

The coffee bean nitrogenous compounds. A critical review of contemporary scientific literature

Ostilio R. Portillo, PhD.¹, Ana C. Arévalo, PhD.²

¹Universidad Nacional Autónoma de Honduras, Honduras, oportillo@unah.edu.hn

²Universidad Nacional Autónoma de Honduras, Honduras, ana.arevalo@unah.edu.hn

Abstract – *Two species have gained economic importance in coffee production: Coffea arabica L. (Arabica coffee) & Coffea canephora Pierre ex A. Froehner var. Robusta, with 65 and 35% of world production attributed to C. arabica L. & C. canephora P. respectively. The seeds chemical composition of C. arabica and C. canephora, before roasting, differs with respect to their primary and secondary metabolites content, which serve as precursors for the synthesis of volatile compounds during the roasting process. For this reason, there are marked organoleptic differences between the roasted grain of both species.*

Unfortunately, although they play an important role during the seed germination, coffee proteins are described as nutritionally deficient due to their lack of essential amino acids. Additionally, most coffee amino acids, polypeptides and proteins are difficult to access because they are trapped in the insoluble waste (bagasse) recovered during the industrial production of instant coffees.

The following discussion is the product of an extensive review of current scientific literature which aims to describe the most salient topics associated with the coffee beans' nitrogenous compounds (primary metabolites) before and after roasting, differences linked to the involved species, their influence on cup quality, solubility, and extraction, as well as a discussion on the analytical techniques used for their determination.

Keywords- synthesis, extraction, amino acids, sensory properties.

I. INTRODUCTION

Peptides (~ 4-10 kDa), oligopeptides [short amino acids chains (2-20) covalently linked together], polypeptides (amino acids chains considered the basic units that make up proteins), proteins [covalently linked amino acids chains (> 50)] and free amino acids are considered as nitrogenous compounds; which are responsible for 9-16% of the chemical composition of the raw coffee bean [1-3]; However, the raw grain's proteins constitute ~ 11-15% of its dry weight [3-5], percentage that is calculated through the determination of the N_{total} (NT) which is multiplied by 6.25 ($NT * 6.25$) [6-9].

Although coffee contains other nitrogenous compounds such as caffeine, trigonelline, theobromine and theophylline [2] when calculating the grain's protein content, generally only the amounts of nitrogen contributed by caffeine and

trigonelline are subtracted [$Protein = (NT * 6.25) - caffeine - trigonelline$] [6,8,10] resulting in a protein content ranging between 8.7-12.2% [6,10] of which ~ 45-50% corresponds to 11S Globulin type storage proteins [5,11-13] which present high (~ 32 kDa) and low (~ 22 kDa) molecular weight subunits, thus constituting ~ 5-7% of the dry weight of the coffee bean [12,13].

The 11S type storage proteins are synthesized in the endosperm [5] and stored in vacuoles [6] constituting an important source of amino acids and nitrogen during seed germination [13,14]. On the other hand, although the grains' protein content of Arabica varieties is slightly lower than that observed in the Robusta varieties [15] these differences are not considered significant [7,8].

In general, only 50% of the coffee bean proteins are water soluble [85% globulins & 15% albumins] [10,11] while other authors state that this percentage is lower since only 33% of the raw bean proteins are solubilized by hot water [12]. In addition, some authors state that coffee bean proteins are nutritionally deficient due to their lack of essential amino acids [16] such as lysine and hydrophobic amino acids such as methionine and tryptophan being the presence or absence of the latter determined through its autofluorescence emission pattern [17].

In addition, tryptophan is present in very small amounts in Arabica coffees; In contrast, Robusta coffees present higher concentrations of it, which in turn is responsible for the development of unpleasant odors associated with 3-methylindole [3].

In contrast, there is evidence which suggest the opposite, for example, approximately 42-49% of the amino acids present in the coffee bagasse correspond to essential amino acids (Table 1) [12,18]. The problem with the coffee bagasse's amino acids and polypeptides is that these are difficult to access because they are trapped in the insoluble waste recovered during the industrial production of instant coffees.

To take advantage of these amino acids, it is necessary to hydrolyze the bagasse chemically and enzymatically, which is not representative of the process of preparing a cup of coffee, would increase production costs and could affect the organoleptic properties of the drink.

On the other hand, Arabica coffees are characterized by having a lower concentration of free amino acids compared to Robusta types (Table 2) [3,9,19]; However, despite the fact that free amino acids and peptides constitute only 0.3-0.6% and 0.4-0.6% on a dry basis, respectively [14,20,21], they probably constitute the most important group in defining the sensory characteristics of the beverage, since together with

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polypeptides and proteins they interact with carbohydrates during the non-enzymatic Maillard reaction, producing the characteristic aroma and color of roasted coffee [3,10,12,22].

In general, the free amino acids present in the raw coffee bean are: proline, alanine [10], asparagine, glutamic acid, and aspartic acid [3,10]. These are the precursors of volatile compounds such as furans, pyridines, pyrazines, pyrroles, aldehydes, and melanoidins [16].

During the grain roasting, most of the peptides and proteins are degraded, being able to find only traces of them (< 1%) in the roasted grains [3,22]. In studies conducted by Reference [22] it was calculated that during roasting serine and cysteine are reduced by more than 50% while arginine completely disappeared.

Table 1. Coffee bagasse's nitrogenous compounds composition (%).

Analyte	Reference [23]	Reference [18]	Reference [12]
Caffeine.	-	0.20	-
Protein.	13.6	11.20	-
Aspartic acid.	-	5.10 ± 0.71	0.2-1.9
Glutamic acid.	-	4.13 ± 0.56	11.5-13.8
Arginine.	-	0.01 ± 0.01	0.1-0.2
Alanine.*	-	2.34 ± 0.71	4.8-5.4
Cysteine.	-	0.15 ± 0.01	5.1
Phenylalanine.*	-	1.18 ± 0.22	0.5-6.7
Glycine.	-	2.68 ± 0.17	2.4-7.9
Histidine.*	-	0.39 ± 0.08	0.1-5.3
Isoleucine.*	-	0.94 ± 0.13	5.1-5.3
Leucine.*	-	2.49 ± 0.37	10.6-10.9
Lysine.*	-	0.59 ± 0.10	1.9-2.3
Methionine.*	-	0.26 ± 0.03	1-1.9
Proline.	-	1.63 ± 0.29	3.1-4.7
Serine.	-	0.57 ± 0.10	0.9-1.2
Threonine.*	-	4.71 ± 1.01	0.3-2.2
Tyrosine.	-	0.33 ± 0.09	2.9-4
Valine.*	-	1.69 ± 0.14	6-6.8

The observed discrepancies between reported values can be attributed to: the beverage preparation methods, the species involved, and the techniques used for the extraction & determination (identification and quantification) of analytes.

* Essential amino acids.

It should be noted that when the objective is to determine the content and type of amino acids present in a coffee sample (raw or roasted), acid hydrolysis of the samples is generally performed to degrade the nitrogenous compounds (i.e., peptides, polypeptides, & proteins) by exposing them to hydrochloric acid (6 M HCl) for 20-24 h at 110 °C [9]. Using this approach, acid hydrolysis of raw and roasted samples of Colombian and Angolan coffee type Robusta revealed a decrease in the concentrations of arginine, cysteine, lysine, serine and threonine.

Cysteine is very importance since it is considered the source of many sulfur compounds responsible for the aroma of roasted coffee. However, Reference [24] states that attention should be paid to high levels of cysteine as it imparts unpleasant odors and flavors to food; presumably due to the sulfur compounds resulting from its decomposition.

In contrast, glutamic acid, leucine, phenylalanine, and valine levels increased after roasting. From all this it can be

concluded that the most thermostable amino acids are alanine, glutamic acid, glycine, leucine, valine [9,22], tyrosine and phenylalanine [9]; While the most unstable are arginine, cysteine, lysine and serine [11].

The amino acids present in the coffee drink are: glutamic acid (the most abundant), the aromatic amino acids phenylalanine and tyrosine, γ -aminobutyric acid (GABA) which is a non-protein amino acid; Also, glycine, aspartic acid, alanine, leucine, valine, isoleucine, serine, threonine, and histidine (Fig. 3) [22].

Curiously, the evidence suggests that during coffee drying there is an increase in GABA concentration, which is why it is considered a marker of water stress [25]. This is produced through the enzymatic decarboxylation reaction of glutamic acid catalyzed by glutamate decarboxylase (Fig. 1). However, at the end of drying, GABA concentrations are higher in grains processed through dry milling than those found in grains processed through wet milling.

According to Reference [26], "glycine has the property of increasing the typical brown coloration of the compounds resulting from the Maillard reaction, in addition to reacting with α -dicarbonyls".

Table 2. Raw coffee beans' free amino acid content.

Amino acid	Reference [27]		Reference [5]
	<i>C. arabica</i> (%)	<i>C. arabica</i> (%)	<i>C. canephora</i> (%)
L-glutamic acid.	0.13	0.102	0.047
L-Asparagine.	0.05	-	-
4-Aminobutyric acid.	0.05	0.028	0.047
L-Alanine.	0.05	0.025	0.034
L-Aspartic acid.	0.05	0.033	0.033
L-Serine.	0.03	0.017	0.016
L-Proline.	0.03	-	-
L-Phenylalanine.	0.02	0.017	0.021
L-Valine.	0.01	0.009	0.017
L-Isoleucine.	0.01	0.005	0.008
L-Tryptophan.	0.01	-	-
L-Leucine.	0.01	0.006	0.010
L-Lysine.	0.01	0.006	0.011
L-Tyrosine.	0.01	0.005	0.011
L-Glutamine.	0.01	-	-
L-Arginine.	0.01	0.008	0.018
Glycine.	0.01	0.003	0.006
L-Threonine.	0.01	0.003	0.005
L-Histidine.	0.01	0.004	0.004
Methionine.	-	0.002	0.001

The observed discrepancies between the reported values can be attributed to: the species involved and the techniques used for the extraction & determination (identification and quantification) of analytes. Values were calculated based on dry matter.

II. COFFEE SAMPLES DISCRIMINATION BASED ON NITROGENOUS COMPOUNDS

Serotonin (5-hydroxytryptamine, 5-HT) is a neurotransmitter derived from L-tryptophan which plays an important role in regulating a number of physiological functions such as sleep regulation and energy balance [28]. Both serotonin and its metabolic derivative melatonin (*N*-Acetyl-5-methoxytryptamine) have been found in raw coffee bean

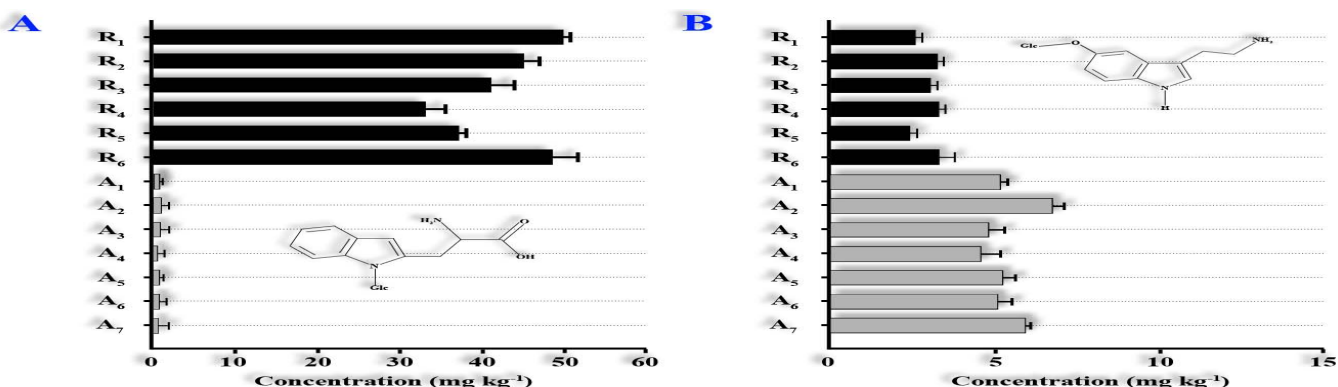


Fig. 2. Levels of (A) tryptophan-N-glucoside and (B) serotonin 5-O-β-glucoside in Robusta and Arabica coffee beans. R and A represent Robusta and Arabica respectively. Each bar represents the mean obtained from four repetitions with its corresponding standard deviation. Adapted from Reference [28].

concentration during the processing (wet or dry) of coffee beans. One of the methodologies used involves derivatization with *o*-phthalaldehyde prior to HPLC analysis. Fig. 3 presents a chromatogram of the derivatives obtained, where it is evident that GABA is accumulated when coffee beans are processed by the dry method.

According to Reference [32] finding that GABA is accumulated in coffee beans during the dry process allows a chemical differentiation of the different processes used in the green coffee bean. The authors report that this accumulation clearly shows the different metabolic reactions that take place in coffee beans during processing.

IV. ROLE OF NITROGENOUS COMPOUNDS IN THE DEVELOPMENT OF ROASTED COFFEE'S ORGANOLEPTIC PROPERTIES

One of the most aromatic food products is roasted coffee (CAS: 68916-18-7), its aroma is the product of a complex mixture of volatile compounds [33] which are formed through the Strecker degradation and the non-enzymatic Maillard reaction during the roasting of the coffee bean. The aroma of coffee is the product of the synergistic effect of compounds that are inherently present in the raw bean (e.g., sugars and amino acids) and those that are formed during the roasting process (e.g., aldehydes, ketones, mercaptans, pyrazines, pyridines, pyrroles, thiazoles, thiophenes, oxasols), roasting's temperature and time, the reactions involved, the grain's pH and moisture [3].

The Strecker degradation is a reaction between a free amino acid and a dicarbonyl group (i.e., diketone) resulting in the release of ammonia, CO₂ and an aldehyde which has one less carbon in its molecular formula than the amino acid from which it is derived.

Aldehydes are produced due to the amino acids degradation (i.e., deamination and decarboxylation) through the Strecker reaction [3,34], but also as a consequence of alcohols auto-oxidation [16,35]. They impart aromatic tones such as malt or honey [3,36,37]. They are considered one of

the most abundant fractions of coffee [37], especially in *Coffea arabica* [38], and during the grain roasting their concentration increases significantly, but they tend to degrade as the coffee roasting profile progresses (Fig. 4).

Ketones are heterocyclic volatile compounds of thermal origin that constitute 10.5% of the identified compounds in roasted coffee [22] and are the product of the interaction between sugars and nitrogenous compounds during the non-enzymatic Maillard reaction [3,14,39] imparting butter-like tones [3]. They are considered one of the most abundant fractions of coffee [37], especially in *Coffea arabica* [38], and during the grain roasting their concentration increases significantly, but they tend to degrade as the roasting profile progresses (Fig. 5).

Mercaptans (aka thiols) are heterocyclic volatile compounds in which a hydroxide group (HO-) is replaced by a SH functional group [40] during the grain roasting since they are not detected in the raw coffee bean [16]. They result from the interaction between sugars and nitrogenous compounds (sulfur amino acids) during the non-enzymatic Maillard reaction [14,33,40] reaching their maximum concentration in well-roasted or black coffees [40]. However, from this point onwards its production slows down as well as inducing the thermal degradation of those already formed [40].

Some of them have a low aromatic threshold, so they have a strong odor, contributing significantly to the aroma of roasted coffee; Unfortunately, they are also highly susceptible to auto-oxidative degradation, which turns them into disulfides even at low temperatures [40] and their concentrations tend to decrease in the presence of melanoidins.

On the other hand, they are not perceived with the same intensity in the infusion due to the low extraction rates during the drink preparation [40] caused by variations between the proportion of water and coffee, the water's temperature and pressure and the extraction time.

Pyrazines are heterocyclic and volatile compounds of thermal origin that contain four carbon and two nitrogen atoms and constitute between 11.8-14% of the volatile compounds identified in roasted and ground coffee [22,41].

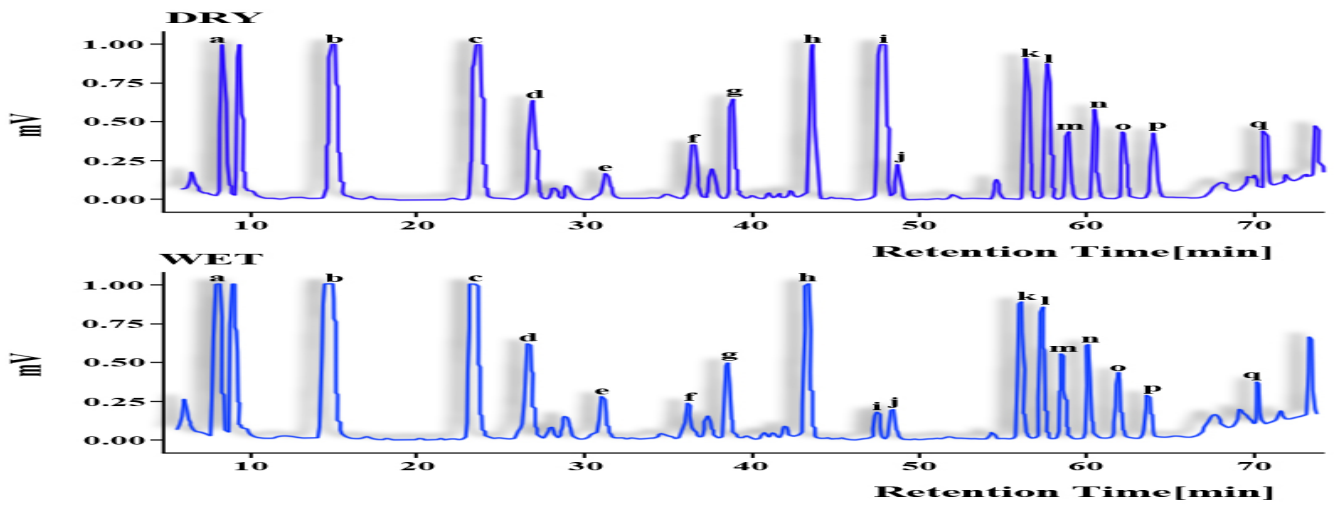


Fig. 3. Free amino acids derivatives chromatography (HPLC) using *o*-phthaldialdehyde from coffee processed through the dry and wet method. Adapted from Reference [32].

- a. Aspartate | b. Glutamate | c. Asparagine (Asn) | d. Serine (Ser)
- e. Histidine (His) | f. Glycine (Gly) | g. Arginine (Arg) | h. Alanine (Ala)
- i. γ -Aminobutyric acid (GABA) | j. Tyrosine (Tyr)
- k. Methionine (Met)/Valine (Val) | l. Norvaline | m. Tryptophan (Trp)
- n. Phenylalanine (Phe) | o. Isoleucine (Ile) | p. Leucine (Leu) | q. Lysine (Lys)

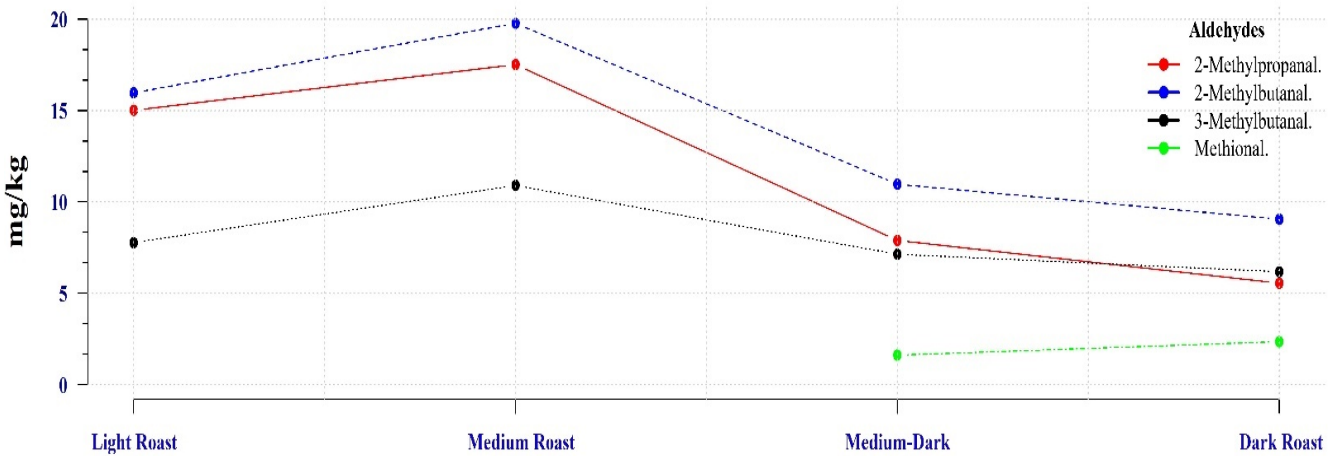


Fig. 4. Coffee's aldehydes concentration at different roasting profiles. Adapted from Reference [36].

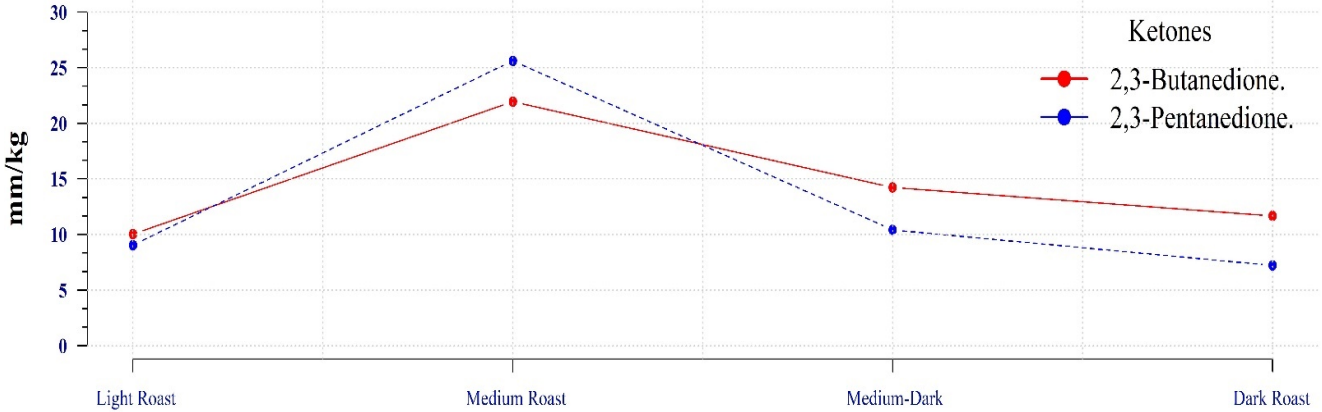


Fig. 5. Coffee's ketones concentration at different roasting profiles. Adapted from Reference [36].

in addition to imparting to the coffee's aroma toasted or nuts tones [15,42,43]. They result from the interaction between sugars and nitrogenous compounds (e.g., free amino acids, peptides, and proteins) during the non-enzymatic Maillard reaction [3,14,39,44]. During roasting, the released ammonia interacts with pyrazine precursors giving rise to their formation [45] which are produced more abundantly in Robusta coffees varieties due to their higher concentration of free amino acids [19,38,41,46].

However, despite being compounds of thermal origin, they tend to be thermo-degraded as the roasting profile progresses (e.g., black or well-roasted coffees) [47]. Likewise, when oxidized, cysteine is transformed into other amino acids such as cysteine sulfonic acid and cysteic acid, which are precursors of pyrazines, of which there are more than 80 molecules.

Alkylpyrazines are produced through different mechanisms and from various precursors; They present odors that vary between nutty and coffee-like [34]. For example, during the drink preparation, hydroxy-amino acids (e.g., serine and threonine) react with other compounds, thus producing alkylpyrazines [12]. In contrast, in the presence of a high content of glycine, alanine, and reducing sugars, the synthesis of alkylpyrazines also occurs [48]. Consequently, the amino acid profile of the raw coffee bean will have a direct effect on the amount and type of pyrazines that are produced during the non-enzymatic Maillard reaction.

Pyridines are volatile organic compounds of thermal origin formed by a heterocyclic aromatic ring containing five carbon atoms and one nitrogen [14,16,49] formed by the interaction between sugars and nitrogenous compounds (e.g., free amino acids, peptides, and proteins) during the Maillard reaction [14,43,44,46] or due to trigonelline's thermal degradation [38,46,50,51]. Pyridines have a pungent, nauseating odor [35], but when diluted impart burnt or smoky aromas to the coffee beverage [35,37,42,52] as well as bitter and astringent flavors [52]. However, some authors do not consider them potent odorants of the drink because their aromatic threshold (when they are diluted in the air) is high [42]. Finally, pyridines register their greatest synthesis at the beginning of the Maillard reaction, but as the pyrolysis of the grain continues their concentrations decrease [46].

Pyrroles are heterocyclic volatile compounds of thermal origin with antioxidant capacity [37,44,53] that constitute 11% of the identified compounds in roasted coffee [22]. In addition, they tend to be produced in greater quantities during the roasting of Robusta coffees varieties [38] and they result from the interaction between sugars and nitrogenous compounds (amino acids) during the Maillard reaction [14,39,46] or due to trigonelline's thermal degradation [38]. Pyrroles impart burnt aromas to the coffee beverage [42], but despite their high contribution, some authors do not consider them potent odorants of the coffee beverage because their aromatic threshold (when diluted in air) is high [42].

Thiazoles are heterocyclic volatile compounds with antioxidant capacity [53] formed during roasting since they

are not detected in the raw coffee grain [16]; They result from the interaction between sugars and nitrogenous compounds (sulfur amino acids) during the non-enzymatic Maillard reaction [3,14,33,44].

Thiophenes are volatile organic compounds with a heterogeneous aromatic ring made up of one sulfur and four carbon atoms. They have antioxidant capacity [37,53]; However, since they are found in low concentrations, their contribution to the drink's antioxidant capacity is minimal [37]. Thiophenes are formed during roasting since they are not detected in the raw coffee grain [16] as they are the product of the interaction between sugars and sulfur-containing amino acids (e.g., cystine, cysteine and methionine) during the non-enzymatic Maillard reaction [14,33,44,54].

Oxasols are produced as a result of the interaction of sugars with amino acids during the non-enzymatic Maillard reaction [14]. According to Reference [33], "during Strecker degradation, amino acids interact with α -dicarbonyl groups producing amino-ketones which, when reacting with an aldehyde, give rise to the formation of oxazoles".

V. CONCLUSION

The chemical composition of *C. arabica* and *C. canephora* seeds, before roasting, differs with respect to their content of primary and secondary metabolites, which serve as precursors for the synthesis of volatile compounds during the roasting process [55]. For this reason, there are marked aromatic differences between the roasted beans of both species.

The nitrogenous compounds, especially the free amino acids, play an important role in defining the sensory properties of both the roasted coffee bean and the beverage; However, for this to occur, interaction with other primary metabolites such as carbohydrates is required; Otherwise, in the absence of carbohydrates the non-enzymatic Maillard reaction would simply not occur and many of the analytes responsible for the organoleptic properties of coffee would not be produced during roasting.

For this reason, the organoleptic properties of the roasted coffee bean are conditioned, to a certain extent, by the differences in the concentration of the primary metabolites that act as precursors of the aromatic compounds produced during the roasting of the bean.

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REFERENCES

- [1] P. Esquivel, and V. M. Jiménez, "Functional properties of coffee and coffee by-products," *Food Res Int*, vol. 46, no. 2, pp. 488-95, May 2012.
- [2] I. A. Ludwig, M. N. Clifford, M. E. Lean, H. Ashihara, and A. Crozier, "Coffee: biochemistry and

- potential impact on health,” *Food Funct*, vol. 5, no. 8, pp. 1695-717, August 2014.
- [3] L. Poisson, I. Blank, A. Dunkel, and T. Hofmann, “The chemistry of roasting - Decoding flavor formation,” in *The craft and science of coffee*, vol. B. Folmer, Eds.: Academic Press, 2017. pp. 273-309.
- [4] T. Joet, A. Laffargue, J. Salmona, S. Doubeau, F. Descroix, et al., “Metabolic pathways in tropical dicotyledonous albuminous seeds: *Coffea arabica* as a case study,” *New Phytol*, vol. 182, no. 1, pp. 146-62, April 2009.
- [5] S. Oestreich-Janzen, “Chemistry of coffee,” in *Comprehensive natural products II*, vol. Oxford: Elsevier, 2010. pp. 1085-117.
- [6] R. Acuña, R. Bassüner, V. Beilinson, H. Cortina, G. Cadena-Gómez, et al., “Coffee seeds contain 11S storage proteins,” *Physiol Plant*, vol. 105, no. 1, pp. 122-31, January 2002.
- [7] A. S. Franca, J. C. F. Mendonça, and S. D. Oliveira, “Composition of green and roasted coffees of different cup qualities,” *LWT - Food Science and Technology*, vol. 38, no. 7, pp. 709-15, November 2005.
- [8] L. S. Oliveira, A. S. Franca, J. C. F. Mendonça, and M. C. Barros-Júnior, “Proximate composition and fatty acids profile of green and roasted defective coffee beans,” *LWT - Food Science and Technology*, vol. 39, no. 3, pp. 235-39, July 2006.
- [9] S. Casal, E. Mendes, M. B. P. P. Oliveira, and M. A. Ferreira, “Roast effects on coffee amino acid enantiomers,” *Food Chem*, vol. 89, no. 3, pp. 333-40, February 1 2005.
- [10] X. Wang, and L.-T. Lim, “Physicochemical characteristics of roasted coffee,” in *Coffee in health and disease prevention*, vol. San Diego: Academic Press, 2015. pp. 247-54.
- [11] A. S. Moreira, F. M. Nunes, M. R. Domingues, and M. A. Coimbra, “Coffee melanoidins: structures, mechanisms of formation and potential health impacts,” *Food Funct*, vol. 3, no. 9, pp. 903-15, September 2012.
- [12] R. Campos-Vega, G. Loarca-Piña, H. A. Vergara-Castañeda, and B. D. Oomah, “Spent coffee grounds: a review on current research and future prospects,” *Trends Food Sci Technol*, vol. 45, no. 1, pp. 24-36, September 2015.
- [13] K. G. D. Livramento, F. M. Borem, A. C. Jose, A. V. Santos, D. E. D. Livramento, et al., “Proteomic analysis of coffee grains exposed to different drying process,” *Food Chem*, vol. 221, no. pp. 1874-82, April 15 2017.
- [14] C.-F. Hwang, C.-C. Chen, and C.-T. Ho, “Contribution of coffee proteins to roasted coffee volatiles in a model system,” *Int J Food Sci Technol*, vol. 47, no. 10, pp. 2117-26, October 2012.
- [15] W. B. Sunarharum, D. J. Williams, and H. E. Smyth, “Complexity of coffee flavor: a compositional and sensory perspective,” *Food Res Int*, vol. 62, no. pp. 315-25, August 1 2014.
- [16] A. Farah, “Coffee constituents,” in *Coffee*, vol. Y.-F. Chu, Eds. Oxford, UK: Wiley-Blackwell, 2012. pp. 21-58.
- [17] P. Bandyopadhyay, A. K. Ghosh, and C. Ghosh, “Recent developments on polyphenol-protein interactions: effects on tea and coffee taste, antioxidant properties and the digestive system,” *Food Funct*, vol. 3, no. 6, pp. 592-605, June 2012.
- [18] N. Martinez-Saez, A. T. Garcia, I. D. Perez, M. Rebollo-Hernanz, M. Mesias, et al., “Use of spent coffee grounds as food ingredient in bakery products,” *Food Chem*, vol. 216, no. pp. 114-22, Feb 1 2017.
- [19] S. Rocha, L. Maeztu, A. Barros, C. Cid, and M. A. Coimbra, “Screening and distinction of coffee brews based on headspace solid phase microextraction/gas chromatography/principal component analysis,” *J Sci Food Agric*, vol. 84, no. 1, pp. 43-51, January 2004.
- [20] E. Ludwig, U. Lipke, U. Raczek, and A. Jäger, “Investigations of peptides and proteases in green coffee beans,” *Eur Food Res Technol*, vol. 211, no. 2, pp. 111-16, July 2000.
- [21] F. Wei, and M. Tanokura, “Chemical changes in the components of coffee beans during roasting,” in *Coffee in health and disease prevention*, vol. San Diego: Academic Press, 2015. pp. 83-91.
- [22] I. Flament, and Y. Bessièrre-Thomas, *Coffee flavor chemistry*. 1 ed. West Sussex, PO19 1UD, England: John Wiley & Sons; 2001, pp. 424.
- [23] S. I. Mussatto, E. M. S. Machado, S. Martins, and J. A. Teixeira, “Production, composition, and application of coffee and its industrial residues,” *Food Bioproc Tech*, vol. 4, no. 5, pp. 661-72, July 2011.
- [24] L. S. Jackson, and F. Al-Taher, “Effects of consumer food preparation on acrylamide formation,” in *Chemistry and safety of acrylamide in food*, vol. M. Friedman, and D. Mottram, editors. Boston, MA: Springer US, 2005. pp. 447-65.
- [25] M. Kleinwächter, G. Bytof, and D. Selmar, “Coffee beans and processing,” in *Coffee in health and disease prevention*, vol. San Diego: Academic Press, 2015. pp. 73-81.
- [26] T. M. Amrein, B. Schönbächler, F. Escher, and R. Amadó, “Factors influencing acrylamide formation in gingerbread,” in *Chemistry and safety of acrylamide in food*, vol. 561, M. Friedman, and D. Mottram, editors. Boston, MA: Springer US, 2005. pp. 431-46.
- [27] F. Wei, and M. Tanokura, “Organic compounds in green coffee beans,” in *Coffee in health and disease prevention*, vol. San Diego: Academic Press, 2015. pp. 149-62.

- [28] L. Servillo, A. Giovane, R. Casale, D. Cautela, N. D'Onofrio, et al., "Glucosylated forms of serotonin and tryptophan in green coffee beans," *Lwt*, vol. 73, no. pp. 117-22, November 2016.
- [29] S. Özcan, and H. Z. Şenyuva, "Improved and simplified liquid chromatography/atmospheric pressure chemical ionization mass spectrometry method for the analysis of underivatized free amino acids in various foods," *Journal of chromatography A*, vol. 1135, no. 2, pp. 179-85, December 1 2006.
- [30] B. M. Silva, S. Casal, P. B. Andrade, R. M. Seabra, M. B. Oliveira, and M. A. Ferreira, "Development and evaluation of a GC/FID method for the analysis of free amino acids in quince fruit and jam," *Anal Sci*, vol. 19, no. 9, pp. 1285-90, Sep 2003.
- [31] V. Pereira, M. Pontes, J. S. Camara, and J. C. Marques, "Simultaneous analysis of free amino acids and biogenic amines in honey and wine samples using in loop orthophthalaldehyde derivatization procedure," *J Chromatogr A*, vol. 1189, no. 1-2, pp. 435-43, May 2 2008.
- [32] G. Bytof, S.-E. Knopp, P. Schieberle, I. Teutsch, and D. Selmar, "Influence of processing on the generation of γ -aminobutyric acid in green coffee beans," *Eur Food Res Technol*, vol. 220, no. 3-4, pp. 245-50, March 2004.
- [33] R. A. Buffo, and C. Cardelli-Freire, "Coffee flavour: an overview," *Flavour Fragr J*, vol. 19, no. 2, pp. 99-104, March/April 2004.
- [34] N. Caporaso, A. Genovese, M. D. Canela, A. Civitella, and R. Sacchi, "Neapolitan coffee brew chemical analysis in comparison to espresso, moka and American brews," *Food Res Int*, vol. 61, no. pp. 152-60, July 1 2014.
- [35] A. T. Toci, and A. Farah, "Volatile compounds as potential defective coffee beans' markers," *Food Chem*, vol. 108, no. 3, pp. 1133-41, June 1 2008.
- [36] S. Y. Kim, J. A. Ko, B. S. Kang, and H. J. Park, "Prediction of key aroma development in coffees roasted to different degrees by colorimetric sensor array," *Food Chem*, vol. 240, no. pp. 808-16, Feb 1 2018.
- [37] I. A. Ludwig, L. Sánchez, M. P. De Peña, and C. Cid, "Contribution of volatile compounds to the antioxidant capacity of coffee," *Food Res Int*, vol. 61, no. pp. 67-74, July 1 2014.
- [38] C. Liu, N. Yang, Q. Yang, C. Ayed, R. Linforth, and I. D. Fisk, "Enhancing Robusta coffee aroma by modifying flavour precursors in the green coffee bean," *Food Chem*, vol. 281, no. pp. 8-17, May 30 2019.
- [39] O. Gonzalez-Rios, M. L. Suarez-Quiroz, R. Boulanger, M. Barel, B. Guyot, et al., "Impact of "ecological" post-harvest processing on coffee aroma: II. roasted coffee," *J Food Compos Anal*, vol. 20, no. 3-4, pp. 297-307, May 2007.
- [40] N. Dulsat-Serra, B. Quintanilla-Casas, and S. Vichi, "Volatile thiols in coffee: a review on their formation, degradation, assessment and influence on coffee sensory quality," *Food Res Int*, vol. 89, no. pp. 982-88, November 1 2016.
- [41] C. Liu, Q. Yang, R. Linforth, I. D. Fisk, and N. Yang, "Modifying robusta coffee aroma by green bean chemical pre-treatment," *Food Chem*, vol. 272, no. pp. 251-57, January 30 2019.
- [42] M. W. Cheong, K. H. Tong, J. J. M. Ong, S. Q. Liu, P. Curran, and B. Yu, "Volatile composition and antioxidant capacity of Arabica coffee," *Food Res Int*, vol. 51, no. 1, pp. 388-96, April 1 2013.
- [43] D. Bressanello, E. Liberto, C. Cordero, P. Rubiolo, G. Pellegrino, et al., "Coffee aroma: chemometric comparison of the chemical information provided by three different samplings combined with GC-MS to describe the sensory properties in cup," *Food Chem*, vol. 214, no. pp. 218-26, January 1 2017.
- [44] I. Steen, S. S. Waehrens, M. A. Petersen, M. Munchow, and W. L. Bredie, "Influence of serving temperature on flavour perception and release of Bourbon Caturra coffee," *Food Chem*, vol. 219, no. pp. 61-68, Mar 15 2017.
- [45] A. Ciampa, G. Renzi, A. Taglienti, P. Sequi, and M. Valentini, "Studies on coffee roasting process by means of nuclear magnetic resonance spectroscopy," *J Food Qual*, vol. 33, no. 2, pp. 199-211, April 2010.
- [46] N. Caporaso, M. B. Whitworth, C. Cui, and I. D. Fisk, "Variability of single bean coffee volatile compounds of Arabica and robusta roasted coffees analysed by SPME-GC-MS," *Food Res Int*, vol. 108, no. pp. 628-40, June 2018.
- [47] S. Schenker, and T. Rothgeb, "The Roast - Creating the beans' signature," in *The craft and science of coffee*, vol. B. Folmer, Eds.: Academic Press, 2017. pp. 245-71.
- [48] L. W. Lee, M. W. Cheong, P. Curran, B. Yu, and S. Q. Liu, "Coffee fermentation and flavor - an intricate and delicate relationship," *Food Chem*, vol. 185, no. pp. 182-91, Oct 15 2015.
- [49] C. Somporn, A. Kamtuo, P. Theerakulpisut, and S. Siriamornpun, "Effects of roasting degree on radical scavenging activity, phenolics and volatile compounds of Arabica coffee beans (*Coffea arabica* L. cv. Catimor)," *Int J Food Sci Technol*, vol. 46, no. 11, pp. 2287-96, November 2011.
- [50] C. A. B. De Maria, L. C. Trugo, F. R. A. Neto, R. F. A. Moreira, and C. S. Alviano, "Composition of green coffee water-soluble fractions and identification of volatiles formed during roasting," *Food Chem*, vol. 55, no. 3, pp. 203-07, March 1 1996.
- [51] A. N. Gloess, A. Vietri, F. Wieland, S. Smrke, B. Schönbacher, et al., "Evidence of different flavour formation dynamics by roasting coffee from different

origins: on-line analysis with PTR-ToF-MS,” *Int J Mass spectrom*, vol. 365-366, no. pp. 324-37, May 15 2014.

- [52] P. Wongsu, N. Khampa, S. Horadee, J. Chaiwarith, and N. Rattanapanone, “Quality and bioactive compounds of blends of Arabica and Robusta spray-dried coffee,” *Food Chem*, vol. 283, no. pp. 579-87, June 15 2019.
- [53] P. Muriel, and J. Arauz, “Coffee and liver health,” in *Coffee*, vol. Y.-F. Chu, Eds. Oxford, UK: Wiley-Blackwell, 2012. pp. 123-39.
- [54] O. G. Vitzthum, and P. Werkhoff, “Steam volatile aroma constituents of roasted coffee: neutral fraction,” *Z Lebensm Unters Forsch*, vol. 160, no. 3, pp. 277-91, 1976.
- [55] P. R. A. B. Toledo, L. Pezza, H. R. Pezza, and A. T. Toci, “Relationship between the different aspects related to coffee quality and their volatile compounds,” *Compr Rev Food Sci Food Saf*, vol. 15, no. 4, pp. 705-19, July 2016.