 ± 0.01	0.05 ± 0	0.01 ± 0	1.21 ± 0.02	0.69	0.52	0.75
			Air	0.37 ± 0.01	0.08 ± 0	0.06 ± 0	0.24 ± 0.01	t	0.03 ± 0	0.01 ± 0	0.80 ± 0.03	0.49	0.31	0.63
		5 °C	N_2	0.50 ± 0	0.12 ± 0	0.10 ± 0	0.40 ± 0.01	0.01 ± 0	0.05 ± 0	0.01 ± 0	1.20 ± 0.01	0.69	0.51	0.74
			Air	0.45 ± 0	0.11 ± 0	0.08 ± 0	0.33 ± 0.01	0.01 ± 0	0.05 ± 0.01	0.01 ± 0.01	1.04 ± 0	0.61	0.42	0.69
	3 months	30 °C	N_2	0.69 ± 0	0.16 ± 0	0.13 ± 0	0.53 ± 0	0.01 ± 0	0.06 ± 0.01	0.02 ± 0.01	1.60 ± 0.01	0.93	0.68	0.73
			Air	0.62 ± 0	0.15 ± 0	0.13 ± 0	0.51 ± 0	0.01 ± 0	0.06 ± 0	0.02 ± 0	1.50 ± 0	0.85	0.65	0.76
		5 °C	N_2	0.63 ± 0.02	0.14 ± 0	0.13 ± 0	0.50 ± 0	0.01 ± 0	0.06 ± 0	0.02 ± 0	1.49 ± 0.03	0.85	0.64	0.75
			Air	0.63 ± 0.04	0.15 ± 0.01	0.12 ± 0.01	0.49 ± 0.04	0.01 ± 0	0.06 ± 0	0.01 ± 0	1.47 ± 0.09	0.85	0.62	0.74
	4 months	30 °C	N_2	0.52 ± 0	0.13 ± 0	0.10 ± 0	0.40 ± 0.02	0.01 ± 0	0.05 ± 0	0.01 ± 0	1.22 ± 0.03	0.70	0.51	0.73
			Air	0.53 ± 0.02	0.13 ± 0	0.11 ± 0	0.43 ± 0.02	0.01 ± 0	0.05 ± 0	0.01 ± 0	1.27 ± 0.04	0.72	0.55	0.76
		5 °C	N_2	0.47 ± 0.02	0.12 ± 0	0.08 ± 0.02	0.33 ± 0.09	0.01 ± 0	0.05 ± 0	0.01 ± 0	1.07 ± 0.14	0.65	0.41	0.63
			Air	0.48 ± 0	0.12 ± 0	0.09 ± 0.01	0.37 ± 0.03	0.01 ± 0	0.05 ± 0	0.01 ± 0	1.13 ± 0.05	0.66	0.47	0.71
	5 months	30 °C	N_2	0.56 ± 0.01	0.14 ± 0	0.11 ± 0	0.45 ± 0.01	0.01 ± 0	0.05 ± 0	0.02 ± 0	1.33 ± 0.02	0.76	0.57	0.74
			Air	0.55 ± 0	0.14 ± 0	0.11 ± 0	0.44 ± 0.02	0.01 ± 0	0.05 ± 0	0.03 ± 0	1.33 ± 0	0.77	0.56	0.73
		5 °C	N_2	0.52 ± 0.01	0.13 ± 0	0.08 ± 0.01	0.33 ± 0.05	0.01 ± 0	0.05 ± 0	0.01 ± 0	1.13 ± 0.05	0.71	0.42	0.59
			Air	0.52 ± 0.01	0.13 ± 0	0.10 ± 0	0.39 ± 0.01	0.01 ± 0	0.05 ± 0	0.01 ± 0	1.20 ± 0.03	0.71	0.49	0.70
	6 months	30 °C	N_2	0.63 ± 0.02	0.17 ± 0.03	0.17 ± 0.01	0.90 ± 0.03	0.02 ± 0.01	0.04 ± 0.01	0.01 ± 0.01	1.95 ± 0.02	0.86	1.09	1.26
			Air	0.73 ± 0.04	0.18 ± 0.01	0.19 ± 0.01	1.01 ± 0.06	0.03 ± 0	0.04 ± 0	0.01 ± 0	2.18 ± 0	0.95	1.23	1.29
		5 °C	N_2	0.66 ± 0.04	0.17 ± 0.02	0.17 ± 0.01	0.89 ± 0.05	0.02 ± 0.01	0.04 ± 0	0.01 ± 0	1.97 ± 0.13	0.88	1.09	1.23
			Air	0.73 ± 0.05	0.18 ± 0.01	0.19 ± 0.01	1.04 ± 0.09	0.03 ± 0	0.05 ± 0	0.01 ± 0	2.23 ± 0.17	0.97	1.26	1.30

(±) Standard deviation from triplicate values; saturated fatty acids (SFA); unsaturated fatty acids (UFA).
t – traces.

a monomolecular or bimolecular reaction can be responsible for the initiation step of the oxidative chain in coffee, through hydroperoxide decomposition. It depends on the initial concentration of these compounds, as observed in other products. So, during the first months, the initially low hydroperoxide concentration, as also observed by Ortalá et al. (1998) for roasted coffee, favors the monomolecular initiation and, when a critical value is attained, in line with the reaction progress, the bimolecular mechanism becomes more controlled.

3.2. FFA fraction

In the light-medium control sample, FFA content was 0.4 mg/100 g (Table 4). In the dark-medium control sample, FFA fraction was not detected (Table 5). These values are in disagreement with Vila, Andueza, Paz de Peña, and Cid (2005), Trugo (2003) and Nikolova-Damyanova et al. (1998), who have reported amounts around 0.5 g/100 g for roasted coffee. This may possibly be due to the differences in initial samples' composition as well as roasting methods and degrees, or perhaps some of these studies might not have conducted the analyzes immediately after roasting, which could have caused an increase in the FFA contents. Like with the TAG fraction, the roasting degree directly influenced the content of FFA in roasted samples.

The total content of FFA increased dramatically during the 1st month of storage, from 0.4 mg/100 g to 95.1 mg/100 g, in the light-medium sample (Table 4), and from non-detected to 1.1 mg/100 g in the dark-medium sample (Table 5). In both light-medium and dark-medium samples, the total contents of FFA increased continuously up to the 3rd month of storage (Fig. 2). These results are consistent with TAG hydrolysis, and with the decreases observed in the TAG contents in light and dark-medium samples after 1 and 2 months of storage, respectively (Fig. 2). However, FFA contents decreased after 3 months of storage, in both light medium and dark medium samples, indicating that other chemical transformations might have affected FFA contents during this storage period. It is possible that during this storage period the rate of loss overcame the rate of FFA production through TAG hydrolysis. Oxidation of FFA could explain the loss of FFA, since this lipid fraction is more susceptible to oxidation than esterified FA in TAG molecules (Kim & Min, 2008). It is expected that oxidation of FFA was already occurring before 2 months of storage, as verified below.

When individual FA were considered, the percent loss seemed to increase with the number of double bonds. 24%, 40%, 42% and 45% decreases were observed in 18:0, 18:1n-9, 18:2n-6 and 18:3n-3, respectively, after three months storage of the light-medium sample (Table 4). These results are consistent with the hypothesis that FFA are at least partially degraded through oxidative reactions, since the relative rates of unsaturated FA oxidation are directly associated with the number of double bonds (Frankel, 2005).

In the samples roasted to both roasting degrees, the total content of UFA was lower than that of SFA, an inverse behavior in relation to the TAG fraction (Fig. 2). Once again, oxidation reactions may explain this phenomenon, since the UFA in the free fraction is more susceptible to oxidation (Kim & Min, 2008). In the light-medium sample, the highest values of Σ UFA/SFA were showed after 2, 3 and 6 months of storage (ranged from 0.63 to 0.90), indicating the decrease in the difference between the contents of UFA and SFA (Table 4). In these periods, there was a slight increase in UFA content while the SFA content remained constant (Fig. 2) and coincided with TAG stability period, which indicates a decrease in oxidative reactions (Fig. 2). In the dark-medium sample, up to the 5th month, the Σ UFA/SFA ratios were similar to those observed in the light-medium degree sample, ranging from 0.58 to 0.75 (Table 5). Nonetheless, in the 6th month of storage there was a complete inversion in the UFA and SFA, leading to a change in Σ UFA/SFA ratio from 1.23 to 1.30 (Table 5), like with the TAG fraction. This phenomenon is better visualized in the Fig. 2.

Storage temperature and atmosphere alone had no significant influence on FFA contents in both roasting degrees (Table 3). As in TAG fraction, only storage time, the interaction between storage time and temperature (in both roasting degrees) and the interaction between storage time and atmosphere (in the dark-medium sample) influenced significantly the FFA results (Table 3). The interaction between temperature and storage time produced a significant difference in the levels of FFA only during the 1st storage month (light-medium sample – Table 4) and in the 5th storage month (dark-medium sample – Table 5), where the low-estrelease of FFA at 5 °C was observed in the main UFA (Fig. 2). The highest FFA contents were observed at 30 °C (Tables 4 and 5), which is in conformity with data from Speer and Kolling-Speer (2006), who reported similar results for raw coffees. Only after the 2nd storage month the interaction between atmosphere and storage time influenced significantly the contents of FFA in the dark-medium sample (Table 5), with the highest contents in the inert atmosphere. These results show that the inert atmosphere contributed to a slower loss of FFA.

4. Conclusion

In the present study, we confirmed the hypothesis of hydrolysis of triacylglicerols and oxidation of free fatty acids during storage of roasted coffee. Both atmosphere and temperature influenced these changes when associated with storage time. The use of inert atmosphere and low temperature contributed to a slower loss of free fatty acids.

The changes observed in the ratio between unsaturated and saturated fatty acids (Σ UFA/SFA) from both triacylglycerols and free fatty acids fractions during coffee storage might potentially be used as a tool to establish the shelf life for ground roasted coffee. However, the sensorial implications of these changes should also be investigated before shelf life reevaluation.

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