Food Reviews International

ISSN: (Print) (Online) Journal homepage: https://www.tandfonline.com/loi/lfri20

^{B}*N*-Alkanoyl-5-Hydroxytryptamines (C_n-5HTs) in Coffee: A Review

A. L. M. Brand, F. A. Lima, N. A. B. Tinoco, J. C. Mota, I. G. S. Moreira, F. J. M. Novaes, R. Garrett, T. B. S. Giorno, P. D. Fernandes & C. M. Rezende

To cite this article: A. L. M. Brand, F. A. Lima, N. A. B. Tinoco, J. C. Mota, I. G. S. Moreira, F. J. M. Novaes, R. Garrett, T. B. S. Giorno, P. D. Fernandes & C. M. Rezende (2022): ^{B}*N*-Alkanoyl-5-Hydroxytryptamines (C_n-5HTs) in Coffee: A Review, Food Reviews International, DOI: 10.1080/87559129.2022.2059807

To link to this article: https://doi.org/10.1080/87559129.2022.2059807

Published online: 19 Apr 2022.

Submit your article to this journal

View related articles

View Crossmark data

Full Terms & Conditions of access and use can be found at
https://www.tandfonline.com/action/journalInformation?journalCode=lfri20

FOOD REVIEWS INTERNATIONAL
https://doi.org/10.1080/87559129.2022.2059807

Taylor & Francis
Taylor & Francis Group

Check for updates

^{B}*N*-Alkanoyl-5-Hydroxytryptamines (C_n-5HTs) in Coffee: A Review

A. L. M. Brand[a], F. A. Lima[a], N. A. B. Tinoco[a], J. C. Mota[a], I. G. S. Moreira[a], F. J. M. Novaes[a], R. Garrett[a], T. B. S. Giorno[b], P. D. Fernandes[b], and C. M. Rezende[a]

[a]Chemistry Institute, Federal University of Rio de Janeiro, Rio de Janeiro, Brazil; [b]Biological Science Institute, Federal University of Rio de Janeiro, Rio de Janeiro, Brazil

ABSTRACT

^{B}*N*-alkanoyl-5-hydroxytryptamines (C_n-5HTs) are serotonin amides that contain saturated or unsaturated alkyl long chains. They can be found in the waxy layer that covers the coffee bean and are also present in the coffee brew, one of the most popular beverages in the world. These substances exhibit relevant biological activities, such as antinociceptive and neuroprotective, despite being responsible for the stomach irritation observed in some individuals after drinking coffee. This review depicts the extraction and quantification of C_n-5HTs in green and roasted coffee beans, and coffee brew. Additionally, it illustrates the biological activities, synthetic strategies, and products formed during the roasting of the coffee bean, regarding the serotonin amides.

KEYWORDS

Coffee; C_n-5HTs; *N*-acylated serotonin; stomach-friendly coffee

Introduction

The coffee plant is a shrub that belongs to the Rubiaceae family, genus *Coffea*. Only two coffee species are economically relevant: Robusta coffee (*Coffea canephora* P.) and Arabica coffee (*C. arabica* L),[1] the latter is responsible for more than 70% of the global market.[2] The coffee sector generated U$ 83 billion and employed 125 million people in 2019, making coffee one of the most valuable commodities in the world.[2]

$^{\beta}$*N*-alkanoyl-5- hydroxytryptamides (C_n-5HTs), also known as serotonin amides, are substances present in coffee beans, more specifically in coffee wax.[3,4] During the brewing process C_n-5HTs are extracted from the coffee beans to the brew and become available for human consumption.[5,6] Though they can induce stomach irritation in sensitive individuals, C_n-5HTs present many interesting biological properties such as anti-inflammatory,[7,8] antinociceptive,[9,10] antidepressant,[11] anxiolytic,[12] anticonvulsant,[13] and protection against Parkinson's and Alzheimer's diseases.[14,15]

Thus, this work aims to present the serotonin amides found in coffee, their thermal degradation products, methods of isolation from the bean, quantification in coffee brews, synthetic strategies, and biological activities.

$^{\beta}$N-alkanoyl-5- hydroxytryptamides (C_n-5HTs) in coffee

A thin layer of lipid material, called wax, covers the coffee bean. This wax corresponds to 0.2–0.3% of the bean's total weight and its main component are the C_n-5HTs.[3]

Coffee contains thirteen different C_n-5HTs (Table 1). They consist of a serotonin moiety conjugated to a fatty acid through an amide link. $^{\beta}$*N*-arachidoyl-5-hydroxytryptamide (C_{20}-5HT) is the most abundant serotonin amide in coffee beans (Fig. 1).[4]

CONTACT C. M. Rezende claudia.rezendeufrj@gmail.com Chemistry Institute, Aroma Analysis Laboratory, Federal University of Rio de Janeiro, Athos da Silveira Ramos Avenue, 149, 6thfloor, 626A - Cidade Universitária, Riode Janeiro - RJ 21941-909, Brazil

© 2022 Taylor & Francis

Table 1. C_n-5HTs present in coffee beans, their molecular formulas, molar mass, and reference.

Name	Molecular formula	Molar mass (g mol^{-1})	Reference
$^{\beta}N$-araquidoyl-5-hydroxytryptamide (C_{20}-5HT)	$C_{30}H_{50}N_2O_2$	470.3872	[18]
$^{\beta}N$- behenoyl –5- hydroxytryptamide (C_{22}-5HT)	$C_{32}H_{54}N_2O_2$	498.4185	[18]
$^{\beta}N$- lignoceroyl –5- hydroxytryptamide (C_{24}-5HT)	$C_{34}H_{58}N_2O_2$	526.4498	[18]
$^{\beta}N$- stearoyl –5- hydroxytryptamide (C_{18}-5HT)	$C_{28}H_{46}N_2O_2$	442.3559	[3]
$^{\beta}N$-hydroxyaraquidoyl-5- hydroxytryptamide (C_{20}(OH)-5HT)	$C_{30}H_{50}N_2O_3$	486.3821	[19]
$^{\beta}N$-hydroxybehenoyl-5- hydroxytryptamide (C_{22}(OH)-5HT)	$C_{32}H_{54}N_2O_3$	514.4134	[19]
$^{\beta}N$- heneicosanoyl –5- hydroxytryptamide (C_{21}-5HT)	$C_{31}H_{52}N_2O_2$	484.4028	[20]
$^{\beta}N$- tricosanoyl –5- hydroxytryptamide (C_{23}-5HT)	$C_{33}H_{56}N_2O_2$	512.4341	[20]
$^{\beta}N$- palmitoyl –5- hydroxytryptamide (C_{16}-5HT)	$C_{26}H_{42}N_2O_2$	414.3246	[4]
$^{\beta}N$ - linoleyl –5- hydroxytryptamide ($C_{18:2}$-5HT)	$C_{28}H_{42}N_2O_2$	438.3246	[4]
$^{\beta}N$- eicosenoyl –5- hydroxytryptamide ($C_{20:1}$-5HT)	$C_{30}H_{48}N_2O_2$	468.3715	[4]
βN-hydroxyeicosenoyl-5- hydroxytryptamide ($C_{20:1}$(OH)-5HT)	$C_{30}H_{48}N_2O_3$	484.3659	[4]
$^{\beta}N$-hydroxydocosenoyl-5- hydroxytryptamide ($C_{22:1}$(OH)-5HT)	$C_{32}H_{52}N_2O_3$	512.3972	[4]

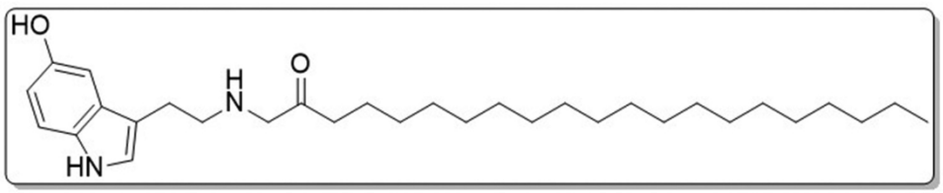

Figure 1. Chemical structure of C_{20}-5HT, the main serotonin amide found in the coffee bean.

Analogs of C_n-5HTs are also present in cocoa, almonds, walnuts, Brazil nuts, and hazelnuts.[16,17]

C_n-5HTs in coffee beans

Green coffee beans

Harms and Wuzier pioneered the identification and quantification of C_n-5HTs in green coffee beans.[18] The procedure, published in 1968, employed the extraction of ground green coffee beans with methanol under reduced pressure, followed by purification by column chromatography (CC), and separation by circular thin-layer chromatography (TLC) sprayed with Gibbs reagent. The resulting bands were scraped off and eluted from the adsorbent with benzene. The quantification was performed through spectrophotometry at 580 nm. The analyzed samples presented 500–1000 mg kg^{-1} of total C_n-5HTs. C_{20}-5HT, C_{22}-5HT, and C_{24}-5HT were characterized through UV, IR, and ^{1}H RMN.

Folstar et al. developed a new method to isolate C_n-5HTs from whole green coffee beans. The procedure involved two successive extractions of the beans with chloroform and petroleum ether, concentration of the insoluble in petroleum ether fraction with CC, and identification through high-performance liquid chromatography (HPLC) equipped with UV-vis detector (HPLC-UV-Vis).[3] This work was the first to describe C_{18}-5HT. In 1980, Folstar et al. updated the previous method and introduced new hydroxylated derivatives of C_n-5HTs: C_{20}(OH)-5HT and C_{22}(OH)-5HT.[19]

To obtain serotonin molecules, Kele e Ohmacht performed alkaline hydrolysis of C_n-5HTs in green coffee wax. The analysis used HPLC-UV-Vis to determine the total amount of serotonin (Table 2).[21] The authors indicated that C_n-5HTs can be a source of serotonin molecules.

Ambient mass spectrometry (MS) techniques have also been used to study C_n-5HTs in coffee beans. Garrett et al. identified C_{18}-5HT, C_{20}-5HT, and C_{22}-5HT on the surface of intact green coffee beans using desorption electrospray ionization (DESI-MS) and ambient sonic spray ionization mass spectrometry (EASI-MS). Both techniques required no sample preparation and did not employ any solvents.[23]

Table 2. Sample preparation and analytical methods for C_n-5HTs and free serotonin determination in coffee beans.

Reference	Coffee sample	Sample preparation	Analytical method	Content (mg kg^{-1})
[18]	Grounded green coffee beans	Extraction with methanol, purification with CC, separation with TLC, and derivatization with Gibbs reagent	Spectrophotometry	500–1000
[3]	Green coffee beans	Extraction with chloroform, and purification with CC.	HPLC-UV-Vis	259 – 273[a]
[19]	Green coffee beans	Extraction with dichloromethane, and purification with CC.	HPLC-UV-Vis	Not quantified
[22]	Grounded green coffee beans	Soxhlet extraction and ultrasound, both with methanol.	HPLC-FL	520
	Grounded roasted coffee beans			517
	Decaffeinated grounded green coffee beans			77
	Dewaxed grounded green coffee beans			200
[21]	Green coffee wax	Alkaline hydrolysis	HPLC-UV-Vis	4100[b]
[28]	Green coffee beans	Soxhlet extraction with methanol, isolation with CC and TLC, derivatization with Gibbs reagent	Spectrophotometry	1108
	Dry green coffee beans			810-934
	Moist green coffee beans			107
	Roasted coffee beans (convection)			473-533
	Roasted coffee beans (microwave)			501-542
[20]	Grounded roasted coffee beans	Extraction with THF and purification with solid-phase extraction	LC-MS/MS and HPLC-FL	426.13–775.57
[6]	Grounded roasted coffee beans	Extraction with THF	LC-MS/EMS and HPLC-FL	428.3–898.1
	Dewaxed grounded roasted coffee beans			330.9–577.0
[23]	Green coffee beans	None	EASI and DESI	Not quantified
[29]	Roasted coffee beans	None	EASI	Not quantified
		Extraction with 1% formic acid in methanol solution.	HPLC-FL HPLC-DAD	
[24]	Grounded green coffee beans	Extraction with THF	HPLC-FL	899–1347

[a]only C_{22}-5HT.
[b]Free serotonin.

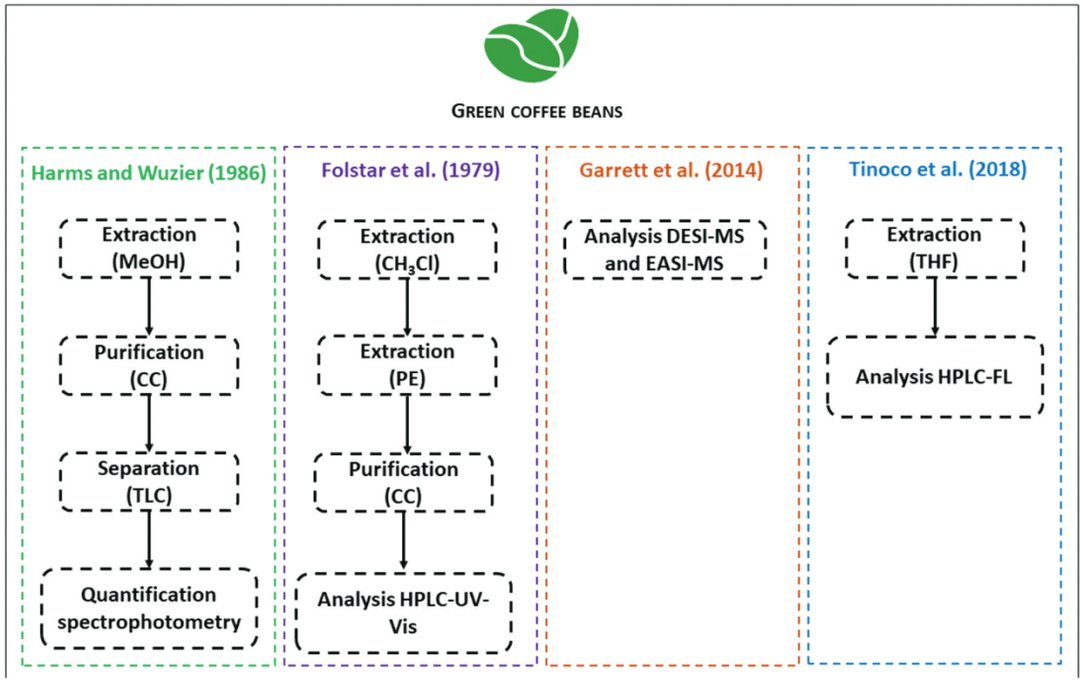

Figure 2. Scheme of the methods of isolation and quantification of C_n-5HTs from green coffee beans. Methanol (MeOH); column chromatography (CC); thin-layer chromatography (TLC); chloroform (CH_3Cl); PE (petroleum ether); THF (tetrahydrofuran); high-performance liquid chromatography equipped with UV-vis detector (HPLC-UV-Vis); high-performance liquid chromatography equipped with fluorescence detector (HPLC -FL); DESI-MS (desorption electrospray ionization); EASI-MS (ambient sonic spray ionization mass spectrometry).

Tinoco et al. studied the metabolism of C_n-5HTs after the fermentation of green coffee beans with *Saccharomyces cerevisiae* yeasts (bakery, white and sparkling wines) as starter cultures. The ground beans were extracted with tetrahydrofuran and analysed using HPLC coupled to a fluorescence detector (HPLC-FL). The authors observed a reduction of 41% and 29% on the content of C_{20}-5HT e C_{22}-5HT, respectively, after the fermentation process (Table 2).[24]

The methods of isolation and quantification of C_n-5HTs of green coffee beans are represented in Fig. 2.

Roasted coffee beans

Roasting is a crucial step in coffee processing that makes the beans suitable for brewing. The heating (150-230°C) induces chemical, physical, and structural changes that lead to the formation of substances responsible for the sensory qualities of the beverage.[25] Many substances in the green coffee bean, such as chlorogenic acids and diterpenes, undergo significant transformation during the roasting process depending on the temperature.[26,27] Thus, many authors have studied the effect of roasting on C_n-5HTs.

Nebesby and Budryn compared two roasting methods, convection, and microwave, on green coffee beans to understand the effect of coffee roasting on total C_n-5HTs content.[28] Green coffee beans showed the highest amounts of C_n-5HTs, followed by microwave roasted coffee and convection roasted coffee (Table 2). During the convection roasting process, the surface of the coffee beans – where the wax is found – presents higher temperatures than microwave roasting. Thus, the study concludes that C_n-5HTs are susceptible to thermal degradation and that higher temperatures induce more degradation.

Lang and Hoffman evaluated the C_n-5HT content in roasted Arabica and Robusta coffee beans from Colombia and Brazil.[20] Sample preparation was comprised of extraction with tetrahydrofuran and purification with solid-phase extraction. The C_n-5HTs were quantified and identified employing HPLC-FL and high-performance liquid chromatography equipped with mass spectrometry (HPLC-MS). In all samples both C_{20}-5HT and C_{22}-5HT were the main amides, representing 85% of total C_n-5HT content. While Robusta coffee presented higher amounts of C_{20}-5HT, Arabica coffee had more C_{22}-5HT. In contrast, the minor C_n-5HTs were not strongly influenced by the coffee variety. The authors also described two new serotonin amides: C_{21}-5HT and C_{23}-5HT.

In 2016, Rosa et al employed EASI-MS to investigate C_n-5HTs in light, medium, dark, and very dark roasted coffee beans.[29] The total content of C_n-5HTs decreased as the roast degree intensified. In addition, C_n-5HTs were considered markers for light roast coffee beans.

The method of isolation and quantification of C_n-5HTs from roasted coffee beans are showed in Fig. 3.

These results corroborate that C_n-5HTs are thermolabile substances that suffer important transformations during the coffee roasting process; furthermore, higher degrees of roasting cause greater thermolysis.

Viani and Horman were the first to study the thermolysis reaction of C_{20}-5HT using a column connected to a GC-MS system.[30] The experiment heated the sample at 180-230°C for 15 minutes in a sealed tube. They observed a series of alkylindoles and alkylindanes as degradation products. The first conclusive work was done by Zham and Speer using a pyrolyzer-GC-MS tandem system to

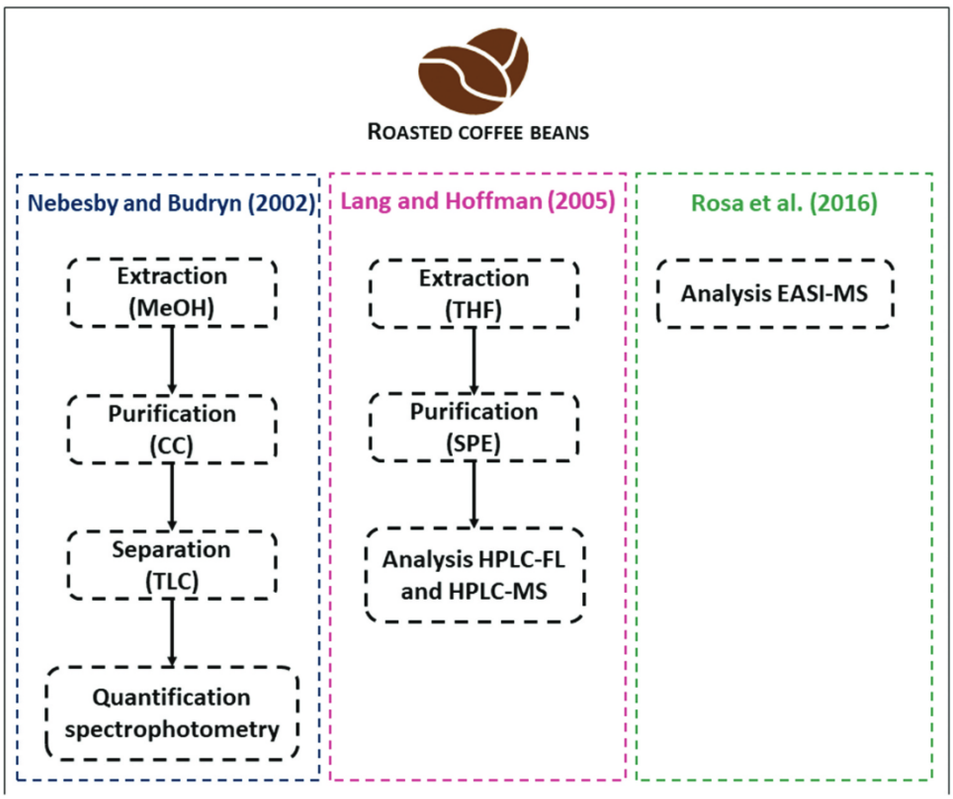

Figure 3. Scheme of the methods of isolation and quantification of C_n-5HTs from roasted coffee beans. Methanol (MeOH); column chromatography (CC); thin-layer chromatography (TLC); THF (tetrahydrofuran); HPLC-MS (high-performance liquid chromatography equipped with a mass spectrometry detector); HPLC-FL (high-performance liquid chromatography equipped with fluorescence detector); EASI-MS (ambient sonic spray ionization mass spectrometry).

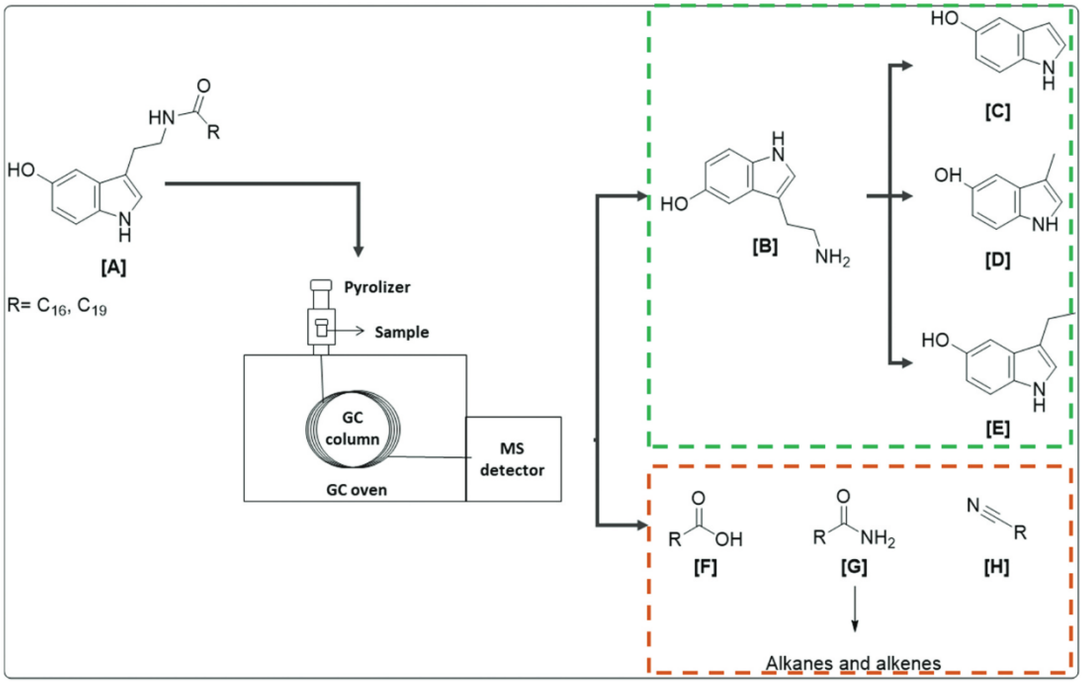

Figure 4. Pyrolysis for C_n-5HTs.[31] ([A] C_{18}-5HT, C_{21}-5HT; [B] Serotonin; [C] 5-hydroxyindole; [D] 3-methyl-5-hydroxyindole; [E] 3-ethyl-5-hydroxyindole; [F] Fatty acid; [G] Fatty acid amide; [H] Nitrile).

investigate the pyrolysis of C_{18}-5HT.[31] The pyrolysis temperatures ranged from 358 to 740°C. They identified 5-hydroxyindole, 3-methyl-5-hydroxyindole, 3-ethyl-5-hydroxyindole, serotonin, octadecanonitrile, octadecanoic acid, and octadecanoamide as thermal degradation products of C_{18}-5HT. The degradation pathway suggested by the authors includes the α-cleavage to serotonin, 5-hydroxyindole, 3-methyl-5-hydroxyindole, 3-ethyl-5-hydroxyindole, and the corresponding fatty acid, fatty acid nitrile, and fatty acid amide. An increase in temperature causes the formation of alkanes and alkenes (Fig. 4). As the pyrolysis temperatures are higher than those employed during coffee roasting, the authors heated a small amount of C_{18}-5HT at 200°C for 15 minutes. After HS-SPME-GC/MS and GC/MS analysis, the authors observed the same degradation products as for the pyrolysis experiment. C_{21}-5HT had similar degradation products.

"Stomach–friendly" coffee beans

Although coffee is one of the most popular beverages in the world, some individuals can experience symptoms of gastric irritation or heartburn after coffee consumption.[6,32,33]

"Stomach-friendly" coffee brews are prepared using dewaxed coffee beans. The dewaxing process was initially developed to reduce caffeine and chlorogenic acid in coffee, as these substances were known for causing stomach irritation.[34] However, as C_n-5HTs are the main component of the coffee wax their concentration diminishes also.[5,6,22]

The coffee wax can be removed through steam and organic solvents. The steaming technique was developed by Lendrich in 1920 and improved by Darboven in 1997.[35,36] Similarly, Ludwig Roselius previously developed the solvent-based decaffeination method,[37] where the green coffee beans are steamed and immersed in organic solvents, such as dichloromethane and ethyl acetate. After the extraction, the beans are steam-treated again to remove the solvent.[38] These methods use similar

procedures mainly employing steam and it must be clarified that decaffeination can also occur only through contact with different solvents, as well as with hot water, differing from the processes mentioned above.

Laganà et al. compared the performance of Soxhlet (1,2,3, and 4 hours) and ultrasound (30 minutes), both with methanol, on the extraction of C_n-5HTs from green, roasted, decaffeinated, and dewaxed coffees. The analysis was performed using HPLC-FL.[22] There were no significant differences between the extraction methods studied. Green coffee beans presented the highest amount of C_n-5HTs followed by roasted, dewaxed, and decaffeinated beans (authors do not describe decaffeination nor dewaxed procedures employed) (Table 2). These results confirm that C_n-5HTs are susceptible to thermal degradation and their content reduces with wax removal or decaffeination. Van der Stegen observed a similar pattern when analyzing C_n-5HTs in green and dewaxed coffee beans.[5]

In 2010, Lang et al. used tetrahydrofuran extraction and HPLC-MS to analyze the influence of steam treatment on the C_n-5HT content in Arabica and Robusta roasted coffee beans from Brazil and Vietnam.[6] The most abundant major amides were C_{20}-5HT and C_{22}-5HT. Concerning total C_n-5HT content, Arabica coffee had more amides than Robusta coffee and steamed beans less than regular ones.

Therefore, the content of C_n-5HTs in coffee beans can be reduced through wax removal processes. However, studies show that the decaffeination process – that employs organic solvents- is the most efficient one.

Table 2 presents a summary of the extraction and analysis techniques employed in the investigation of C_n-5HTs in coffee beans.

The methods applied in the investigation of C_n-5HTs in coffee beans have evolved a lot since the first one described in 1968. Currently, these substances can be identified using simple isolation procedures, liquid chromatography, and solvent-free methods, such as DESI-MS and EASI-MS.

Moreover, the total C_n-5HT content in coffee beans decreases with roasting, decaffeination, and wax removal.

C_n-5HTs in coffee brews

Approximately 2.25 billion cups of coffee are consumed daily worldwide and C_n-5HTs are biologically active substances present in this drink.[6,33] Thus, the study of these substances on coffee brew is of great importance for human health.

In 1979, Van der Stegen started the research on C_n-5HTs in coffee brews, analyzing filtered and boiled coffee prepared with regular and dewaxed roasted coffee beans.[5] As expected, the boiled brew prepared with dewaxed beans had a lower amount of C_n-5HT than the one prepared with regular beans. Independently of the bean used, filtered brews did not present C_n-5HTs (Table 3). Similar

Table 3. C_n-5HTs content found in different types of coffee brews according to bean treatment.

Reference	Type of coffee bean	Type of coffee Brew	Content (mg L^{-1})
[5]	Dewaxed	Filtered	-
		Boiled	0.50
	Regular	Filtered	-
		Boiled	2.30
[22]	Decaffeinated	Moka	0.31
	Dewaxed		0.56
	Regular		1.02
[6]	Regular	Filtered	0.14
		Moka	0.99
		French Press	3.50
		Espresso (machine)	8.41
		Espresso (manual)	1.21

results are observed for other lipophilic substances in coffee. The diterpene cafestol is also found in lower concentrations in filtered coffee.[39–41] During the brewing process the hot water extracts lipids from the beans forming an emulsion. Due to the low solubility of the lipid fraction in water these substances are retained by the filter in filtered coffee preparation, thus this type of brew has low diterpene content.[41]

Laganà et al. studied the content of C_n-5HT in Moka brews prepared with dewaxed, decaffeinated, and regular coffee beans using solid-phase extraction and HPLC-FL.[22] The wax removal and decaffeination provided brews with a lower concentration of C_n-5HTs, however, the second treatment was more efficient (Table 3).

Lang et al. studied C_n-5HTs in filtered, French press, Moka, and espresso coffee prepared manually or with a machine, using HPLC-FL and HPLC-MS.[6] Espresso coffee (machine) had a higher amount of C_n-5HTs, followed by the French press. In contrast to Van der Stegen's results,[5] it was possible to identify C_n-5HTs in filtered coffee (Table 3).

Figure 5 shows a schematic representation of methods applied for the quantification of C_n-5HTs on coffee brews.

Thus, C_n-5HTs are extracted from the coffee bean to the brew and become available for human consumption. The type of bean and the method of preparation used directly influence the concentration of these substances in the final drink, with the lowest levels found in the

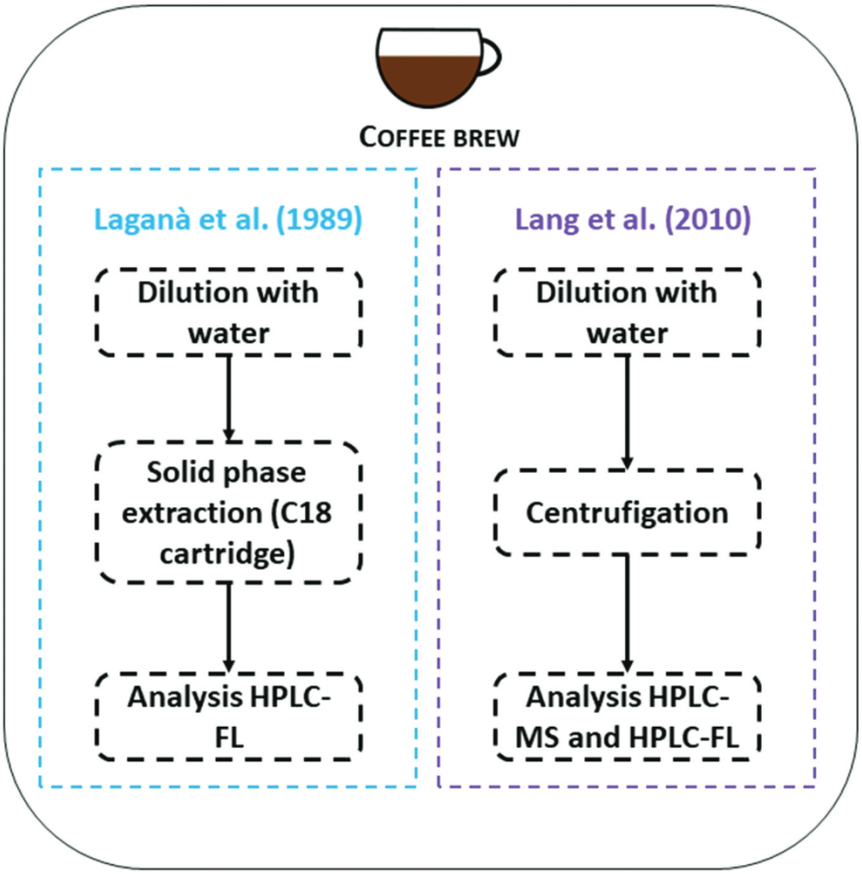

Figure 5. Scheme of isolation and quantification of C_n-5HTs in coffee brews. (HPLC-MS (high-performance liquid chromatography equipped with a mass spectrometry detector); high-performance liquid chromatography equipped with fluorescence detector (HPLC-FL).

filtered and the highest in espresso coffee. The previous decaffeination or wax removal of the bean also leads to a reduction of these substances in the beverages, confirming that the "stomach-friendly" coffees have a lower content of C_n-5HTs. The total content of C_n-5HT described in coffee brews by the authors is presented in Table 3.

Synthetic approaches to obtain C_n-5HTs

The isolation of C_n-5HTs from green coffee beans is a laborious process that uses harmful solvents. It also provides low yields of a complex mixture, in the order of 0.05–0.1%.[18] C_n-5HTs synthesis enables higher yields, increased scale, and safer methodologies for obtaining these substances.

In 1977, Hubert et al. synthesized the serotonin amides of arachidic (C_{20}: 0), behenic (C_{22}: 0), and lignoceric ($C_{24:0}$) acids through the fatty acid chlorides and 5-benzyloxytryptamine followed by palladium-catalyzed hydrogenation to obtain the serotonin amides.[42] Bezuglov et al. employed serotonin hydrochloride (5HT · HCl), triethylamine and isobutyl chloroformate providing the arachidonic acid serotonin amide ($C_{20:4}$-5HT).[43] In a second methodology for eicosapentaenoic acid ($C_{20:5}$) , they used 1,1-carbonyldiimidazole (CDI) on the acid for the formation of the reactive acylated species, showing that both methodologies stand out for enabling the synthesis of serotonin amides with polyunsaturated fatty acid chains. Both provide yields between 60 and 70%.

Lang and Hoffmann and Lang et al. used thionyl chloride ($SOCl_2$) and covered amides from C_{18} to C_{24}, obtained in yields that varied between 37–57%.[6,20]

Ortar et al. synthesized C_n-5HTs via carbodiimide using 1-(3-dimethylaminopropyl)-3-ethyl carbodiimide (EDC) and 1-hydroxybenzotriazole (HOBt) (Fig. 5).[44] Reddy et al. expanded the scope of this method while studying the properties of several serotonin amides.[45] Together, the two works carried out the amidation of chain acids between C_{11} and C_{20}.[46] Jai et al. also employed EDC and HOBt to synthesize C_{18}-5HT obtaining a yield higher than 90%.[46] Giorno et al. (2021) exploited a slightly different approach to obtain C_{18}-5HT using EDC and 4-dimethylaminopyridine (DMAP) with a yield of 78%.[47]

Amorim et al. also used EDC and DMAP to synthesize C_{20}-5HT and C_{22}-5HT on a mechanochemical reactor (planetary ball mill). Mechanochemistry is a technique that allows the synthesis of compounds using the energy provided by the shock between the spheres in a ball mill. These reactions are usually performed in a single step and the absence of solvents. The authors obtained yields of 59% and 64% for C_{20}-5HT and C_{22}-5HT, respectively.[48]

Figure 6 shows a schematic representation of the synthetic approaches described.

The synthetic approaches to obtain C_n-5HTs have received important contributions since the first published work. Different scopes of saturated and unsaturated fatty acids were evaluated, safer methodologies were developed and different amidation pathways were explored. However, there is still room for improvement with new technologies such as microwave, and ultrasound reactors, flow chemistry, biocatalysis, and the use of "greener" solvents.

Biological activities related to C_n-5HTs

The role of C_n-5HTs in stomach irritation

Fehlau and Netter were the first to make the correlation between C_n-5HTS and stomach irritation by feeding rats with water (control), untreated coffee brew, waxless coffee brew, and pellets enriched with C_n-5HTs.[49] The total amount of C_n-5HTs in the pellets was comparable to untreated coffee. The interventions lasted 1, 2, 3, 8, 13, and 20 weeks. The animal's gastric mucosa was analysed, and the numbers of ulcers counted. The animals fed with the pellets presented more ulcers than the control group in week 1 and had the same number of lesions as the ones that received the normal coffee brew after week 8. These results indicate that C_n-5HTs had a pro ulcerogenic effect in the

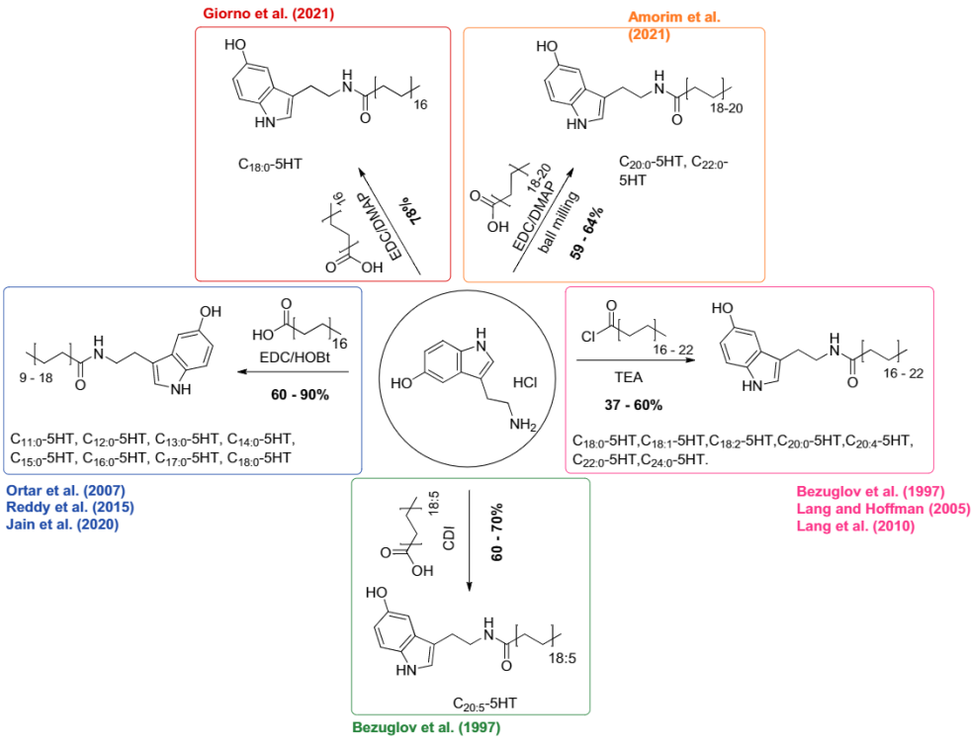

Figure 6. Scheme of the synthetic approaches to produce C_n-5HTs (TEA, triethylamine; EDC, 1-(3-dimethylaminopropyl)-3-ethyl carbodiimide; HOBt, 1-hydroxybenzotriazole (HOBt), and DMAP, 4-dimethylaminopyridine.

gastric mucosa of the animals. However, after week 13, the animals fed with the regular coffee brew presented the highest number of ulcers, which suggests that other substances in coffee contribute to this effect.

Weiss et al. used human gastric tumor cells (HGT-1) to study the influence of lyophilized water extracts from unprocessed and decaffeinated coffee brews on proton secretion.[32] The regular drink extract elevated the intracellular pH more than the decaffeinated one – an increase in intracellular pH indicates transport of protons to the extracellular medium. A principal component analysis indicated that a group of substances present in coffee, including C_n-5HTs, are responsible for stomach discomfort. However, the study did not investigate the effect of these substances separately.

Rubach et al. followed Weiss's study by fractionating a coffee brew with water, ethyl acetate, chloroform, and pentane.[50] HGT-1 cells were treated with each fraction and the effect on the intracellular pH was observed. The only fraction that caused a reduction in proton secretion was the aqueous fraction, which had the highest C_n-5HTs content. However, in this fraction *N*-methylpyridinium (*N*-MP) was also found. This substance is known for reducing proton secretion. Besides, only the pentane fraction did not present C_n-5HTs.

In 2012, Rubach et al. used the same HGT-1 cells model to investigate the action of chlorogenic acid, caffeine, C_n-5HTs, *N*-MP, pyrogallol, and catechol standards, individually and combined, on proton secretion.[51] A mixture of C_n-5HT was employed (C_{18}-5HT (2 nM), C_{20}-5HT (75 nM) and C_{22}-5HT (180 nM) and C_{24}-5HT (25 nM). Among the substances studied, C_n-5HTs had the greatest impact on proton secretion, indicating that these substances favour the reduction of stomach pH.

Lang et al. also employed HGT-1 cells to study the structure-activity relationship of C_n-5HTs and increased acid secretion.[6] The authors synthesized amides with different sizes of alkyl chains and degrees of unsaturation (C_{18}-5HT, $C_{18:\,1}$-5HT, $C_{18:\,2}$-5HT, and C_{20}-5HT). The size of the alkyl chain

had a positive correlation with proton secretion, and the degree of unsaturation had no significant influence. Also, the mixture of C_n-5HTs stimulated more proton secretion than each amide individually.

Rubach et al. used healthy human volunteers to study the influence of coffee components on stomach pH.[52] 200 mL of two different coffee brews were administered: one prepared from medium roast coffee beans, and the other from dark roast ones. Both had similar caffeine content but differed in their concentrations of C_n-5HTs, chlorogenic acids, trigonelline, and *N*-methylpyridinium. The medium roast brew, with a higher content of C_n-5HTs, reduced the stomach pH of the volunteers more effectively.

The studies show that C_n-5HTs are substances capable of inducing the secretion of stomach protons, causing a consequent reduction in pH in vitro cell models, animals, and humans. Coffee brews prepared from beans with reduced C_n-5HTs content, like "stomach-friendly" and decaffeinated, had a milder impact on acid secretion.

C_n-5HTs in the context of the endocannabinoid system

The endocannabinoid system was discovered in 1988 by Devane et al.[53] It consists of cannabinoid receptors (CB1 and CB2), endocannabinoids (ECs), and enzymes responsible for the synthesis and degradation of these substances.[54] Cannabinoids are capable of interacting with other types of receptors beyond CBs, such as the vanilloid 1 transient potential receptor (TRVP1).[55]

Endocannabinoids are endogenous agonists of CBs. The most studied ECs are anandamide (*N*-arachidonoylethanolamide) (Fig. 7) and 2-arachidonoylglycerol (2-AG). The key enzyme for EC metabolism is fatty acid amide hydrolase (FAAH), which degrades anandamide into arachidonic acid and ethanolamine and 2-AG into arachidonic acid and glycerol. The endocannabinoid system has become an important pharmacological target for the treatment of several disorders, such as chronic pain, depression and anxiety, epilepsy, among others.[55]

C_n-5HTs and the treatment of pain

Anandamide is an agonist of CB and TRVP1 receptors, both involved in the nociception process. While CB receptors are related to reduced sensitivity to pain, TRVP1 receptors have a pro-analgesic effect when activated.[56–58] Selective inhibition of increases the endogenous concentration of anandamide, stimulating both CB and TRVP1 receptors. Therefore, substances that inhibit FAAH and antagonize the TRVP1 receptor have great potential for the treatment of chronic pain.[59,60]

In 1998 Bisogno et al. found that C_{20}-5HT can inhibit FAAH (IC50 12 μM) without acting on the CB1 receptor in neuroblastoma cells N18TG2.[61] Arachidonoyl-histamine and arachidonoyl-dopamine were also tested and showed inferior inhibitory activity than C_{20}-5HT, indicating that the 5-hydroxy-indole nucleus of the serotonin molecule would allow its interaction with the active enzyme site through hydrogen bonding. The replacement of the arachidonoyl group by eicosapentaenoyl reduced the potency of the inhibition as well.

Fowler et al. used followed up on the previous study, evaluating the activity of C_{20}-5HT analogs in the inhibition of FAAH in rat brain cells *in vitro*.[62] The removal of the phenolic hydroxyl group of the serotonin molecule reduced the inhibition of FAAH, confirming the importance of the 5-hydroxy

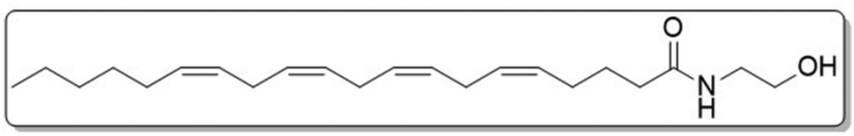

Figure 7. Structure of the endocannabinoid anandamide.

indole group for the stabilization of the enzyme-inhibitor complex. Beyond that, the substitution of the amide group by an ester group reduced the potency of the inhibition even further. The replacement of the arachidoyl unit by linoleoyl did not affect the amide activity.

Maione et al. demonstrated that C_{20}-5HT is not only capable of inhibiting FAAH activity, but also has antagonistic action on the TRPV1 receptor in HEK-293 cells overexpressing the human or the rat recombinant TRPV1 receptor, and *in vivo*, in rats and mice.[10] In the same year, Ortar et al. observed the same activities for an $^{\beta}N$-araquidoyl-5-hydroxytryptamide (AA-5HT) (C_{20}-Δ^4-5HT) analog both *in vitro* and *in vivo* employing similar models to the previous study.[44]

Novellis et al. and Costa et al. confirmed the C_{20}-5HT activities on both CB and TRVP1 receptors using an *in vivo* model employing mice.[63,64] The second study showed that the systemic administration of C_{20}-5HT reduced the edema and pain associated with inflammation, while the local administration had no effect. While the TRVP1 receptor was responsible for the anti-inflammatory effect, both receptors, TRPV1, and CB1 were involved in the anti-hyperalgesic activity. C_{20}-5HT did not induce undesired effects on the animals' walking ability and body temperature.

In 2016, Malek et al. demonstrated that C_{20}-5HT indirectly activates the CB2 receptor, using a neuropathic pain model in rats.[65] The intrathecal administration of the amide increased the pain threshold for mechanical and thermal stimuli in animals.

Giorno et al. evaluated the antinociceptive activity of C_{18}-5HT in mice.[9] The amide showed central and peripheral antinociceptive activities that involved the endocannabinoid, opioid, and serotonergic systems. The authors also observed an anti-hyperalgesic effect; as C_{18}-5HT decreased the pain of inflammatory origin, by reducing the animals' latency time in the model of thermal hyperalgesia induced by carrageenan. In 2021, the same group used a similar model to investigate the antinociceptive effect of C_{20}-5HT and C_{22}-5HT. Opioidergic, muscarinic, cannabinoid, and serotoninergic pathways are involved in the antinociceptive activity.[48]

In this context, it is possible to observe that C_{18}-5HT and C_{20}-5HT, serotonin amides present in coffee, have antinociceptive activity confirmed by *in vitro* and *in vivo* models. Their mechanism of action involves FAAH inhibition and TRVP1 receptor antagonism. Therefore, C_n-5HTs have great potential for the treatment of chronic pain.

C_n-5HTs and aspects related to anxiety and depression

The CB and TRPV1 receptors are considered new targets for the development of anxiolytic and antidepressant drugs. However, activation of the CB1 receptor and TRPV1 channel seems to modulate emotional responses in opposite ways: the first inhibit anxiety and reduces the response to fear, and the second promotes anxiety and conditioned fear when activated.[66,67] Hence, the C_{20}-5HT has great potential for the treatment of this type of disorder due to its dual capacity of FAAH inhibition and TRVP1 antagonism.

Micale et al. evaluated the anxiolytic activity of C_{20}-5HT in rats, using the elevated plus-maze test, after its systemic administration.[12] The amide reduced animal anxiety more efficiently than selective FAAH and TRPV1 inhibitors. However, this study does not indicate the site of action of amide in the central nervous system.

In 2012, John and Currie showed that the anxiolytic action of C_{20}-5HT occurs in the basolateral intra-amygdala of rats, using the elevated plus-maze test.[68] The mechanism of action is related to the increased binding of endocannabinoids to CB1 receptors, due to the inhibition of FAAH, and to the antagonism of TRPV1 receptors. The systemic administration of C_{20}-5HT also reduced behavioural despair in the animals after the forced swim test. Navarria et al. confirmed the previous study results in a similar work.[69]

Kirkedal et al. also employed the forced swim test to assess the influence of C_{20}-5HT on the depressive behavior of rats.[11] The administration of C_{20}-5HT on the animals' prefrontal cortex resulted in an antidepressant effect that was partially attenuated by the local blockage CB1 receptor.

In 2017, Gobira et al. hypothesized that C_{20}-5HT would reduce the fear response due to greater activation of the CB1 receptor in the dorsal hippocampus of mice.[70] Both systemic and local administration inhibited the fear response in the EPM test. The administration of a CB1 antagonist in the hippocampus inhibited the action of C_{20}-5HT. Therefore, C_{20}-5HT inhibits the fear response due to the activation of the CB1 receptor by endocannabinoids in the dorsal hippocampus.

Thus, C_{20}-5HT reduces anxiety and depression in rodents through action on the basolateral intra-amygdala and prefrontal cortex, respectively. These results indicate the study of C_n-5HTs for the treatment of disorders is of great relevance.

The anticonvulsant activity of C_{20}-5HT

The blockage of anandamide hydrolysis through FAAH inhibition has anticonvulsant effects mediated by CB1 receptors.[71] However, anandamide also activates TRPV1 receptors that have the opposite effect.[72] Therefore, the dual-action described for FAAH inhibitor and TRPV1 antagonist C_{20}-5HT can be promising for the treatment of seizures.

Vilela et al. tested this hypothesis and used C_{20}-5HT in a rat seizure model *in vivo*.[13] They observed that C_{20}-5HT reduced the duration and intensity of seizures and that this effect is related to the CB1 receptor.

C_n-5HTs and neurodegenerative diseases

C_n-5HTs analogs have also been studied for their neuroprotective activity against Parkinson's and Alzheimer's diseases.

Lee et al. and Lee et al. conducted studies to access C_{20}-5HT activity against Parkinson's disease.[15,73] In the first study, transgenic α-Synuclein mice on a diet supplemented with C_{20}-5HT showed reduced α-Synuclein phosphorylation and aggregation in the brain due to PP2A methylation. These biochemical changes were associated with enhanced neural and motor activity. The second study demonstrated that four-week diet supplementation with C_{20}-5HT conferred neuroprotection on mice treated with MPTP through many mechanisms that involve anti-inflammatory and anti-oxidant activities.

Basurto-Islas et al. studied C_{20}-5HT neuroprotectant activity against Alzheimer's disease in rats.[14] Dietary supplementation with C_{20}-5HT for 6 to 12 months decreased the formation of neurofibrillary tangles and neuritic plaques, hallmarks of Alzheimer's disease, due to the demethylation of the protein phosphatase 2A PP2Ac.

Min et al. evaluated the effect of C_{16}-5HT in learning and memory deficits models induced by scopolamine in mice.[74] Scopolamine is often used as a memory and learning impairment inducer in experimental Alzheimer´s disease models. C_{16}-5HT significantly improved the amnesia of mice in the behavioral assessment, as well as suppressing oxidative stress by increasing levels of glutathione peroxidase (GPx), glutathione reductase (GR), or NAD (P) H quinine oxidoreductase-1 (NQO −1) and reduced level of malonaldehyde (MDA) and decreased the death of neuronal cells in the hippocampus, with the restoration of BDNF and *p*-CREB levels.

In 2014, Jin et al. showed the C_n-5HTs analogs (C_{16}-5HT, C_{18}-5HT, $C_{18:1}$-5HT, C_{20}-5HT, and $C_{22:6}$-5HT) diminished glutamate-related cytotoxicity in HT-22 cells *in vitro*.[75] Glutamate neurotoxicity is involved in neuronal cell death associated with many neurodegenerative disorders. They were also able to reduce oxidative stress by decreasing the production of reactive oxygen species (ROS) and increasing glutathione production. The authors suggest that C_n-5HTs attenuated glutamate-induced cytotoxicity through PI3K/PDK-1/Akt activation and p38 MAPK-dependent Nrf2 signaling in the early phase, as well as suppression of the MEK/ERK pathway in the late phase.

Hence, C_n-5HTs show neuroprotectant activities towards Alzheimer's and Parkinson´s diseases *in vitro* and *in vivo* animal models, mainly by reducing inflammation and oxidative stress in brain cells.

Table 4. Biological activities of C_n-5HTs.

Bioactivity	Research design	Conclusion	Reference
Stomach irritation	*In vivo* (rats)	C_n-5HTs have a pro ulcerogenic effect in the gastric mucosa.	[49]
Stomach irritation	*In vitro* (HGT-1 cells)	Coffee brews with higher C_n-5HT content elevated proton secretion.	[32]
Stomach irritation	*In vitro* (HGT-1 cells)	C_{18}-5HT, $C_{18:1}$-5HT, $C_{18:2}$-5HT, and C_{20}-5HT increased acid secretion.	[6]
Stomach irritation	*In vitro* (HGT-1 cells)	Water fraction of coffee brews high in Cn-5HTs and *N*-MP reduced acid secretion.	[50]
Stomach irritation	*In vitro* (HGT-1 cells)	C_{18}-5HT, C_{20}-5HT, C_{22}-5HT, and C_{24}-5HT increased acid secretion.	[51]
Stomach irritation	*In vivo* (humans)	Coffee brew with the highest C_n-5HT content reduced the stomach pH of volunteers more effectively.	[52]
Antinociception	*In vitro* (N18TG2 cells)	C_{20}-5HT inhibits FAAH without acting on the CB1 receptor.	[61]
Antinociception	*In vitro* (rat brain cells)	C_{20}-5HT inhibits FAAH without acting on the CB1 receptor. The amide group and 5-hydroxy-indole moiety are relevant to the inhibition.	[62]
Antinociception	*In vitro* (HEK-293 cells)	C_{20}-5HT inhibits FAAH and has antagonistic action on the TRVP1 receptor.	[10]
Antinociception	*In vitro* (HEK-293 cells) and *in vivo* (mice)	C_{20}-5HT analog inhibits FAAH and has antagonistic action on the TRVP1 receptor.	[44]
Antinociception	*In vivo* (mice)	C_{20}-5HT antinociceptive effect is mediated by TRVP1 and CB1 receptors.	[63]
Antinociception	*In vivo* (mice)	C_{20}-5HT antinociceptive effect that is related to TRVP1 antagonism and CB1 receptor activation. The TRVP1 receptor is responsible for the anti-inflammatory effect of C_{20}-5HT.	[64]
Antinociception	*In vivo* (rats)	C_{20}-5HT antinociceptive effect that involves CB2 receptor activation.	[65]
Antinociception	*In vivo* (mice)	C_{18}-5HT has central and peripheral antinociceptive activities that involve the endocannabinoid, opioid, and serotonergic systems.	[9]
Antinociception	*In vivo* (mice)	C_{20}-5HT and C_{22}-5HT antinociceptive effects are linked to opioidergic, muscarinic, cannabinoid, and serotoninergic pathways.	[48]
Anxiolytic	*In vivo* (rats)	C_{20}-5HT reduced animal anxiety after systemic administration.	[12]
Anxiolytic	*In vivo* (rats)	C_{20}-5HT anxiolytic activity occurs in the basolateral intra-amygdala of rats due to indirect activation of the CB1 receptor and antagonism of TRVP1.	[68]
Anxiolytic	*In vivo* (rats)	C_{20}-5HT improves behavioral stress in rats due to blockade of FAAH and TRPV1	[69]
Antidepressive	*In vivo* (rats)	C_{20}-5HT acts on the CB1 receptor on the animals' prefrontal cortex producing an antidepressive effect.	[11]
Reduced fear response	*In vivo* (rats)	C_{20}-5HT inhibits the fear response due to the activation of the CB1 receptor by endocannabinoids in the dorsal hippocampus.	[70]
Anticonvulsant	*In vivo* (rats)	C_{20}-5HT reduced the duration and intensity of seizures and this effect is related to the CB1 receptor.	[13]
Neuroprotective (Parkinson´s disease)	*In vivo* (mice)	C_{20}-5HT reduced α-Synuclein phosphorylation and aggregation in the brain due to PP2A methylation	[73]
Neuroprotective (Parkinson´s disease)	*In vivo* (mice)	C_{20}-5HT showed neuroprotection on mice treated with MPTP through anti-inflammatory and antioxidant activities.	[15]
Neuroprotective (Alzheimer´s disease)	*In vivo* (mice)	C_{20}-5HT decreased the formation of neurofibrillary tangles and neuritic plaques due to the demethylation of the protein phosphatase 2A PP2Ac.	[14]

(*Continued*)

Table 4. (Continued).

Bioactivity	Research design	Conclusion	Reference
Neuroprotective (Alzheimer´s disease)	*In vivo* (mice)	C_{16}-5HT improved amnesia suppressed oxidative stress, and decreased the death of neuronal cells in the hippocampus, with the restoration of BDNF and *p*-CREB levels.	[74]
Neuroprotective	*In vitro* (HT-22 cells)	C_{16}-5HT, C_{18}-5HT, $C_{18:1}$-5HT, C_{20}-5HT, and $C_{22:6}$ -5HT reduced glutamate-related cytotoxicity through PI3K/PDK-1/Akt activation and p38 MAPK-dependent Nrf2 signaling in the early phase, and suppression of the MEK/ERK pathway in the late phase.	[75]
Anti-allergic	*In vitro* (RBL-2H3 cells)	C_{20}-5HT reduces allergic reaction by inhibition of the FcεRI receptor cascade and reduced the IgE-mediated degranulation of mast cells.	[7]
Anti-inflammatory	*In vitro* (RAW 264.17 cells)	$C_{22:6}$-5HT attenuated the LPS induced IL-23-IL17 signaling cascade in murine macrophages, downregulated LPS induced genes involved in the CD4+ Th17 response.	[8]
Anti-inflammatory	*In vitro* (human peripheral blood mononuclear cells)	C_n-5HTs inhibited IL-17 and CCL-20; and regulated IL-1/Th17	[80]
Visceral pain and intestinal motility	*In vivo* (mice)	C_{20}-5HT reduced intestinal contractility via a TRVP1 receptor mechanism and inhibited colonic motility due to a CB1 mediated response.	[81]
Anti-inflammatory	*In vivo* (mice) and *in vitro* (RAW 264.7 cells)	C_{18}-5HT inhibited the production of NO and pro-inflammatory cytokines *in vitro* and reduced leukocyte migration into the SAP, protein extravasation, NO production, and cytokines levels *in vivo*.	[47]

C_n-5HT and inflammatory diseases

Yoo et al. investigated the anti-allergic action of C_{20}-5HT in IgE-mediated degranulation in RBL-2H3 cells *in vitro*.[7] The results suggested that C_{20}-5HT can inhibit allergic reaction by direct inhibition of the FcεRI receptor cascade through the suppressing the activation of Syk, Gab2, PI3K, Akt, LAT, ERK1/2, p38, JNK, PLCγ1/2, and PKCδ. The amide also inhibited the IgE-mediated degranulation of mast cells, evidenced by lower levels of TNF-α, PGD_2, and LTB_4 in the cells. This suggests that C_{20} -5HT also suppresses allergic inflammation.

Poland et al. showed that $^{\beta}$*N*-docosahexaenoyl-5-hydroxytryptamide ($C_{22:6}$-5HT) has a potent anti-inflammatory activity as it attenuated the LPS induced IL-23-IL17 signaling cascade in murine macrophages (RAW 264.17).[8] Though $C_{22:6}$-5HT is not found in coffee, its structure resembles other C_n-5HTs. Many studies and clinical trials have shown that the IL-23-IL-17 cascade has great importance in autoimmune diseases.[76]

Beyond that, $C_{22:6}$-5HT downregulated LPS induced genes, especially those involved in the CD4+ Th17 response. Hence, low concentrations of $C_{22:6}$-5HT lowered the levels of macrophage-produced mediators that activate Th1 cells, such as PGE2, IL-6, IL-1β, and IL-23. CD4+ Th17 cells are currently the focus of many studies targeting inflammatory diseases, such as psoriasis, inflammatory bowel disease, and multiple sclerosis.[76–78] $C_{22:6}$-5HT, also inhibited RAW 264.7 migration; and decreased chemokines levels (MCP-1 and CCL-20), and the gene expression of CCL-22 and various metalloproteinases. In 2011, the same group detected endogenous C_n-5HT analogs in swine (*ex vivo)* and rats (*in vivo*) intestines.[79] They observed that the level of C_n-5HT was related to local serotonin supply was increased. The identity of the alkyl chain portion varied according to the animals' diet: the formation of $C_{22:6}$-5HT and $C_{20:5}$-5HT was elevated in mice fed with fish oil.

Later, Wang et al. identified a series of C_n-5HTs in the human colon *ex vivo*.[80] C_{16}-5HT, C_{18}-5HT, $C_{18:\ 1}$-5HT were detected at higher levels than C_{20}-5HT, and $C_{22:6}$-5HT, $C_{20:5}$-5HT could not be quantified. The authors tested the amide's ability to inhibit the release of IL-17 and CCL-20 in human peripheral blood mononuclear cells stimulated by Concanavalin A (ConA-PBMCs). $C_{22:6}$-5HT was the best inhibitor of IL-17 and CCL-20; Th17 pro-inflammatory mediators involved in inflammatory intestinal disorders, such as Crohn's disease and ulcerative colitis. Thus, $C_{22:6}$-5HT is a gut-specific endogenous mediator capable of regulating IL-1/Th17.

In this context, Bashashati et al. studied the effect of C_{20}-5HT in visceral pain and intestinal motility in mice.[81] The intracolonic administration of the amide reduced intestinal contractility via a TRVP1 receptor mechanism. C_{20}-5HT had an inhibitory effect on colonic motility due to a CB1 mediated response. The authors suggested that this amide has the potential to be employed in the treatment of irritable bowel syndrome.

Giorno et al. characterized the anti-inflammatory effects of C_{18}-5HT using a carrageenan-induced cellular migration in a subcutaneous air pouch model (SAP) in mice and RAW264.7 cells activated with LPS (*in vitro*).[47]

The *in vitro* assays indicated that C_{18}-5HT inhibited the production of NO and pro-inflammatory cytokines, such as IL-1β. An increase in the levels of IL-10, an immunomodulatory cytokine, was observed.

The animals treated orally with C_{18}-5HT (0.1, 1, and 10 mg kg^{-1}) presented lower leukocyte migration into the SAP as well less protein extravasation and lower production of nitric oxide (NO). There was also a reduction in cytokines (TNF-α, IL-1, and IFN- γ) levels in the SAP exudates after treatment with C_{18}-5HT. There was also increased IL-10 production and accumulation in the exudate. Hence, C_{18}-5HT showed potent *in vivo* and *in vitro* anti-inflammatory effects.

The biological activities of C_n-5HTs are presented in Table 4.

Disclosure statement

No potential conflict of interest was reported by the author(s).

Funding

Authors would like to acknowledge Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) [142180/2020-3; 141330/2017-1; 304394/2017-3], Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) [88887.320176/2019-00], Fundação de Amparo à Pesquisa Estado do Rio de Janeiro (FAPERJ) [E-26/202.941/2017; E-26/202.763/2018; E-26/202.283/2019; E-26/200.862/2021], and Embrapa Café for financial support.

References

[1] Durán, C. A. .; Tsukui, A.; dos Santos, F. K. F.; Martinez, S. T.; Bizzo, H. R.; de Rezende, C.M. Coffee: General Aspects and Its Use Beyond Drink. *Rev. Virtual Quím.* 2017, *9*(1), 107–134. DOI: 10.21577/1984-6835.20170010.

[2] International Coffee Organization. Exports of Coffee by Exporting Countries. http://www.ico.org/trade_statistics.asp?section=Statistics (accessed Dec 27, 2020).

[3] Folstar, P.; Van der Plas, H. C.; Pilnik, W.; Schols, H. A.; Melger, P. Liquid Chromatographic Analysis of N-Beta-Alkanoyl-5-Hydroxytryptamine (C-5-HT) in Green Coffee Beans. *J. Agric. Food. Chem.* 1979, *27*(1), 12–15. DOI: 10.1021/jf60221a031.

[4] Speer, K.; Kölling-Speer, I. The Lipid Fraction of the Coffee Bean. *Braz. J. Plant Physiol.* 2006, *18*(1), 201–216. DOI: 10.1590/S1677-04202006000100014.

[5] Van der Stegen, G. The Effect of Dewaxing of Green Coffee on the Coffee Brew. *Food Chem.* 1979, *4*(1), 23–29. DOI: 10.1016/0308-8146(79)90027-X.

[6] Lang, R.; Bardelmeier, I.; Weiss, C.; Rubach, M.; Somoza, V.; Hofmann, T. Quantitation of β N -Alkanoyl -5-Hydroxytryptamides in Coffee by Means of LC-MS/MS-SIDA and Assessment of Their Gastric Acid Secretion Potential Using the HGT-1 Cell Assay. *J. Agric. Food. Chem.* 2010, *58*(3), 1593–1602. DOI: 10.1021/jf903612h.

[7] Yoo, J. M.; Sok, D. E.; Kim, M. R. Effect of Endocannabinoids on IgE-Mediated Allergic Response in RBL-2H3 Cells. *Int. Immunopharmacol.* 2013, *17*(1), 123–131. DOI: 10.1016/j.intimp.2013.05.013.

[8] Poland, M.; ten Klooster, J. P.; Wang, Z.; Pieters, R.; Boekschoten, M.; Witkamp, R.; Meijerink, J. Docosahexaenoyl Serotonin, an Endogenously Formed N-3 Fatty Acid-Serotonin Conjugate Has Anti-Inflammatory Properties by Attenuating IL-23–IL-17 Signaling in Macrophages. *Biochim. Biophys. Acta - Mol. Cell Biol. Lipids*. 2016, *1861*(12), 2020–2028. DOI: 10.1016/j.bbalip.2016.09.012.

[9] Giorno, T. B. S.; Moreira, I. G. D. S.; Rezende, C. M.; Fernandes, P. D. New BN-Octadecanoyl-5-Hydroxytryptamide: Antinociceptive Effect and Possible Mechanism of Action in Mice. *Sci. Rep*. 2018, *8*(1), 10027. DOI: 10.1038/s41598-018-28355-4.

[10] Maione, S.; Petrocellis, L.; Novellis, V.; Moriello, A. S.; Petrosino, S.; Palazzo, E.; Rossi, F. S.; Woodward, D. F.; Marzo, V. Analgesic Actions of N -Arachidonoyl-Serotonin, a Fatty Acid Amide Hydrolase Inhibitor with Antagonistic Activity at Vanilloid TRPV1 Receptors. *Br. J. Pharmacol*. 2007, *150*(6), 766–781. DOI: 10.1038/sj.bjp.0707145.

[11] Kirkedal, C.; Wegener, G.; Moreira, F.; Joca, S. R. L.; Liebenberg, N. A Dual Inhibitor of FAAH and TRPV1 Channels Shows Dose-Dependent Effect on Depression-Like Behaviour in Rats. *Acta Neuropsychiatr*. 2017, *29*(6), 324–329. DOI: 10.1017/neu.2016.68.

[12] Micale, V.; Cristino, L.; Tamburella, A.; Petrosino, S.; Leggio, G. M.; Drago, F.; Di Marzo, V. Anxiolytic Effects in Mice of a Dual Blocker of Fatty Acid Amide Hydrolase and Transient Receptor Potential Vanilloid Type-1 Channels. *Neuropsychopharmacology*. 2009, *34*(3), 593–606. DOI: 10.1038/npp.2008.98.

[13] Vilela, L. R.; Medeiros, D. C.; de Oliveira, A. C. P.; Moraes, M. F.; Moreira, F. A. Anticonvulsant Effects of N -Arachidonoyl-Serotonin, a Dual Fatty Acid Amide Hydrolase Enzyme and Transient Receptor Potential Vanilloid Type-1 (TRPV1) Channel Blocker, on Experimental Seizures: The Roles of Cannabinoid CB1 Receptors and TRPV1 Channels. *Basic Clin. Pharmacol. Toxicol*. 2014, *115*(4), 330–334. DOI: 10.1111/bcpt.12232.

[14] Basurto-Islas, G.; Blanchard, J.; Tung, Y. C.; Fernandez, J. R.; Voronkov, M.; Stock, M.; Zhang, S.; Stock, J. B.; Iqbal, K. Therapeutic Benefits of a Component of Coffee in a Rat Model of Alzheimer's Disease. *Neurobiol. Aging*. 2014, *35*(12), 2701–2712. DOI: 10.1016/j.neurobiolaging.2014.06.012.

[15] Lee, K. W.; Im, J. Y.; Woo, J. M.; Grosso, H.; Kim, Y. S.; Cristovao, A. C.; Sonsalla, P. K.; Schuster, D. S.; Jalbut, M. M.; Fernandez, J. R., et al. Neuroprotective and Anti-Inflammatory Properties of a Coffee Component in the MPTP Model of Parkinson's Disease. *Neurotherapeutics*. 2013, *10*(1), 143–153.

[16] Wurziger, J.; Harms, U. Über Carbonsäurehydroxy-Tryptamide in Ölhaltigen Samen. *Fette. Seifen. Anstr*. 1973, *75*(2), 121–126. DOI: 10.1002/lipi.19730750214.

[17] Speer, K.; Zahm, A. Sterols and Carboxylic Acid-5-Hydroxytrypt-Amides in Selected Tree Nuts. *Lipid Technol*. 2011, *23*(6), 127–130. DOI: 10.1002/lite.201100112.

[18] Harms, U.; Wurziger, J. Carbonsäure-5-(Hydroxy)-Tryptamide in Kaffeebohnen. *Z. Lebensm. Unters. Forsch*. 1968, *138*(2), 75–80. DOI: 10.1007/BF02341897.

[19] Folstar, P.; Schols, H. A.; Van der Plas, H. C.; Pilnik, W.; Landheer, C. A.; Van Veldhuizen, A. New Tryptamine Derivatives Isolated from Wax of Green Coffee Beans. *J. Agric. Food. Chem*. 1980, *28*(4), 872–874. DOI: 10.1021/jf60230a022.

[20] Lang, R.; Hofmann, T. A Versatile Method for the Quantitative Determination of BN-Alkanoyl-5-Hydroxytryptamides in Roasted Coffee. *Eur. Food Res. Technol*. 2005, *220*(5–6), 638–643. DOI: 10.1007/s00217-004-1123-y.

[21] Kele, M.; Ohmacht, R. Determination of Serotonin Released from Coffee Wax by Liquid Chromatography. *J. Chromatogr. A*. 1996, *730*(1–2), 59–62. DOI: 10.1016/0021-9673(95)01186-2.

[22] Laganà, A.; Curini, L.; de Angelis Curtis, S.; Marino, A. Rapid Liquid Chromatographic Analysis of Carboxylic Acid-5-Hydroxytryptamides in Coffee. *Chromatographia*. 1989, *28*(11–12), 593–596. DOI: 10.1007/BF02260684.

[23] Garrett, R.; Schwab, N. V.; Cabral, E. C.; Henrique, B. V. M.; Ifa, D. R.; Eberlin, M. N.; Rezende, C. M. Ambient Mass Spectrometry Employed for Direct Analysis of Intact Arabica Coffee Beans. *J. Braz. Chem. Soc*. 2014, *25*(7), 1172–1177. DOI: 10.5935/0103-5053.20140094.

[24] Tinoco, N. A. B.; Pacheco, S.; Godoy, R. L. O.; Bizzo, H. R.; de Aguiar, P. F.; Leite, S. G. F.; Rezende, C. M. Reduction of N-Alkanoyl-5-Hydroxytryptamides and Diterpenes by Yeast Supplementation to Green Coffee During Wet Processing. *Food. Res. Int*. 2019, *115*(June), 487–492. DOI: 10.1016/j.foodres.2018.10.007.

[25] Fabbri, A.; Cevoli, C.; Alessandrini, L.; Romani, S. Numerical Modeling of Heat and Mass Transfer During Coffee Roasting Process. *J. Food Eng*. 2011, *105*(2), 264–269. DOI: 10.1016/j.jfoodeng.2011.02.030.

[26] Farah, A.; de Paulis, T.; Trugo, L. C.; Martin, P. R. Effect of Roasting on the Formation of Chlorogenic Acid Lactones in Coffee. *J. Agric. Food. Chem*. 2005, *53*(5), 1505–1513. DOI: 10.1021/jf048701t.

[27] Dias, R. C. E.; de Faria-Machado, A. F.; Mercadante, A. Z.; Bragagnolo, N.; Benassi, M. D. T. Roasting Process Affects the Profile of Diterpenes in Coffee. *Eur. Food Res. Technol*. 2014, *239*(6), 961–970. DOI: 10.1007/s00217-014-2293-x.

[28] Nebesny, E.; Budryn, G. Effect of the Roasting Method on the Content of 5-Hydroxytryptamides of Carboxylic Acids in Roasted Coffee Beans. *Nahrung/food*. 2002, *46*(4), 279. DOI: 10.1002/1521-3803(20020701)46:4<279:AID-FOOD279>3.0.CO;2-R.

[29] da Rosa, J. S.; Freitas-Silva, O.; Rouws, J. R. C.; da Silva Moreira, I. G.; Novaes, F. J. M.; de Almeida Azevedo, D.; Schwab, N.; de Oliveira Godoy, R. L.; Eberlin, M. N.; de Rezende, C. M. Mass Spectrometry Screening of Arabica Coffee Roasting: A Non-Target and Non-Volatile Approach by EASI-MS and ESI-MS. *Food. Res. Int.* 2016, *89*, 967–975. DOI: 10.1016/j.foodres.2016.03.021.
[30] Viani, R.; Horman, I. Thermal Behavior of Trigonelline. *J. Food Sci.* 1974, *39*(6), 1216–1217. DOI: 10.1111/j.1365-2621.1974.tb07357.x.
[31] Zahm, A.; Peters, J.; Speer, K. Identification of C-5-HT Degradation Products Using Pyrolysis-GC/MS. ASIC 23rd International Conference on Coffee Science, Paris, 2010; pp 244–247.
[32] Weiss, C.; Rubach, M.; Lang, R.; Seebach, E.; Blumberg, S.; Frank, O.; Hofmann, T.; Somoza, V. Measurement of the Intracellular PH in Human Stomach Cells: A Novel Approach to Evaluate the Gastric Acid Secretory Potential of Coffee Beverages. *J. Agric. Food. Chem.* 2010, *58*(3), 1976–1985. DOI: 10.1021/jf903614d.
[33] Cordoba, N.; Pataquiva, L.; Osorio, C.; Moreno, F. L. M.; Ruiz, R. Y. Effect of Grinding, Extraction Time and Type of Coffee on the Physicochemical and Flavour Characteristics of Cold Brew Coffee. *Sci. Rep.* 2019, *9*(1), 1–12. DOI: 10.1038/s41598-019-44886-w.
[34] Toci, A.; Farah, A.; Trugo, L. C. Effect of Decaffeination Using Dichloromethane on the Chemical Composition of Arabica and Robusta Raw and Roasted Coffees. *Quim. Nova.* 2006, *29*(5), 965–971. DOI: 10.1590/s0100-40422006000500015.
[35] Lendrich, K. Process of Improving Coffee Beans. 1931, 1822227. DOI: 10.1145/178951.178972.
[36] Darboven, A. *Process for Improving the Quality of Green Coffee by Treatment with Steam and Water*, 1997.
[37] Ludwig, R.; Hans-Albert Kurzhals, H.; Peter Hubert, B. Method for the Selective Extraction of Caffeine from Vegetable Materials. US4255458, 1981.
[38] Meyer, J. F.; Roselius, L.; Wimmer, K.; Bremen, O. F. *Treatment of Coffee*, 1908; pp 897733.
[39] Zhang, C.; Linforth, R.; Fisk, I. D. Cafestol Extraction Yield from Different Coffee Brew Mechanisms. *Food. Res. Int.* 2012, *49*(1), 27–31. DOI: 10.1016/j.foodres.2012.06.032.
[40] Moeenfard, M.; Silva, J. A.; Borges, N.; Santos, A.; Alves, A. Quantification of Diterpenes and Their Palmitate Esters in Coffee Brews by HPLC-DAD. *Int. J. Food. Prop.* 2015, *18*(10), 2284–2299. DOI: 10.1080/10942912.2014.933351.
[41] Rendón, M. Y.; dos Santos Scholz, M. B.; Bragagnolo, N. Physical Characteristics of the Paper Filter and Low Cafestol Content Filter Coffee Brews. *Food. Res. Int.* 2018, *108*(January), 280–285. DOI: 10.1016/j.foodres.2018.03.041.
[42] Hubert, P.; Kwasny, H.; Werkhoff, P.; Turner, U. Analytik von Carbonsaurehydroxytryptamiden in Kaffee. *Fresenius Z. Für Anal. Chem.* 1977, *285*(3–4), 242–250. DOI: 10.1007/BF00453570.
[43] Bezuglov, V. V.; Manevich, E. M.; Archakov, A. V.; Bobrov, M. I.; Kuklev, D. V.; Petrukhina, G. N.; Makarov, V. A.; Buznikov, G. A. Artificially Functionalized Polyenoic Fatty Acids–a New Lipid Bioregulators. *Bioorg. Khim.* 1997, *23*(3), 211–220.
[44] Ortar, G.; Cascio, M. G.; De Petrocellis, L.; Morera, E.; Rossi, F.; Schiano-Moriello, A.; Nalli, M.; De Novellis, V.; Woodward, D. F.; Maione, S., et al. New N -Arachidonoyl serotonin Analogues with Potential "Dual. *Mechanism of Action Against Pain* 2007, 6554–6569.
[45] Reddy, S. T.; Swamy, M. J. Synthesis, Physicochemical Characterization and Membrane Interactions of a Homologous Series of N-Acylserotonins: Bioactive, Endogenous Conjugates of Serotonin with Fatty Acids. *Biochim. Biophys. Acta - Biomembr.* 2015, *1848*(1), 95–103. DOI: 10.1016/j.bbamem.2014.09.012.
[46] Jain, P.; Bhagat, S.; Tunki, L.; Jangid, A. K.; Singh, S.; Pooja, D.; Kulhari, H. Serotonin-Stearic Acid Bioconjugate-Coated Completely Biodegradable Mn3o4 Nanocuboids for Hepatocellular Carcinoma Targeting. *ACS Appl. Mater. Interfaces.* 2020, *12*(9), 10170–10182. DOI: 10.1021/acsami.0c00331.
[47] Giorno, T. B. S.; Lima, F. A.; Brand, A. L. M.; De Oliveira, C. M.; Rezende, C. M.; Fernandes, P. D. Characterization of BN-Octadecanoyl-5-Hydroxytryptamide Anti-Inflammatory Effect. *Molecules.* 2021, *26*(12), 3709. DOI: 10.3390/molecules26123709.
[48] Amorim, J. L.; Lima, F. A.; Brand, A. L. M.; Cunha, S.; Rezende, C. M.; Fernandes, P. D. Two New β N -Alkanoyl -5-Hydroxytryptamides with Relevant Antinociceptive Activity. *Biomedines.* 2021, *9*(5), 455. DOI: 10.3390/biomedicines9050455.
[49] Fehlau, R.; Netter, K. J. Effect of Untreated and Non-Irritating Purified Coffee and Carbonic Acid Hydroxytryptamides on the Gastric Mucosa in the Rat. *Z. Gastroenterol.* 1990, *28*(5), 234–238.
[50] Rubach, M.; Lang, R.; Skupin, C.; Hofmann, T.; Somoza, V. Activity-Guided Fractionation to Characterize a Coffee Beverage That Effectively Down-Regulates Mechanisms of Gastric Acid Secretion as Compared to Regular Coffee. *J. Agric. Food. Chem.* 2010, *58*(7), 4153–4161. DOI: 10.1021/jf904493f.
[51] Rubach, M.; Lang, R.; Seebach, E.; Somoza, M. M.; Hofmann, T.; Somoza, V. Multi-Parametric Approach to Identify Coffee Components That Regulate Mechanisms of Gastric Acid Secretion. *Mol. Nutr Food Res.* 2012, *56*(2), 325–335. DOI: 10.1002/mnfr.201100453.
[52] Rubach, M.; Lang, R.; Bytof, G.; Stiebitz, H.; Lantz, I.; Hofmann, T.; Somoza, V. A Dark Brown Roast Coffee Blend is Less Effective at Stimulating Gastric Acid Secretion in Healthy Volunteers Compared to a Medium Roast Market Blend. *Mol. Nutr Food Res.* 2014, *58*(6), 1370–1373. DOI: 10.1002/mnfr.201300890.

[53] Devane, W. A.; Dysarz, F. A.; Johnson, M. R.; Melvin, L. S.; Howlett, A. C. Determination and Characterization of a Cannabinoid Receptor in Rat Brain. *Mol. Pharmacol.* 1988, *34*(5), 605–613.
[54] Lu, H. C.; MacKie, K. An Introduction to the Endogenous Cannabinoid System. *Biol. Psychiatry*. 2016, *79*(7), 516–525. DOI: 10.1016/j.biopsych.2015.07.028.
[55] Pacher, P.; Bátkai, S.; Kunos, G. The Endocannabinoid System as an Emerging Target of Pharmacotherapy. *Pharmacol. Rev*. 2006, *58*(3), 389–462. DOI: 10.1124/pr.58.3.2.
[56] Ross, R. A. Anandamide and Vanilloid TRPV1 Receptors. *Br. J. Pharmacol*. 2003, *140*(5), 790–801. DOI: 10.1038/sj.bjp.0705467.
[57] Tóth, A.; Czikora, Á.; Pásztor, E. T.; Dienes, B.; Bai, P.; Csernoch, L.; Rutkai, I.; Csató, V.; Mányiné, I. S.; Pórszász, R., et al. Vanilloid Receptor-1 (TRPV1) Expression and Function in the Vasculature of the Rat. *J. Histochem Cytochem*. 2014, *62*(2), 129–144.
[58] Barrie, N.; Manolios, N. The Endocannabinoid System in Pain and Inflammation: Its Relevance to Rheumatic Disease. *Eur. J. Rheumatol*. 2017, *4*(3), 210–218. DOI: 10.5152/eurjrheum.2017.17025.
[59] Bisogno, T.; Petrocellis, L.; Marzo, V. Fatty Acid Amide Hydrolase, an Enzyme with Many Bioactive Substrates. Possible Therapeutic Implications. *Curr. Pharm. Des*. 2002, *8*(7), 533–547. DOI: 10.2174/1381612023395655.
[60] Fowler, C.; Naidu, P.; Lichtman, A.; Onnis, V. The Case for the Development of Novel Analgesic Agents Targeting Both Fatty Acid Amide Hydrolase and Either Cyclooxygenase or TRPV1. *Br. J. Pharmacol*. 2009, *156*(3), 412–419. DOI: 10.1111/j.1476-5381.2008.00029.x.
[61] Bisogno, T.; Melck, D.; De Petrocellis, L.; Bobrov, M. Y.; Gretskaya, N. M.; Bezuglov, V. V.; Sitachitta, N.; Gerwick, W. H.; Marzo, V. D. Arachidonoyl serotonin and Other Novel Inhibitors of Fatty Acid Amide Hydrolase. *Biochem. Biophys. Res. Commun*. 1998, *248*(3), 515–522. DOI: 10.1006/bbrc.1998.8874.
[62] Fowler, C. J.; Tiger, G.; López-Rodríguez, M. L.; Viso, A.; Ortega-Gutiérrez, S.; Ramos, J. A. Inhibition of Fatty Acid Amidohydrolase, the Enzyme Responsible for the Metabolism of the Endocannabinoid Anandamide, by Analogues of Arachidonoyl-Serotonin. *J. Enzyme Inhib. Med. Chem*. 2003, *18*(3), 225–231. DOI: 10.1080/1475636031000080216.
[63] de Novellis, V.; Palazzo, E.; Rossi, F.; De Petrocellis, L.; Petrosino, S.; Guida, F.; Luongo, L.; Migliozzi, A.; Cristino, L.; Marabese, I., et al. The Analgesic Effect of N-Arachidonoyl-Serotonin, a FAAH Inhibitor and TRPV1 Receptor Antagonist, Associated with Changes in Rostral Ventromedial Medulla and Locus Coeruleus Cell Activity in Rats. *Neuropharmacology*. 2008, *55*(7), 1105–1113.
[64] Costa, B.; Bettoni, I.; Petrosino, S.; Comelli, F.; Giagnoni, G.; Di Marzo, V. The Dual Fatty Acid Amide Hydrolase/trpv1 Blocker, N-Arachidonoyl-Serotonin, Relieves Carrageenan-Induced Inflammation and Hyperalgesia in Mice. *Pharmacol. Res*. 2010, *61*(6), 537–546. DOI: 10.1016/j.phrs.2010.02.001.
[65] Malek, N.; Kostrzewa, M.; Makuch, W.; Pajak, A.; Kucharczyk, M.; Piscitelli, F.; Przewlocka, B.; Di Marzo, V.; Starowicz, K. The Multiplicity of Spinal AA-5-HT Anti-Nociceptive Action in a Rat Model of Neuropathic Pain. *Pharmacol. Res*. 2016, *111*, 251–263. DOI: 10.1016/j.phrs.2016.06.012.
[66] Huang, W. J.; Chen, W. W.; Zhang, X. Endocannabinoid System: Role in Depression, Reward and Pain Control (Review). *Mol. Med. Rep*. 2016, *14*(4), 2899–2903. DOI: 10.3892/mmr.2016.5585.
[67] Papagianni, E. P.; Stevenson, C. W. Cannabinoid Regulation of Fear and Anxiety: An Update. *Curr. Psychiatr. Rep*. 2019, *21*(6). DOI: 10.1007/s11920-019-1026-z.
[68] John, C. S.; Currie, P. J. N-Arachidonoyl-Serotonin in the Basolateral Amygdala Increases Anxiolytic Behavior in the Elevated Plus Maze. *Behav. Brain Res*. 2012, *233*(2), 382–388. DOI: 10.1016/j.bbr.2012.05.025.
[69] Navarria, A.; Tamburella, A.; Iannotti, F. A.; Micale, V.; Camillieri, G.; Gozzo, L.; Verde, R.; Imperatore, R.; Leggio, G. M.; Drago, F., et al. The Dual Blocker of FAAH/TRPV1 N-Arachidonoylserotonin Reverses the Behavioral Despair Induced by Stress in Rats and Modulates the HPA-Axis. *Pharmacol. Res*. 2014, *87*, 151–159. DOI: 10.1016/j.phrs.2014.04.014.
[70] Gobira, P. H.; Lima, I. V.; Batista, L. A.; de Oliveira, A. C.; Resstel, L. B.; Wotjak, C. T.; Aguiar, D. C.; Moreira, F. A. N-Arachidonoyl-Serotonin, a Dual FAAH and TRPV1 Blocker, Inhibits the Retrieval of Contextual Fear Memory: Role of the Cannabinoid CB1 Receptor in the Dorsal Hippocampus. *J. Psychopharmacol*. 2017, *31*(6), 750–756. DOI: 10.1177/0269881117691567.
[71] Cheung, K. A. K.; Peiris, H.; Wallace, G.; Holland, O. J.; Mitchell, M. D. The Interplay Between the Endocannabinoid System, Epilepsy and Cannabinoids. *Int. J. Mol. Sci*. 2019, *20*(23). DOI: 10.3390/ijms20236079.
[72] Cho, S. J.; Vaca, M. A.; Miranda, C. J.; N'-Gouemo, P. Inhibition of Transient Potential Receptor Vanilloid Type 1 Suppresses Seizure Susceptibility in the Genetically Epilepsy-Prone Rat. *CNS Neurosci. Ther*. 2018, *24*(1), 18–28. DOI: 10.1111/cns.12770.
[73] Lee, K. W.; Chen, W.; Junn, E.; Im, J. Y.; Grosso, H.; Sonsalla, P. K.; Feng, X.; Ray, N.; Fernandez, J. R.; Chao, Y., et al. Enhanced Phosphatase Activity Attenuates α-Synucleinopathy in a Mouse Model. *J. Neurosci*. 2011, *31*(19), 6963–6971.
[74] Min, A. Y.; Doo, C. N.; Son, E. J.; Sung, N. Y.; Lee, K. J.; Sok, D. E.; Kim, M. R. N-Palmitoyl Serotonin Alleviates Scopolamine-Induced Memory Impairment via Regulation of Cholinergic and Antioxidant Systems, and Expression of BDNF and P-CREB in Mice. *Chem. Biol. Interact*. 2015, *242*, 153–162. DOI: 10.1016/j.cbi.2015.09.016.

[75] Jin, M. C.; Yoo, J. M.; Sok, D. E.; Kim, M. R. Neuroprotective Effect of N-Acyl 5-Hydroxytryptamines on Glutamate-Induced Cytotoxicity in HT-22 Cells. *Neurochem. Res.* 2014, *39*(12), 2440–2451. DOI: 10.1007/s11064-014-1448-2.

[76] Gaffen, S. L.; Jain, R.; Garg, A. V.; Cua, D. J. The IL-23-IL-17 Immune Axis: From Mechanisms to Therapeutic Testing. *Nat. Rev. Immunol.* 2014, *14*(9), 585–600. DOI: 10.1038/nri3707.

[77] Rostami, A.; Ciric, B. Role of Th17 Cells in the Pathogenesis of CNS Inflammatory Demyelination. *J. Neurol. Sci.* 2013, *333*(1–2), 76–87. DOI: 10.1016/j.jns.2013.03.002.

[78] Mease, P. J. Inhibition of Interleukin-17, Interleukin-23 and the TH17 Cell Pathway in the Treatment of Psoriatic Arthritis and Psoriasis. *Curr. Opin. Rheumatol.* 2015, *27*(2), 127–133. DOI: 10.1097/BOR.0000000000000147.

[79] Verhoeckx, K. C. M.; Voortman, T.; Balvers, M. G. J.; Hendriks, H. F. J.; Wortelboer, M.; Witkamp, H.; Presence, R. F. Formation and Putative Biological Activities of N-Acyl Serotonins, a Novel Class of Fatty-Acid Derived Mediators, in the Intestinal Tract. *Biochim. Biophys. Acta - Mol. Cell Biol. Lipids.* 2011, *1811*(10), 578–586. DOI: 10.1016/j.bbalip.2011.07.008.

[80] Wang, Y.; Balvers, M. G. J.; Hendriks, H. F. J.; Wilpshaar, T.; van Heek, T.; Witkamp, R. F.; Meijerink, J. Docosahexaenoyl Serotonin Emerges as Most Potent Inhibitor of IL-17 and CCL-20 Released by Blood Mononuclear Cells from a Series of N-Acyl Serotonins Identified in Human Intestinal Tissue. *Biochim. Biophys. Acta - Mol. Cell Biol. Lipids.* 2017, *1862*(9), 823–831. DOI: 10.1016/j.bbalip.2017.05.008.

[81] Bashashati, M.; Fichna, J.; Piscitelli, F.; Capasso, R.; Izzo, A. A.; Sibaev, A.; Timmermans, J.-P.; Cenac, N.; Vergnolle, N.; Di Marzo, V., et al. Targeting Fatty Acid Amide Hydrolase and Transient Receptor Potential Vanilloid-1 Simultaneously to Modulate Colonic Motility and Visceral Sensation in the Mouse: A Pharmacological Intervention with N-Arachidonoyl-Serotonin (AA-5-HT). *Neurogastroenterol. Motil.* 2017, *29*(12), e13148.