Coffee Bean' Lipids. A critical review of contemporary scientific literature

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Abstract: *The coffee bean´s lipids are energy reserves stored in the endosperm in the form of oil bodies intended to be used for the development of the embryo during the germination process. However, due to its low concentration in relation to the seed´s dry weight, the coffee bean is not considered an important source of vegetable oil for industrial purposes. However, when the bean is subjected to supercritical extraction processes during the production of decaffeinated coffees, as well as during the instant coffee production, the oil is isolated, but it must be subjected to subsequent purification processes to separate its different fractions to use those with industrial value. This essay focuses on the study of coffee beans' lipids (primary metabolites) before and after roasting, their influence on cup quality, biosynthesis and differences linked to the involved species, their metabolism, solubility and extraction, as well as a discussion on the analytical techniques used for its determination.*

Keywords-- *coffee oil, saponifiable lipids, unsaponifiable lipids, fatty acids, triglycerides.*

INTRODUCTION

Lipids are plant chloroplasts and mitochondria' constituents, found in seeds and fruits and constitute an energy reserve used during seed germination [1]. Lipids are characterized by their solubility in apolar solvents & their extraction is carried out with organic solvents, thus separating them from the water-soluble compounds present in plant tissue.

Vegetable lipids' chemical composition is divided in two groups: a saponifiable fraction (e.g., triglycerides, partial glycerides, fatty acid esters and free fatty acids), and a non-saponifiable fraction (~ 2%); this fraction is made up of terpenic alcohols, hydrocarbons, phenols, flavonoids, sterols, pigments, tocopherols and volatile compounds [2,3].

The qualitative & quantitative composition is determined by different analytical techniques, and it is used as a quality control tool to determine the authenticity and quality of vegetable oils [4].

In the coffee beans, lipids are located in the endosperm [2] specifically in the cytoplasm in oleaginous bodies located along the cell wall [5]. The grain lipids represent around 8-18% in a dry weight basis [5,6]. The lipid fraction is made of 75% triacyl glycerides (saponifiable fraction), 19–20% free or esterified diterpene alcohols (i.e.; cafestol, kahweol, & 16-O-methylcafestol), 5% sterols (i.e.; stigmasterol, sitosterol, & campesterol) found as free sterols (~ 2.2%) or esterified with acids (~ 3.2%), 0.05% of tocopherols and other hydrocarbons that make up the unsaponifiable fraction [6-13].

The lipid fraction prevents the compounds volatilization and the loss of flavour during the roasting process, in addition to contributing to the beverage viscosity [14].

EXTRACTION OF THE COFFEE BEAN OIL

The oil can be extracted from the raw coffee beans by cold expression. This is the most used technique to obtain marketable oil. Another alternative is the Soxhlet extraction technique that uses organic solvents, for several hours, such as petroleum ether, hexane, diethyl ether, EtOH or mixtures of them [2,14-16], but it can also be extracted through supercritical fluids using CO_2 as a solvent.

One of the advantages of working with hydro or anhydride EtOH is that it is less toxic, for the person handling it and for the environment, compared to other organic solvents [17]. Although the use of hydro EtOH results in a lower rate of lipids extraction compared to anhydride EtOH, the oil extracted with the latter one requires the use of molecular sieves to remove the water, resulting in a higher market price.

One of the problems found when organic solvents are used is the presence of lipases (lipolytic enzymes) present in the grain or produced by microorganisms, which hydrolyse the extracted lipids, increasing the percentage of free fatty acids [18]. Although the roasting process inactivates the hydrolytic enzymes, if one chooses to work with raw coffee beans, then the recommendation is to use isopropanol because, unlike other organic solvents, it inactivates lipases, thereby the lipid degradation is prevented during the extraction process [18].

Unfortunately, isopropanol requires a greater amount of energy to reach its boiling point, which makes the extraction more expensive [17]. However, the use of organic solvents with low boiling points, although they require less energy, also renders the extraction expensive since they tend to volatilize except when the extractions are carried out in hermetic devices as in the case of CO_2-based supercritical fluid extraction.

Organic solvents are not selective since they allow the extraction of pigments and other non-lipid compounds [2,19]. In addition, the Soxhlet method consumes a large amount of time and organic solvents [16]. In contrast, purification and extraction with supercritical fluids allows the extraction of better-quality oil, free of caffeine and pigments, which can be used in applications such as food processing, cosmetics, and pharmaceutical formulations, since it constitutes an important source of sterols, terpenes and tocopherols [2,8,19,20].

The supercritical fluid extraction (SFE) is the most expensive process used by industry, but it removes metabolites from the grain endosperm through supercritical fluids using different solvents that act selectively and has the advantage of a very low residual effect thus eliminating the problem associated with other methods.

Since the process is applied to raw grains, the compounds responsible for the characteristic smell & taste of roasted coffee are not removed, so the sensory qualities of roasted and ground coffee, as well as those of the beverage, aren't affected.

The SFE can be used in industrial processes such as decaffeination of caffeine-containing products (eg, coffee, tea, cocoa, mate grass, etc.) or for the recovery of specific secondary metabolites such as caffeine, lipids, and *N*-alkanoyl-5-hydroxytryptamides (C5HTs) [21,22].

CO_2 is the solvent of choice for the extraction from coffee beans because; although it doesn't dissolve it as efficiently as other solvents [23]: it is inert, it isn't corrosive, it isn't combustible, it isn't explosive, it is abundant and cheap, it isn't toxic, it is a harmless ingredient in drinks and food, it is easily separated from the metabolite of interest after extraction, it has a low molecular weight, a low surface tension and easily diffuses into the sample [24].

Raw coffee beans are soaked in water until they reach a 45% humidity to then be placed in a sealed container. Then CO_2 is injected into it at a pressure of approximately 200 atm. CO_2 is specific for caffeine and lipids, so it is removed leaving behind all other secondary metabolites thus increasing their relative concentration [24].

Then the mixture of CO_2, caffeine and lipids is transferred to a chamber where the pressure is released, while the CO_2 returns to its gaseous state, the caffeine and precipitated lipids are passed through an activated carbon filter [24] (Fig 1).

However, when CO_2-based SFE is performed in conjunction with an organic co-solvent (EtOH), the lipid extraction efficiency is even higher [25] meaning the amount of oil extracted is increased and the time required is reduced.

Despite a high triglyceride content, coffee's oil is not refined for human or animal consumption due to its high unsaponifiable lipids content (e.g., diterpenes, sterols, squalenes, & tocopherols) which would increase the refining costs [26]. The green beans extracted oil is used in cosmetics for its moisturizing properties and as sunscreen because of the linoleic acid's ability to absorb ultraviolet light [8,16].

THE COFFEE BEAN'S SAPONIFIABLE FRACTION

The saponifiable fraction is composed of fatty acids esterified with glycerol in the form of triglycerides; these represent 75% of the total lipids [2]. 43.5% of saturated fatty acids while the remaining 56.5% are unsaturated, of which a high proportion are polyunsaturated, a characteristic that makes it different from most vegetable oils where a higher concentration of monounsaturated fatty acids prevails (Table 1) [3].

The presence of free fatty acids is associated with the triacyl glycerides degradation due to poor storage conditions or contamination by fungi; In addition, a prolonged storage can activate the enzymes that favour free fatty acids oxidation [18] therefore preserving its integrity is vital to avoid its rancidity, thus prolonging its shelf life [11].

COFFEE BEANS' FATTY ACIDS CONTENT

Unsaturated linoleic acid is the most important fatty acid in coffee, but it is very susceptible to oxidation [18] and saturated palmitic acid represents most of the fatty acids present in the coffee beans endosperm [3,13,19,27]; however, small amounts of unsaturated oleic and linolenic acids are also found in addition to the saturated stearic, arachidic, lignoceric, and behenic acids (Table 1) [28-30].

The total lipid content varies considerably depending on the species [11]. In coffee, the lipid content is a highly heritable quantitative trait (*sensu stricto* heritability = 0.74) as it is governed by additive effects inherited from the father in intraspecific crosses [31].

The lipid content in *C. arabica* is approximately 14 g/100 g; that is, approximately 15% of the dry matter [2,32-34], a concentration twice as high compared to *C. canephora* grains [11,13] which registers 9.8% of total lipids [2,15,33,34].

COFFEE SAMPLES DISCRIMINATION BASED ON FATTY ACID PROFILE

The coffee beans fatty acid profile can be used to discriminate between samples based on their botanical origin (species); in addition to its varietal and regional origin [35,36].

According to several authors, Robusta coffee varieties have a higher concentration of oleic acid compared to Arabica varieties [37,38], in contrast, Arabica varieties have a higher linolenic acid concentration than Robusta (Table 1) [37,38].

Based on these parameters, the botanical origin of a series of samples can be identified if they aren't the result of a mixture of species. For example, one can differentiate between Arabica and Robusta samples when using a scatterplot to visualize the relationship between oleic acid and linolenic acid in raw or roasted samples (Fig. 2) [39,40]. Furthermore, lipids are associated with good cup characteristics in such a way that high concentrations of them improve the organoleptic properties of the beverage [10,31].

THE COFFEE BEAN FATTY ACIDS PROFILE DETERMINATION

The first step is the lipids extraction through CO_2-based supercritical extraction or by apolar organic solvents. The most widely used method for fatty acids determination (identification and quantification) in oil and fat samples is performed by derivatization into their corresponding methyl esters and subsequent analysis by capillary gas chromatography using a flame ionization detector [39].

The comparison of fatty acid profiles is a useful tool to identify the samples botanical and geographic origin [41]. Reference [39] reported that Robusta coffee samples contain a higher oleic acid content (12%) than Arabica samples (8.3%); In contrast, linoleic acid is found in a higher proportion in Arabica (1.5%) than in the Robusta samples (0.9%); however, no significant differences are reported between green and roasted coffee.

THE COFFEE BEAN UNSAPONIFIABLE FRACTION

The coffee unsaponifiable fraction can also be used as a fingerprint to identify and authenticate grain samples. Qualitative and quantitative analysis of this lipid fraction includes extraction through saponification, purification, identification and quantification.

Three main groups composed the lipid fraction: diterpenes, sterols and tocopherols [37]; However, the qualitative and quantitative composition is determined by the

Table 1. Coffee bean's fatty acids composition (% ± SD).

Fatty acid	Type	Reference [27]		Reference [18]	Reference [19]
		Arabica	Robusta	Arabica	Undefined species
Linoleic acid ($C_{18:2}$).	Polyunsaturated	45.8 ± 1.4	42.6 ± 1.3	43.6 ± 1.3	43.1
Palmitic acid ($C_{16:0}$).	Saturated	33.0 ± 1.3	32.5 ± 1.4	34.4 ± 0.2	31.1
Oleic acid ($C_{18:1}$).	Monounsaturated	8.7 ± 0.9	12.3 ± 0.7	8.5 ± 0.1	9.6
Stearic acid ($C_{18:0}$).	Saturated	7.3 ± 0.7	7.5 ± 0.3	7.9 ± 0.1	9.6
Arachidic acid[1] ($C_{20:0}$).	Saturated	2.5 ± 0.2	2.9 ± 0.3	3.0 ± 0.1	-
α-linolenic acid ($C_{18:3}$).	Polyunsaturated	1.5 ± 0.1	0.9 ± 0.1	1.7 ± 0.2	1.8
Behenic acid[2] ($C_{22:0}$).	Saturated	0.6 ± 0.1	0.6 ± 0.1	0.8 ± 0.1	-
Eicosenoic acid ($C_{20:1}$).	Monounsaturated	0.3 ± 0.0	0.5 ± 0.2	Traces	-
Palmitoleic acid[3] ($C_{16:1}$).	Monounsaturated	0.2 ± 0.0	0.1 ± 0.0	0.1 ± 0.05	-
Myristic acid ($C_{14:0}$).	Saturated	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.02	-
Saturated fatty acids.	-	43.5	43.4	-	-
Unsaturated fatty acids.	-	56.5	56.6	-	-
Monounsaturated fatty acids.	-	9.2	13.0	-	-
Polyunsaturated fatty acids.	-	47.3	43.6	-	-

The observed discrepancies between values can be attributed to the beverage preparation methods, the species involved, and the techniques used for the analyte's extraction & determination. SD: standard deviation. [1] also known as eicosanoid acid. [2] also known as docosanoic acid. [3] This fatty acid has two isomeric forms (cis & trans) and its concentration is the sum of both [39].

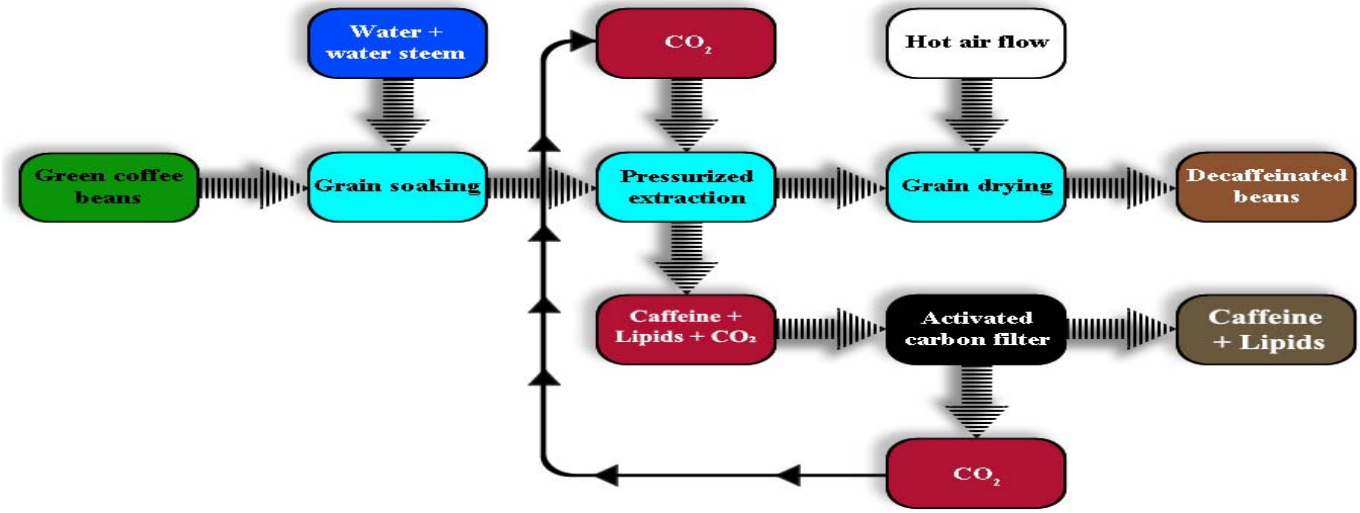

Fig 1. Caffeine and lipids supercritical fluid extraction scheme.

botanical origin (Table 2), where variations attributed to the botanical and geographical origin are evident [2,11,14].

THE COFFEE BEAN'S DITERPENES

As indicated in Table 2, the diterpene fraction is the most abundant for both species (*C. arabica* and *C. canephora*), and kahweol and its dehydro derivative, cafestol, are the most important, plus 16-O-methylcafestol and 16-O-metilkahweol which appear as minor components (Fig. 3).

These compounds occur as esters of palmitic (~ 36-49%) & linoleic (~ 22–30%) acids [14,19,29,42,43] and only small amounts are found in a free form (~ 0.7–3.5%) [43,44]. The rest corresponds to oleic, stearic and eicosanoic acid esters. In general, diterpenes represent ~ 1.3–1.9% on a dry weight basis in *C. arabica* while 0.2–1.5% are found in *C. canephora* [16].

In *C. arabica* the ratio between free diterpenes to total diterpenes (esterified + free) is 0.7–2.5% [44]. More specifically, in *C. arabica* the concentration of free or non-esterified cafestol ranges from 50–200 mg/kg of dry matter while the esterified fraction is approximately 5.2–11.8 g/kg on a dry matter basis [13].

Cafestol and kahweol are thermostable, relatively polar pentacyclic diterpene alcohols which are part of the non-saponifiable lipid fraction found only in coffee [45] in addition to contributing to the bitter taste of the beverage [15,29].

Their quantification is performed jointly, that is, without differentiating the free from those esterified with fatty acids.

This is performed after oil saponification extracted from the coffee bean endosperm. The oil is mixed with a catalyst (e.g., KOH or NaOH) in the presence of a short-chain alcohol (e.g., MeOH or EtOH), which causes the ester bonds alkaline hydrolysis, thus converting the triacylglycerols into potassium or sodium salts, (soap) which are easily removed from the matrix as they are water-soluble [46] leaving behind the unsaponifiable fraction (e.g., diterpenes, sterols and tocopherols) since they aren't affected by the reaction.

Next, a chromatographic analysis of the non-saponifiable fraction is performed through High Performance Liquid Chromatography, using a reverse phase column [2,44].

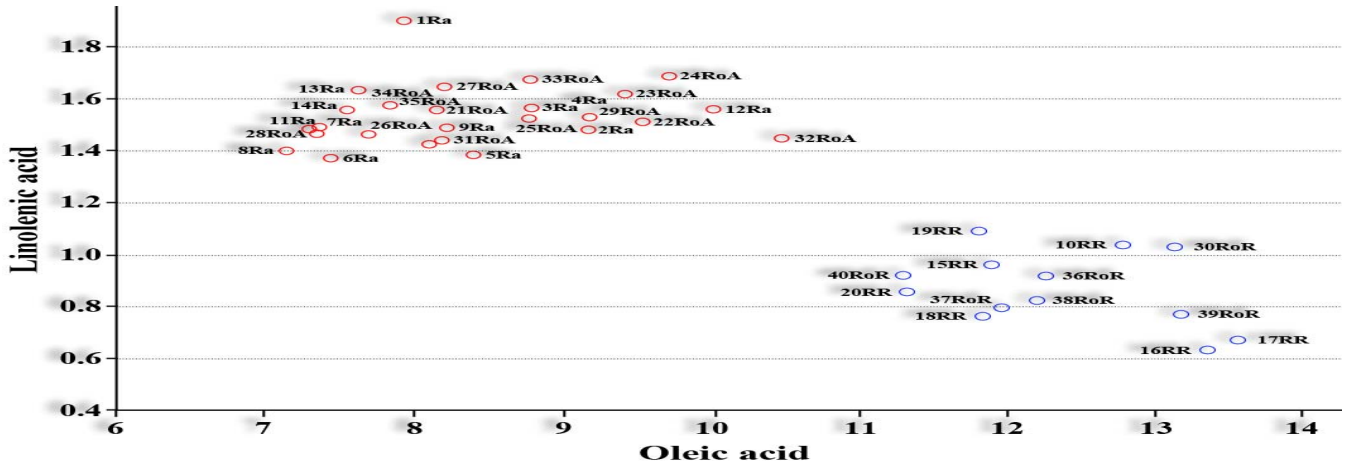

Fig. 2. Scatter plot between oleic acid and linolenic acid retrieved from Arabica and Robusta coffee samples (raw and roasted).
Raw Arabica, Ra; Roasted Arabica, RoA; Raw Robusta, RR & Roasted Robusta, RoR. Adapted from Reference [39].

Table 2. *Coffea arabica* L and *C. canephora* var. Robusta raw grain unsaponifiable composition (% ± SD).

	Arabica coffee				Robusta coffee			
	Guatemala	Colombia	Santo Domingo	Brazil	India*	Ivory Coast	India**	Vietnam
DITERPENES								
Kahweol.	44.8 ± 1.5	43.7 ± 1.8	46.6 ± 2.0	48.9 ± 0.7	0.8 ± 0.2	1.3 ± 0.4	0.5 ± 0.1	3.6 ± 0.6
Cafestol.	41.3 ± 0.0	45.2 ± 0.7	40.7 ± 0.2	39.3 ± 3.1	48.8 ± 0.5	48.5 ± 3.9	46.2 ± 1.3	42.5 ± 1.8
STEROLS								
Campesterol	1.6 ± 0.2	1.3 ± 0.4	1.7 ± 0.2	1.2 ± 0.3	5.0 ± 0.1	3.4 ± 0.6	4.0 ± 0.6	5.1 ± 0.2
Stigmasterol	2.0 ± 0.2	1.7 ± 0.3	1.8 ± 0.4	1.7 ± 0.4	7.6 ± 0.5	4.3 ± 0.1	5.8 ± 0.1	7.4 ± 0.5
β-sistosterol.	4.5 ± 0.3	4.5 ± 0.9	4.6 ± 0.5	4.8 ± 1.4	12.9 ± 0.9	10.9 ± 1.6	12.0 ± 0.4	14.3 ± 0.7
TOCOPHEROLS								
α-Tocopherol	0.8 ± 0.1	0.8 ± 0.1	0.7 ± 0.2	0.4 ± 0.2	0.8 ± 0.2	1.1 ± 0.2	0.4 ± 0.1	0.7 ± 0.2

Analyses were performed using gas chromatography with flame ionization detector. Adapted from Reference [14].
* Analysis performed using the grain plus the parchment or endocarp. ** Analysis performed using the coffee cherry.

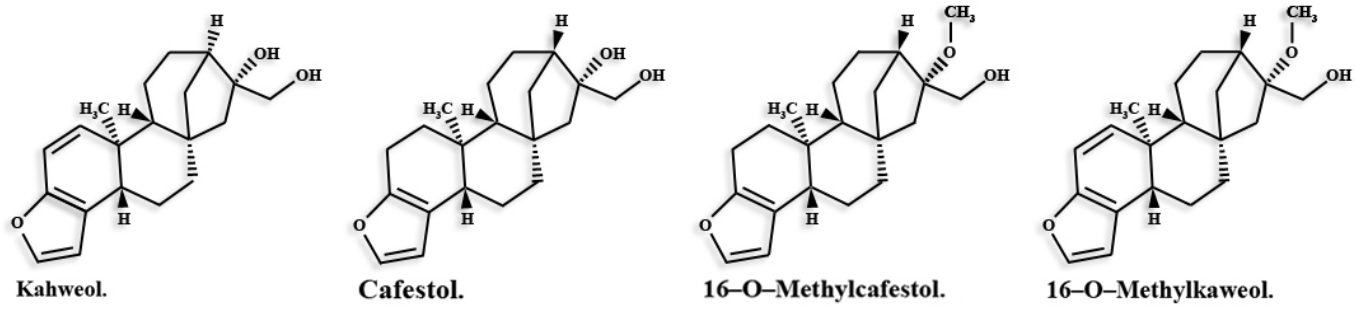

Fig. 3. Coffee's diterpenes.

In *C. arabica*, the free cafestol fraction is generally higher than that of kahweol. When purified, kahweol is more unstable because it is polyunsaturated, more sensitive to heat, oxygen, light and acids, therefore it is less abundant [11,47]; However, its highest concentrations are found in the grain endosperm and perisperm while it is absent from the pericarp [45].

Reference [44] reported free kahweol levels of 40–90 mg/kg on a dry matter basis, while the free cafestol was 45–190 mg/kg of dry matter. Cafestol is responsible for 0.2–0.6% of the coffee bean dry weight [11].

COFFEE GRAIN DISCRIMINATION BASED ON DITERPENES COMPOSITION

Kahweol is specific to Arabica varieties, so it can be used as a marker to discriminate products made from that species or mixtures made between Arabica and Robusta coffees [6,45].

Likewise, the pentacyclic diterpene alcohol 16-O-methylcafestol (16-OMC) is only present in Robusta [6,8,13,37,48] so it can be used to identify products made from that species or mixtures of both [13,38,48] since due to its thermostability isn't degraded during roasting [2,13,28,48].

Curiously, kahweol, cafestol, 16-OMC and 16-OMK share the same molecular structure, differing from each other only by the number and position of double bonds, as well as by the radical located at C-16. Finally, the ability to efficiently

discriminate coffee bean batches based on their lipid composition is important in a global market that imposes significant price differences based on their botanical origin.

ROASTING EFFECT ON THE COFFEE BEAN LIPID COMPOSITION

The roasted grain's lipids constitute approximately 10–15% of its dry weight [49-51] therefore there are no significant variations in the grain's lipid content after roasting [15,52]. However, during roasting, the Robusta varieties fatty acids tend to be more affected, in their composition, than those present in the Arabica varieties [15].

On the other hand, the slight increases in the roasted beans lipid composition reported by some authors can be elucidated as relative increases caused by the thermal degradation of other compounds during roasting [53,54]. The roasted beans lipids correspond to 75% of triglycerides of which 40 to 55% correspond to linoleic acid [50].

During roasting, the pressure built by gas accumulation inside the grain causes the lipids migration to the grain surface, through the small channels or fractures caused by the disintegration of cellular structures [5]. As a result, the oils tend to accumulate on the roasted bean surface giving it a shiny and greasy appearance. However, the speed at which the oil migration occurs is a function of the applied roasting profile; For instance, in roasted beans with a dark profile the speed at which lipids migrate to the grain surface is faster [5].

During roasting, Arabica coffee bean´s aldehydes are formed capable of reacting with other compounds [34], as well as new diterpenes such as dehydrocafestol (isomers: 15, 16- & 13, 16-) and dehydrokahweol (isomers 15, 16- & 13, 16-) which, in addition to being formed from the dehydration of their precursors (cafestol and kahweol) [2,15,37] are also part of the unsaponifiable fraction [37].

In contrast, only dehydrocafestol and its isomers are produced during the roasting of Robusta coffee varieties, since kahweol is only present in Arabica coffee varieties. Likewise, other compounds such as cafestal, kahweal, isokahweol and dehydroisokahweol are formed as degradation products of cafestol and kahweol.

While light roasting favours the concentration of cafestol and kahweol, the amounts of their derivatives depend not only on their precursors' initial concentration, but also on the roasting temperature. So, the higher the roasting temperature, the higher their concentration will be.

According to Reference [43], light roasting favours a higher concentration of diterpenes precursor in *C. arabica* (Table 3). Finally, the lipids in the roasted beans are used in the manufacture of flavouring ingredients [15].

METABOLISM OF COFFEE DITERPENES

Once ingested, only 70% of them are absorbed through the duodenum; afterwards, they are metabolized by the enzymes encoded by the UGTs and SULTs genes [55] and subsequently partially excreted (~ 1%) through the urine in the form of glucuronic acid or sulphate conjugates [42].

a. Phase I metabolism.

Kahweol and cafestol have beneficial and adverse effects in humans and animals. For example, many xenobiotic substances (e.g., carbon tetrachloride, nitrosamines, and polycyclic aromatic hydrocarbons) require prior metabolic activation to exert their carcinogenic effects.

Xenobiotic compounds are toxic to humans and animals, and in nature they are rare or non-existent because they are artificially synthesized and usually enter the body through the diet, by inhalation, or through the skin. Usually, the activation of xenobiotic substances is carried out by Phase I metabolic enzymes, so a disruption in their functioning or a reduction in the expression of their corresponding encoding genes would constitute another protection mechanism against xenobiotic compounds with carcinogenic potential.

The evidence suggests that a prolonged exposure to kahweol and cafestol through the diet causes a reduction in the concentration and activity of several Phase I enzymes such as cytochromes P450 32A (P45032A) and CYP2C11, the latter one being specific for males and which concentration was reduced by 35% compared to what was observed in controls after exposure to both pentacyclic diterpenes [47,56].

CYP2C11 and CYP3A2 are the phase I metabolic enzymes responsible for the aflatoxin B1 (AFB1) activation turning it into AFB1-8,9-epoxide [47,57], a reactive substance which interacts with the DNA forming adducts; consequently, their genes transcription reduction through dietary exposure to kahweol and cafestol constitutes a preventive mechanism in the DNA adducts formation and would probably prevent mutations derived from it.

Both pentacyclic diterpenes are also capable of interfering with the functions of enzymes responsible for the metabolic activation of several xenobiotic substances without affecting the transcription of genes that encode them, which constitutes another protection mechanism against carcinogenic compounds. For instance, kahweol and cafestol affect the function of the hepatic enzyme P4501A1 and CYP2B6 responsible for the metabolic activation of benzo[α]pyrene [B(α)P] and AFB1 respectively, which is why both substances lose their capacity to adhere to genetic material, thus inhibiting the adducts formation [47].

For these reasons, both cafestol and kahweol have been recognized for their chemoprotective effects, since they can be used against the genotoxic effect of B(α)P and AFB1, both carcinogenic agents [6,28,58-61]. Likewise, cafestol and kahweol confer protection against the carcinogenic effect of 7,12-dimethylbenz[α]anthracene [57] and heterocyclic amines such as 2-amino-1-methyl-6-phenylimidazo(4,5-b)pyridine and *N*-Nitrosodimethylamine both normally found in food for human consumption [57,58,61].

Genotoxicity is the ability to cause DNA damage; certain physical (e.g., temperature, UV light, ionizing and electromagnetic radiation), chemical (e.g., alkylating agents, acridine, oxidants, redox agents and aliphatic epoxides) and biological agents are genotoxic since they bind to DNA through covalent bonds or affect the enzymes involved in the

Table 3. Cafestol and kahweol in Arabica and Robusta coffee subjected to different roasting profiles.

Degree of roasting	*C. arabica* (mg/100 g)		*C. canephora* (mg/100 g)	
	Cafestol	Kahweol	Cafestol	Kahweol
Light.	463 ± 4	829 ± 16	163 ± 24	0
Medium.	398 ± 46	744 ± 8	250 ± 8	0
Dark.	420 ± 4	800 ± 28	242 ± 6	0

Both samples are from Brazil (Minas de Gerais). Results are presented as means + standard deviations. Adapted from Reference [43].

genetic material replication (e.g., DNA repair, condensation and de-condensation) causing mutations that may or may not cause cancer. Genotoxic substances are not necessarily carcinogenic, but most carcinogens are genotoxic.

The scientific evidence suggests that cafestol and kahweol mixtures in a 1:1 ratio are capable of inhibiting the ability of 2-amino-1-methyl-6-phenylimidazo(4,5-b)pyridine to form adducts with DNA in animal models (rodents) previously treated with such heterocyclic amine, probably as a result of a change in its metabolism [61].

Heterocyclic amines are considered carcinogens that may contribute to the development of colon cancer through a high consumption of red meat [62]. The 2-amino-1-methyl-6-phenylimidazo(4,5-b)pyridine is the most important since it represents more than 70% of the heterocyclic amines found in cooked red meats, in addition to having been found forming complexes (adducts) covalently linked to DNA in colonic tissues of several species including humans [61,62].

Protein adducts (e.g., albumin) are useful blood markers to determine the degree of exposure to a carcinogenic agent, as well the innate activation and detoxification capacity of the individual. Likewise, the 2-amino-1-methyl-6-phenylimidazo(4,5-b)pyridine is responsible for the development of colon cancers, mammary glands, prostate, and lymphatic system in animal models [61,62].

The metabolic activation of 2-amino-1-methyl-6-phenylimidazo(4,5-b)pyridine is a two-step process. First, the cytochrome P450 (1A1, 1A2 & 1B1) catalyses the oxidation of its amino group into the genotoxic metabolite 2-hydroxyamino-1-methyl-6-phenylimidazo(4,5-b)pyridine.

Next, the *N*-Acetyltransferase and sulfotransferase metabolize it once more producing highly reactive esters with carcinogenic capacity such as *N*-Acetoxy-2-amino-1-methyl-6-phenylimidazo(4,5-b)pyridine (

Fig *4*) [61,62] which forms stable complexes with proteins & DNA.

However, the evidence suggests that kahweol and cafestol confer protection against the carcinogenic action of *N*-Acetoxy-2-amino-1-methyl-6-phenylimidazo(4,5-b)pyridine and its metabolic derivatives by interfering with metabolic processes responsible for its activation and more specifically through the inhibition of *N*-acetyltransferase [47,60,61].

N-acetyltransferases have a double function in the metabolism of xenobiotic substances, since they can turn a metabolite derived from a toxic substance into a compound with a higher or lower degree of activity than the substance from which it was produced.

Studies conducted by Reference [61] provide evidence of both diterpenes ability to interfere with the metabolic activation of 2-Amino-1-methyl-6-phenylimidazo(4,5-b)pyridine through the inhibition of *N*-Acetyltransferase. In their studies, a group of rodents were subjected to a diet with both diterpenes at concentrations of 0.2, 0.1 and 0.02%, in addition to being exposed to 2-Amino-1-methyl-6-phenylimidazo(4,5-b)pyridine. Their results indicate the hepatic activation of 2-Amino-1-methyl-6-phenylimidazo(4,5-b)pyridine by *N*-acetyltransferase was reduced by 80%.

Unfortunately, it is difficult to extrapolate these results since the lower dose (0.02%) of both diterpenes would be equivalent to ingesting daily approximately 30 cups of unfiltered coffee, which is highly unlikely to occur.

b. Phase II metabolism.

In addition, both diterpenes confer protection against carcinogenic compounds due to their ability to induce the expression of genes encoding Phase II metabolic and detoxifying enzymes that prevent the formation of electrophilic and oxidizing compounds or promote their detoxification [16,56,61,63].

Among these enzymes, glutathione S-transferases (GST) causes the glutathione conjugation (GSH) with electrophilic compounds which in turn causes their detoxification [8,58,59,64,65] or the γ-glutamyl cysteine synthetase which is a key enzyme in the synthesis of the endogenous antioxidant GSH [57,61,65], the uridine 5`-diphospho-glucuronosyltransferase and the quinine oxidoreductase [47].

The enzymes expression suggests the existence of a detoxification mechanism as demonstrated in studies with animal models in which both diterpenes have reduced the toxicity of a series of carcinogenic compounds [60,64].

Furthermore, kahweol & cafestol are hypercholesterolemic since they increase the cholesterol levels (low-density lipoprotein "LDL" and very low-density lipoprotein "VLDL") and triglycerides in the blood, which is not recommended for patients with hyperlipidemia or fatty liver disease [7,11,19,42,66-68].

In addition, there is also evidence of their antioxidant properties, that is, they could protect lipids, intracellularly, from oxidative degradation caused by free radicals [69,70]. For this reason, both pentacyclic diterpenes have been negatively correlated with oxidative stress.

Experiences conducted by Reference [65] in which rodent hepatocytes were incubated in the presence of kahweol, cafestol and tert-Butyl hydroperoxide (t-BHT) demonstrated that both diterpenes protect the liver cells from cytotoxicity.

t-BHT (CAS: 75-91-2) is an analogous, short-chain organic hydroperoxide used in a wide range of oxidative processes. Being metabolized into free radicals (e.g., peroxyls

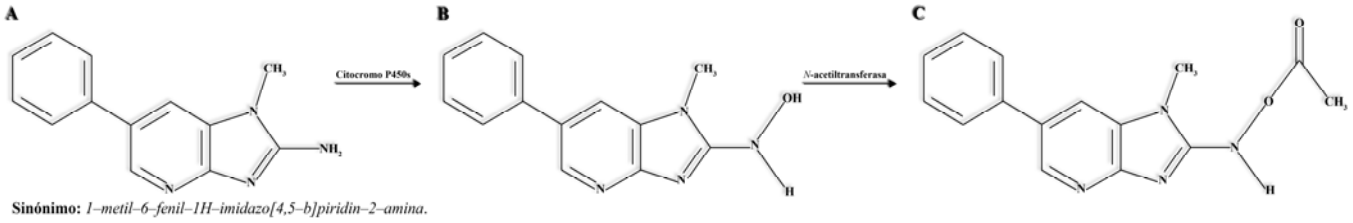

Fig 4. Metabolic activation of 2-amino-1-methyl-6-phenylimidazo(4,5-b)pyridine.
A: 2-Amino-1-methyl-6-phenylimidazo(4,5-b)pyridine. B: 2-Hydroxyamino-1-methyl-6-phenylimidazo(4,5-b)pyridine.
C: *N*-Acetoxy-2-amino-1-methyl-6-phenylimidazo(4,5-b)pyridine. Adapted from Reference [62].

& alkoxides) by cytochrome P450, it can cause cellular damage in hepatocytes by causing the oxidation of unsaturated lipids, as well as decreasing GSH levels.

Such protection was manifested in three ways: a) A significant increase in GSH concentration one of the most abundant antioxidant compounds found in the cytosol which prevents the oxidative stress through the elimination of hydroperoxides and inactivation of free radicals [65]. The increase in GSH concentration is proportional to the kahweol & cafestol concentrations, meaning that the higher the concentration of pentacyclic diterpenes, the higher the GSH level, b) Through a reduction in the malondialdehyde (MDA) synthesis; the main product of unsaturated fatty acids' oxidative degradation causing the rearrangement of their double bonds, which in turn causes the degradation of the cell membrane [57,71] reason why it is considered a biological marker of the lipids peroxidation.

The inhibition is inversely proportional to the kahweol & cafestol concentrations, that is, the higher the pentacyclic diterpenes concentration, the lower the synthesis of MDA & c) Through a reduction in the concentration of blood markers typically associated with hepatic damage or inflammation [e.g., alanine aminotransferase (ALT), & aspartate aminotransferase (AST)] (Fig 5).

The results obtained by Reference [65] are consistent with those reported by Reference [57] who confirmed the synergistic action of kahweol and cafestol could prevent hepatotoxicity in rodents exposed to carbon tetrachloride (CCl_4). CCl_4 was selected as the pro-oxidant compound since once enzymatically activated (CYP2E1) it is metabolized into trichloromethyl free radicals (CCl_3, CCl_3OO) which have hepatotoxic properties [57].

Trichloro-methyl radicals bind to proteins, forming complexes, in addition to causing peroxidation of cell membrane lipids, thus inducing hepatotoxicity manifested through an increase in ALT, AST & MDA concentrations in addition to producing necrosis of the affected cells [57].

The results of the study suggest that the daily administration of kahweol and cafestol at a rate of 10, 50 & 100 mg/kg of body weight prior to exposure to CCl_4 reduced the blood's ALT & AST concentration in the test animals compared to what was observed in the controls [57].

The evidence suggests the administration of diterpenes to test animals exposed to CCl_4 caused a reduction in the blood concentration of MDA with respect to those that were exposed to CCl_4 only (controls) [57]. This result is evidence of a decrease in the peroxidation rate of cell membrane lipids, and this decrease is correlated with the administered dose.

THE METHOD OF DRINK PREPARATION AND THE DITERPENES LEVELS IN SOLUTION

The method of infusion preparation and amount consumed affect the diterpenes´ capacity to raise cholesterol and triglyceride levels in the blood. As a rule, the higher the cafestol and kahweol concentration, the greater the increase in cholesterol and triglycerides in plasma. Evidence indicates that for every 10 mg of cafestol the concentration of blood cholesterol increases by 0.15 mmol/L [72].

The water temperature is important because it helps to solubilize a variety of substances in the roasted and ground coffee [73]. For instance, although the lipid fraction is hydrophobic, it is removed from the roasted beans during brewing due to the water pressure and temperature; and depending on the method of preparation, lipids can be retained or transferred to the infusion as an emulsion as in the case of unfiltered coffees (e.g., French-pressed coffee, espresso coffee, Scandinavian coffee, and Turkish coffee) which have the highest lipids concentration [11,16,74] around 3–6 mg of each diterpene per coffee cup [68,69].

The practice of boiling roasted ground coffee in water and separating the impurities by decanting without the use of a filter increases the cafestol and kahweol concentration in solution. The daily consumption of 8 cups of coffee prepared in this way increased blood cholesterol by 0.79 mmol/L.

Several types of coffee prepared without the use of filters, such as espresso coffee has a lipids concentration of 1–2% in solution; in addition to being one of the preferred coffees in Europe [75]. On the other hand, Scandinavian-type coffee has up to 22% of lipids in solution [2] while French-pressed coffee and Turkish coffee contain 6–12 mg per cup of coffee [29].

However, the preferred option in the USA are filtered coffees [59,75,76] which causes coffee to lose its ability to raise cholesterol levels since the filter paper retains cafestol and kahweol due to its low solubility in water [7,59,75,77,78].

Experiences reported by Reference [78] confirm this effect. A total of 20 healthy volunteers participated in a study to explore the effect of coffee preparation method (filtered vs. non-filtered) on the blood lipid profile. During a period of 4 weeks, the participants ingested boiled but not filtered coffee, with an average consumption of 8.3 dL/day, and then

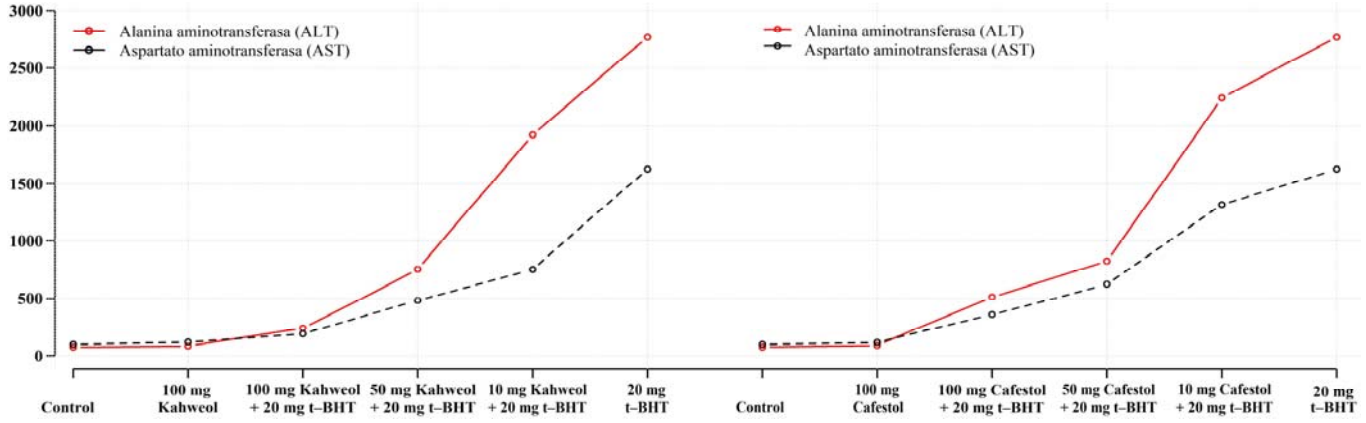

Fig 5. Kahweol and cafestol effects on t-BHT induced hepatotoxicity. Adapted from Reference [65].
The mice were fed with kahweol and cafestol at a rate of 10, 50 and 100 mg/kg of live weight for three days. Controls received only corn oil. Three hours after the last treatment the mice were treated with t-BHT (20 mg/kg of body weight). Hepatotoxicity was evaluated 24 h after exposure to t-BHT by determining ALT (U/L), AST (U/L) and GSH (μmol/g).

underwent another period of the same duration in which they were only allowed to ingest filtered coffee with the same daily average consumption.

At the end of each period, the blood lipid´s profile analyses were performed, and the data was subjected to a T test for dependent populations. The results confirm a significant elevation of total cholesterol, LDL-cholesterol and triglycerides levels attributed to the intake of unfiltered coffee, which in turn decreased during the period in which the participants ingested only filtered coffee. HDL-cholesterol levels were not significantly affected, which is consistent with the results reported by other researchers.

In contrast, there is evidence confirming the consumption of filtered coffee is also related to the increase, in small amounts, in blood cholesterol [79,80] due to an average concentration of less than 0.6 mg of lipids per cup [29].

THE STEROL FRACTION OF COFFEE BEANS

Coffee bean sterols are relatively heat stable and there are three main categories: 4-desmethylsterols (~ 93–98% of the total), 4-methylsterols (2%) and 4,4-dimethylsterols (5%) [11,28,37]. Likewise, the three major sterols (i.e., β-sitosterol, stigmasterol and campesterol) belong to the first category [26].

β-sitosterol is the main sterol in coffee beans [18] since it is responsible for 50% of the total sterols present [2]; while stigmasterol is responsible for 20–22% and campesterol for 18–19% of the total [28].

The differences in the sterol fraction composition between *C. arabica* and *C. canephora* var. Robusta have been used as discrimination parameters between both species [46]. However, to determine these differences, the analytical techniques most used for this purpose are the thin layer chromatography (TLC) combined with gas chromatography (GC) [46].

DISCRIMINATION OF COFFEE SAMPLES BASED ON THE STEROL PROFILE

Studies conducted by Reference [33] provide evidence of the presence of Δ5-Avenasterol and stigmastanol, which represent approximately 1.7 and 9.5% of the total sterols present in the coffee bean (*C. arabica*) respectively.

Reference [27] reported that Δ5-Avenasterol has been suggested as a reliable marker of the presence and proportion of Arabica coffee in blends made with Robusta coffee; since through its concentration the fraction of Arabica coffee can be determined when the mixtures contain between 30–100% of this species [37].

Meanwhile, Reference [46] & Reference [37] proposed sitostanol and Δ5-Avenasterol as markers to identify the grain samples' botanical origin.

Finally, the sterols concentration is almost not affected by the roasting process; In addition, traces of oxidized phytosterol have been inconsistently found after the process [14].

THE WAX OF THE COFFEE BEAN

While most of the lipids are in the endosperm, the wax is located on the seed surface [2,6]. The wax is responsible for 0.2–0.3% of the seed dry weight and its main components are fatty acids chains of variable length with antioxidant properties and 5-hydroxytryptamide's derivatives [2,6,34] which are serotonin amides with different fatty acids, mostly amides formed with arachidic, behenic and lignoceric acids in a 12:12:1 ratio [2,11,28,81,82] which could irritate the gastrointestinal tract of some susceptible individuals [82].

The presence of 5-hydroxytryptamide with stearic, 20-hydroxyarachidic and 20-hydroxybehenoic acids as minor components is also reported [2]. There are differences in the total concentration of this type of compound depending on the species; The work published by Reference [2] indicates that Robusta coffee varieties contain 565–1120 mg/kg of dry matter, that is, a lower concentration compared to Arabica varieties (500–2370 mg/kg); However, these concentrations tend to decrease through long periods of storage.

However, 5-hydroxytryptamide derivatives are denatured when exposed to moisture, which is why the industry has

developed mechanisms through which raw grain batches are exposed to water vapor reducing their concentration being able to sell a more acceptable product, in addition, these are also partially degraded during roasting.

Unfortunately, during roasting other metabolites (e.g., chlorogenic acids) are also extracted or thermally degraded [54,70] thus affecting the organoleptic properties of the bean.

The content of these compounds in the raw coffee bean ranges from 800–2000 mg/kg of dry matter, while in the roasted bean it ranges from 600–1000 mg/kg [82].

There are registered patents for the conversion of 5-hydroxytryptamide derivatives into serotonin [5-hydroxytryptamine, (5-HT)], a neurotransmitter derived from L-tryptophan, an organic compound responsible for the transmission of information from one neuron to another, which is why it plays an important role in psychology and human behaviour due to its influence on sleep pattern and energy balance [26,48,83] in addition to being used in some formulations as an antidepressant [26].

Wax is a thin layer that covers the grain surface removable with chlorinated organic solvents, through grain polishing, steam treatments (e.g., the Lendrich & Darboven processes) [82,84], through decaffeination or during roasting, reducing the concentration of the 5-hydroxytryptamide derivatives [82] by 50%, resulting in a product whose drink is much more digestible [2].

However, the evidence suggests the infusion preparation method also influences the concentration of these compounds in the beverage, since they tend to be retained in the filters used in the preparation of filtered coffees [82].

Wax removal through the aforementioned methods is especially important for those consumers who have stomach problems from wax constituents [26].

CONCLUSIONS

During the coffee drink preparation, especially when using filter coffee machines, as well as during the industrial production of instant coffees, most of the lipids are retained in the bagasse [25]. For this reason, the lipids concentration in soluble coffee is quite low, thus preventing their oxidative rancidity, which in turn extends the product shelf life.

The efficiency of grain lipids recovery depends on the extraction method, the type of organic solvent, the time spent in the process [85,86], the temperature and pressure applied during extraction.

Additionally, the evidence indicates that the higher the polarity of the organic solvent, the greater the number of fat-soluble analytes removed [87]. In this sense, because of their polarity, EtOH, isopropanol, and acetone are more efficient organic solvents than their nonpolar counterparts (e.g., toluene, $CHCl_3$, *n*-hexane, *n*-pentane, ether).

One of the disadvantages of the use of certain organic solvents is their capacity to extract high concentrations of free fatty acids, which are unstable since they tend to oxidize rapidly in the presence of oxygen, thus accelerating the degradation of the extracted oil via oxidative rancidity.

One of the advantages of the fluid supercritical extraction is that the process is performed in a closed environment devoid of O_2 and light, so the possibility of lipid oxidation is reduced [88]. In addition, when coffee bagasse is submitted to supercritical extraction the recovered oil has a slight aroma of coffee and its colour fluctuates between orange and dark coffee without residues of organic solvents [86].

Although the oil is the most important grain-derived substrate due to its easy extraction and high triglyceride content, its refinement is mandatory to be able to use it as a substrate in the formulation of concentrates for animal nutrition. The problem of refining the oil resides in its high content of unsaponifiable lipids which, although they can be isolated from the oil through molecular distillation, the process itself increases production costs [26,89].

The refinement allows the use of analytes with industrial applications such as palmitic acid, which can be used as raw material for the manufacture of soaps and acids [89]; In addition, it also allows the use of other compounds (e.g., caffeine, sterols, terpenes, & tocopherols) with applications in the pharmacological and the cosmetics industries.

Finally, a possible research topic would be the conversion of coffee triglycerides into biodiesel. Considering that ~ 8 million metric tons of coffee bagasse are annually produced worldwide with a lipid content of 10-15%, around 1.3 billion liters of biodiesel could be produced annually [89].

The value of coffee bagasse lies in its triglyceride-rich oil, which makes it an easy substrate to convert into biodiesel [25] and the evidence indicates that the biodiesel produced is of good quality because of its high stability since it remains unchanged for approximately one month [85,90] presumably as a result of a high antioxidant content [85,91].

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