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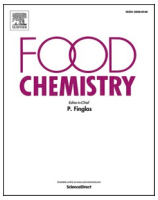

2-Methyloxolane as an effective bio-based solvent for the removal of $^{\beta}$*N*-alkanoyl-5-hydroxytryptamines from Arabica green coffee beans

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ABSTRACT

$^{\beta}$*N*-alkanoyl-5-hydroxytryptamines (C_n-5HTs) are the main constituents of coffee wax and may be responsible for the increased severity of gastric disorders in sensitive consumers. Their removal from green coffee beans can result in a "stomach-friendly" brew. This work presents a green approach to C_n-5HTs extraction using the bio-based solvent 2-methyloxolane (2-MeOx). HPLC/DAD analyses on Arabica Brazil samples show that mild conditions (30 min at 50 °C) extract about 90% of the wax, without affecting the caffeine content of the beans, whereas almost complete removal is achieved in 60 min at reflux. 2-MeOx forms an azeotrope with water, its possible re-use has been demonstrated using aqueous 2-MeOx (95.5%) as the solvent. These preliminary results make 2-MeOx a possible candidate for the replacement of dichloromethane (DCM) in coffee dewaxing.

The importance of fermentation in reducing C_n-5HTs by about 36% has been demonstrated in an analysis of green beans subjected to different post-harvest treatments.

1. Introduction

The origins of the coffee beverage are uncertain, but undoubtedly very remote and distant in time. However, it is only in recent years that the extremely complex nature of this food has been highlighted, with certain components, whose concentrations may vary with factors that include cultivar and post-harvest treatment, being able to manifest biological activity individually (Das, 2021; Preedy, 2015). Despite the beverage's possible health-promoting features, such as antioxidant, anti-inflammatory and antimicrobial activity, and chronic-disease prevention (e.g., type 2 diabetes mellitus), some physiological effects may be adverse for particular consumer types, with these effects including digestive disorders (e.g. heartburn, gastro-oesophageal reflux) (Castaldo, Narváez, Izzo, Graziani, & Ritieni, 2020; Loader, Taylor, Zahradka, & Jones, 2017; Nehlig, 2022; Nieber, 2017).

Serotonin (5-hydroxytriptamine, 5HT) amides, also named $^{\beta}$*N*-alkanoyl-5-hydroxytryptamines (C_n-5HTs), are the principle components of coffee wax; the thin layer located in the cortical part of the beans (Folstar, van der Plas, Pilnik, Schols, & Melger, 1979; Speer & Kölling-Speer, 2006). The qualitative and quantitative profile of these lipid compounds (ranging indicatively from 800 to about 2000 ppm in green beans) varies depending on coffee origin (Nebesny & Budryn, 2002), with higher total amounts found in Arabica samples than in Robusta (Brand et al., 2023; Farah, 2019). Specifically, the C_n-5HTs of arachidic (C_{20}), behenic (C_{22}) and lignoceric (C_{24}) acids are described as being the most abundant, and the analytical data on the total amount of C_n-5HTs in coffee beans is considered an indicator of their wax content (Folstar et al., 1979; Nebesny & Budryn, 2002). A positive correlation between the alkyl chain length of synthesised C_n-5HTs and an increase in stomach-acid secretion, measured as changes in the intracellular proton index, has been demonstrated using gastric cells (HGT-1) (Lang et al., 2010). Although C_n-5HTs are thermolabile, and therefore partially decomposed during roasting (da Rosa et al., 2016), they are found in the beverage as they are extracted during the brewing processes at varying percentages (e.g., 0.3 and 7.2% using cellulose filter and French-press methods, respectively) (Lang et al., 2010).

From the point of view of consumer tolerance, roasted coffee products that have had their wax removed at the green bean stage can be described as "stomach-friendly", proving to be more digestible and less irritating to the gastric mucosa (Baldino, Scognamiglio, & Reverchon,

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2021; Polese et al., 2022).

The partial removal of the waxy layer from green beans can occur during the decaffeination process and in various technological pre-roasting treatments such as polishing and steaming, which allow for up to about 30% to be removed (Farah, 2019). However, a wax reduction of 85–90% can be achieved by optimising processes and changing the organic solvents used (Roselius, Kurzhals, Sylla, & Hubert, 1979; van den Stegen, 1979).

In an industrial dewaxing treatment (Health Authorisation for the production of dewaxed coffee, registration number IT0610100111) in which the green beans are swelled with water and steam before extraction with dichloromethane (DCM), the simultaneous, but partial, removal of the caffeine content also occurs (reduction of about 50%) (Polese et al., 2022), as declared in available commercial products. However, they cannot be defined as decaffeinated as their caffeine content exceeds the reference values indicated by various national and international standards (0.1% in Europe and 3% in the USA) (Pietsch, 2017; Preedy, 2015). In order to prevent any risk to human health, the use of food-grade DCM in coffee processing is regulated by authorities in both Europe and the United States (*European Directive 2009/32/EC (Annex I, Part II),* 2009; *FDA Code of Federal Regulations 2023 (Title 21, Chapter 1, Subch. B, Part 173, Sec. 173.255),* 2023). However, to the best of our knowledge, international food-safety authorities currently issue no guidance on maximum residue limits (MRLs) for C_n-5HTs in dewaxed green coffee beans. In Italy, following the Ministerial Decree (22 June 1983), good industrial practice requires that green coffee must contain <250 ppm (and not >30% of the original wax content) of waxy products (expressed as C_n-5HTs) to be defined as dewaxed.

Growing concern as to the use of organic solvents and their environmental impact in recent years has constantly stimulated the scientific community, including the food sector (Rapinel et al., 2020; Sicaire et al., 2015), to search for more sustainable alternatives to traditional protocols. Thanks to its physical characteristics and wide solvation range, 2-methyloxolane (2-MeOx, 2-methyltetrahydrofuran) is a promising bio-based alternative solvent that can be produced from levulinic acid and furfural, which themselves can be obtained from renewable resources such as cellulose, hemicelluloses and lignin (Calvo-Flores, Monteagudo-Arrebola, Dobado, & Isac-García, 2018; Pace, Hoyos, Castoldi, Domínguez de María, & Alcántara, 2012). Due to its low solubility in water, which decreases with increasing temperature, this solvent can be employed in lipid extraction as a replacement for other organic food-grade solvents, such as DCM and hexane (see Table 1 for solvent properties), including in processes performed under heating (Cascant et al., 2017; Ravi et al., 2019). Stabilisers, such as 3,5-di-*t*-butyl-4-hydroxytoluene (BHT) and tocopherols, are added to 2-MeOx to prevent the possible formation of hydroperoxides during extraction processes.

Table 1
Solvent characteristics of 2-MeOx, DCM and hexane.

Solvent characteristics	2-Methyloxolane (2-MeOx)	*n*-Hexane	Dichloromethane (DCM)
Sourcing	Bio-based	Petro-sourced	Methane + chlorine
Formula	$C_5H_{10}O$	C_6H_{14}	CH_2Cl_2
Molecular weight (g/mol)	86.1	86.2	84.9
Boiling point (°C)	80	68	39.6
Density (20 °C)	0.855	0.661	1.327
Viscosity (cP; 25 °C)	0.60	0.30	0.43
Dielectric constant (25 °C)	7.0	1.9	8.93
Dipole moment (D)	1.38	0.09	1.14
Log $P_{o/w}$	1.85	3.76	1.19
Solubility in H_2O (20 °C; $\%_w$)	14.40	0.95	1.60
H_2O solubility (20 °C; $\%_w$)	4.4	$9{\cdot}10^{-3}$	0.24
Flash point closed cup (°C)	−11	−30	none

A scientific opinion on the safety assessment of the use of 2-MeOx as a food-extraction solvent has been issued for processes in which hexane is currently used (extraction of oil and protein from plant sources, extraction of food additives). Specifically, the following MRLs have been proposed: 1 mg/kg in fat, oil or butter; 10 mg/kg in protein products, flour and other fat-free solid ingredients; 1 mg/kg in food intended for particular nutritional uses as defined in Directive 2009/32/EC and successive amendments (26 January 2023); 1 mg/kg for food-additive extraction. The available toxicological data indicate that 2-MeOx is rapidly metabolised with a low potential for bioaccumulation and does not raise concerns for genotoxicity (European Food Safety Authority, 2022; Commission Directive (EU) 2023/175).

Concerning solvent re-use, it has been shown that 2-MeOx forms an azeotrope with water (10.6% w of water at 71 °C), and a saturated organic phase with 4.5% water is obtained at 55 °C after condensation (2-MeOx 95.5%). The two forms of this solvent (named dry and aqueous) have performed well when used in the production of soya oil and defatted soya flour (Claux et al., 2021).

As far as the coffee matrix is concerned, the efficiency of 2-MeOx has been demonstrated in the defatting of spent coffee grounds (Chemat et al., 2022; Mkhonto & Chetty, 2021) while, to the best of our knowledge, no studies have reported the use of this green solvent in raw-coffee-bean treatment to give a dewaxed coffee product.

The purpose of this work is, therefore, to evaluate the effectiveness of 2-MeOx, both in its dry and aqueous forms, in the extraction of C_n-5HTs from Arabica Brazil green coffee beans. Since C_n-5HT standards are not commercially available, the most abundant homologues in coffee were synthesised for wax quantification via HPLC/DAD in the obtained extracts. The time and temperature variables of the extraction processes with 2-MeOx were taken into account. Since the multi-directional stimulating effects of caffeine on various organs of the human body may be desired by coffee consumers, the selectivity of 2-MeOx for wax vs. 1,3,7-trimethylxanthine was also considered. Finally, the C_n-5HT content in green coffee samples subjected to different post-harvest treatments was determined.

2. Materials and methods

2.1. Materials

Serotonin hydrochloride (98%), heptadecanoic acid (98%), eicosanoic acid (98%), behenic acid (98%), tetracosanoic acid (99%), 2-methyltetrahydrofuran (≥99% GC grade), and *N*,*N*-dimethylformamide (≥99.9%) were purchased from VWR International (International Srl, Milan, Italy). Thionyl chloride (reagent grade 97%), dichloromethane (≥99.9% GC grade), tetrahydrofuran (≥99.9% anhydrous), petroleum ether (ACS reagent bp ≥90% 40–60 °C), methanol (≥99.9% HLC grade), caffeine (analytical standard), triethylamine (≥99%), and magnesium oxide heavy (ACS reagent 97%) were purchased from Sigma-Aldrich (Sigma-Aldrich, Milan, Italy). Dimethyl sulfoxide d6 (99.80%) was purchased from Eurisotop (Eurisotop, Saint-Aubin, Switzerland).

Commercially available samples of green Arabica Brazil coffee beans (natural, semi-wet and wet processed) were investigated.

2.2. Synthesis of $^{\beta}$*N*-alkanoyl-5-hydroxytryptamines

$^{\beta}$*N*-Heptadecanoyl-5-hydroxytriptamine (C_{17}-5HT), $^{\beta}$*N*-Arachinoyl-5-hydroxytriptamine (C_{20}-5HT), $^{\beta}$*N*-behenoyl-5-hydroxytriptamine (C_{22}-5HT) and $^{\beta}$*N*-lignoceroyl-5-hydroxytriptamine (C_{24}-5HT) were synthesised following the procedure described by Lang et al. (2010). The target compounds were obtained as white powders and were characterised using MS(ESI)$^+$ (Micromass® ZQ™ – Waters Corporation, Milford, MA, USA) and ^{1}H NMR (ECZR 600 MHz, JEOL Europe B.V. Nieuw-Vennep, The Netherlands, d_6-DMSO) according to literature data (Amorim et al., 2021; Tinoco et al., 2019). The following yields were

obtained; 86.4% (C_{17}-5HT), 54.6% (C_{20}-5HT), 51.4% (C_{22}-5HT), and 38.4% (C_{24}-5HT).

The products were used for the quantification of both single serotonin amides and total wax via HPLC/DAD.

2.3. Moisture content of green coffee beans

The moisture content of all the Arabica Brazil green coffee beans was calculated according to the procedure described by Tinoco et al. (2019). Measurements were made in triplicate and the average values for the different post-harvest treatments were 9.9%, 9.8% and 10.3% for natural, semi-wet and wet samples, respectively.

2.4. C_n-5HTs extraction

10 g of Arabica Brazil green coffee beans were subjected to the exhaustive Soxhlet extraction of the waxes using 2-MeOx dry in successive extractions (each lasting 2 h at 80 °C, siphoning 50 times) with a matrix/solvent ratio of 1:5. The solvent was renewed for each extraction (for a total of three extractions), and every resulting extract was analysed in order to monitor wax depletion. The second Soxhlet extraction can be considered exhaustive as no more C_n-5HTs (<LOD) were detected.

The same extraction protocol was repeated using DCM as the solvent.

2-MeOx dry extraction was then monitored in terms of time and temperature by applying the following conditions to 10 g of green coffee beans: 15, 30 and 60 min at room temperature (RT); 15, 30 and 60 min at 40 °C; 15, 30 and 60 min at 50 °C; 15, 30 and 60 min at 60 °C; 15, 30 and 60 min at 80 °C (reflux). Again, a matrix/solvent ratio of 1:5 was used.

The procedure at 50 °C for 30 min was repeated with aqueous 2-MeOx (95.5%).

The extraction protocol performed for 30 min at reflux was repeated using DCM as the solvent.

After filtration, each extract obtained was reduced under vacuum and then re-suspended in 50 mL of MeOH, an aliquot (4 mL) of which was filtered (SPHEROS nylon syringe filter, 0.45 μm) prior to HPLC/DAD analysis.

2.5. C_n-5HT recovery experiment

10 g of green coffee powder, obtained by grinding frozen Arabica Brazil (natural) beans, was spiked with 10 mL of a 2-MeOx dry solution containing a known amount of the C_{17}-5HT (internal standard); C_{20}-5HT, C_{22}-5HT, and C_{24}-5HT. The resulting samples were then extracted via the Soxhlet method (2x2h) with 2-MeOx dry.

2.6. Determination of total caffeine content

Evaluations of total caffeine content were performed following the procedure reported by Menzio, Binello, Barge, and Cravotto (2020) on 20 g of Arabica Brazil (natural) green coffee beans. 1 mL of the obtained suspension was filtered (SPHEROS nylon syringe filter, 0.45 μm), and analysed via HPLC/DAD.

2.7. Instrumental analysis

Analytical conditions were optimized based on published data on raw coffee (Brand et al., 2023).

Analyses were performed using an HPLC/DAD system consisting of the following components: a 1525EF pump, a 717plus Autosampler injector and a 2996 DAD detector (operating wavelength range from 190 to 800 nm) (Waters Corporation, Milford, MA, USA).

After sample injection (10 μL), the analytes were separated on an RP-18 column (Kromasil® 100–5-C18 4.0 × 250 mm) at a flow rate of 1 mL/min in isocratic elution (MeOH/H_2O 96:4 *v*/v) for 30 min. C_n-5HTs were detected at λ_{max} = 279.4 nm, while caffeine was detected at λ_{max} = 273.5 nm.

The calibration curves of the HPLC/DAD method were determined using MeOH standard solutions (from 5.0 to 500.0 μg/mL); linear regressions with R^2 = 0.9995 for C_n-5HTs, and R^2 = 0.9998 for caffeine were obtained using Empower software (LOD 1.5 μg/mL, LOQ 5.0 μg/mL).

The reported total C_n-5HTs mg/kg also takes into account minority C_n-5HTs that were identified in UV spectra (λmax = 279.4 nm) and quantified on the basis of the C_{22}-5HT calibration curve, as it is the most abundant.

2.8. Statistical analysis

Each procedure was performed in triplicate and results were expresses as mean values ± standard deviation (SD). The Principal Component Analysis (PCA) of C_n-5HT content in Arabica Brazil green coffee beans that had been submitted to different post-harvest treatments was performed using the Statistical software RStudio (V 4.3.1)© (RStudio, PBC, Boston, MA, USA).

3. Results and discussion

3.1. Evaluation of total C_n-5HT and caffeine content in green coffee beans

The procedure reported by Chemat et al. (2022), related to sustainable strategies for the extraction of lipids from spent coffee grounds with 2-MeOx dry in the conventional Soxhlet system, was used for the determination of the total amount of C_n-5HTs (used as an analytical parameter of wax content) in the green Arabica Brazil (natural) samples. Compared to the above-mentioned work, the matrix/solvent ratio of 1:10 was reduced to 1:5 as preliminary tests showed that it provided the same C_n-5HT extraction yields. Standards of the most abundant serotonin amides in coffee (arachidic (C_{20}), behenic (C_{22}) and lignoceric (C_{24}) derivatives, Fig. 1) (Brand, Silva, Garrett, & Rezende, 2024) were synthesised in order to grant a reliable analytical method. Moreover, C_{17}-5HT, not reported as present in coffee, was also synthetized to be employed as an internal standard for recovery measurements.

As mentioned above, an industrial dewaxing process (IT0610100111) can remove about 50% of the caffeine (Polese et al., 2022). The quantification of this xanthine was therefore carried out on the same Arabica sample with the aim of monitoring the selectivity of 2-MeOx dry towards C_n-5HTs with respect to caffeine. Following the protocol described in paragraph 2.6., a quantity of 16.0 ± 0.1 g/kg was detected by HPLC-DAD.

After three successive Soxhlet extraction procedures with 2-MeOx

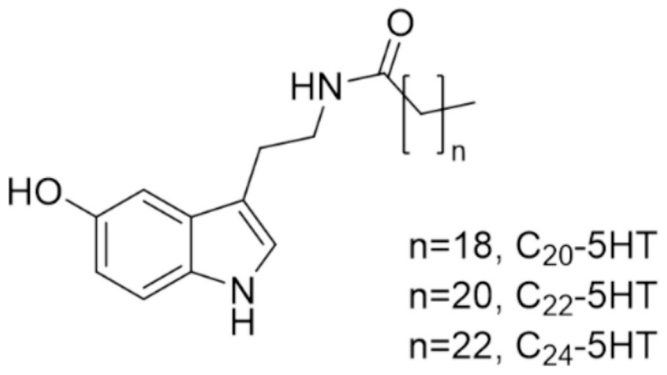


Fig. 1. Chemical structures of the three major C_n-5HTs in green coffee wax: $^{\beta}N$-arachinoyl-5-hydroxytryptamine (C_{20}-5HT), $^{\beta}N$-behenoyl-5-hydroxytryptamine (C_{22}-5HT) and $^{\beta}N$-lignoceroyl-5-hydroxytryptamine (C_{24}-5HT). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

dry (paragraph 2.4., Scheme 1), the average total C_n-5HT content was estimated to be 930.91 ± 12.1 ppm, a value which is in line with the range reported in the literature for Arabica coffee (500–2370 ppm) (Tinoco et al., 2019). Extraction was conducted on whole beans as the possibility of a post-dewaxing roasting process was considered; their appearance remains almost unchanged after the defatting process. It was observed that, unlike the wax (the amount of which decreases over the subsequent steps up to exhaustion after the second extraction), the quantities of caffeine obtained varied relatively little over the procedure; 284.7 ± 15.3, 176.8 ± 18.3 and 184.4 ± 11.7 ppm for the first, second and third extractions, respectively. This can be explained by the fact that the beans, wetted by the solvent of the first extraction, became more permeable to 2-MeOx dry in subsequent steps. This is not the case for wax as it is only present in a thin layer in the cortical part of the bean. However, it is worth noting that only about 4% of the total caffeine content was removed after the third Soxhlet extraction (Fig. 2).

The following values were quantified by HPLC-DAD for individual C_n-5HT in each of the two successive Soxhlet extractions: 214.2 ± 10.3 and 31.6 ± 5.5 ppm for C_{20}-5HT, 527.6 ± 21.6 and 17.6 ± 4.8 for C_{22}-5HT, and 54.3 ± 7.4 and < LOD for C_{24}-5HT.

The evaluation of the recovery and the wax's affinity for 2-MeOx dry were performed on ground green bean samples fortified with C_n-5HT standard solutions, as it is not possible to be certain of their uptake from the whole beans. Following the procedure reported in paragraph 2.5., an average recovery of 99.7% was obtained (considering all C_n-5HTs) by comparing the quantitative results of the spiked samples with those obtained from the same extraction protocol performed on the non-fortified green coffee powder.

For the sake of comparison, the same conditions that led to the depletion of C_n-5HTs using 2-MeOx dry were repeated with DCM, even though they differ substantially from those used in the above-mentioned industrial process (IT0610100111). The results obtained show that, in this case, wax removal is not exhausted by the third Soxhlet extraction, and that about 24% less is obtained (712 ± 10.4 ppm) compared to the procedure performed using 2-MeOx dry.

3.2. 2-MeOx efficiency in C_n-5HT extraction

Growing focus on consumer health is increasingly leading food manufacturers, including the coffee industry, to use processing protocols that are ever safer and more environmentally friendly. Using the above-reported evaluations as a benchmark, experimental parameters were then defined to optimise a sustainable and efficient extraction process using 2-MeOx dry to make it as selective as possible for coffee wax. Several different extraction times and temperature conditions were then tested (Scheme 1), with time and energy savings also being considered. With regards to temperature, a range between room temperature and the solvent boiling point was chosen. The extraction durations started at 15 min and were doubled for each subsequent test (i.e. 30 and 60 min).

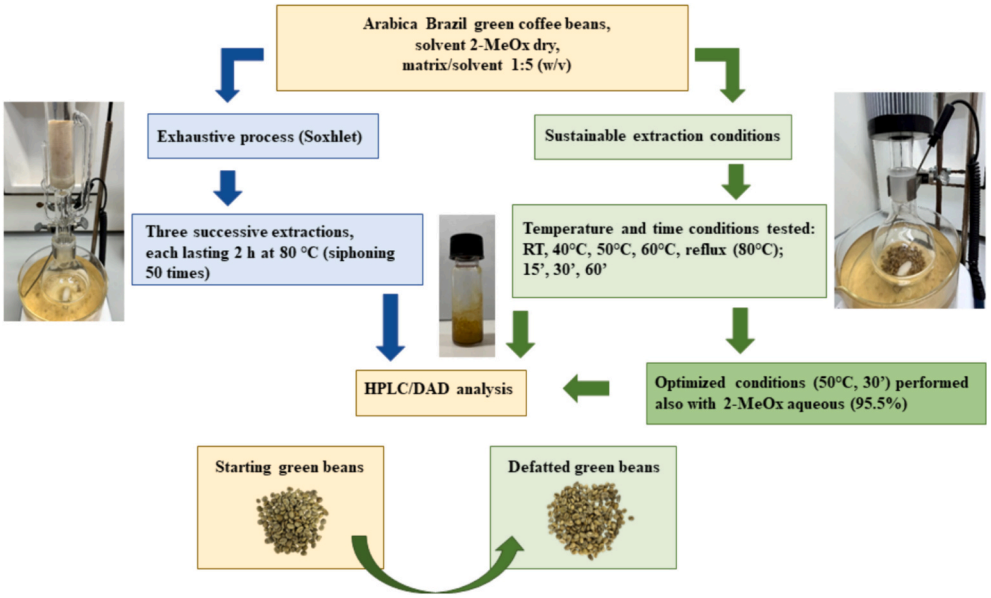
The C_n-5HT and caffeine values reported in Table 2 were determined from the analysis of the extracts obtained without the need for purification steps. Although almost complete dewaxing can be achieved after 60-min of reflux treatment, milder conditions, such as 30 min at 40 °C or 50 °C, give green beans that can be defined as dewaxed (wax residues of around 135 ppm, which is well within the limit of 250 ppm required by good industrial practice). It is worth noting that even the 30-min RT protocol produces a coffee at the limit of tolerance for the presence of waxes. Interestingly, caffeine removal was minimal, ranging from 0.4 to 0.5% in the instances mentioned, under all of the conditions tested, demonstrating that this process has high wax selectivity.

Brand et al. (2023), in their recent review, reported literature data on the extraction and quantification of C_n-5HTs from coffee beans. However, these processes were not aimed at obtaining a dewaxed product, but merely at the quantification of coffee wax, and involved the use of conventional organic solvents such as chloroform, DCM, methanol, petroleum ether and tetrahydrofuran in the protocols, most of which included Soxhlet extractions and subsequent purification procedures prior to qualitative-quantitative analysis (HPLC/UV, HPLC/MS, HPLC/FL).

It should be noted that a US patent describes an invention that makes it possible to reduce C_n-5HTs to <200 ppm without modifying the initial caffeine content of the green beans. However, the process, which requires the use of supercritical fluids, entrainment, a moisture content of 20–35% and an adsorbent pre-loaded with caffeine, appears to be very complex, time consuming and energy intensive (Roselius et al., 1979).

Again, a comparison with DCM was sought, and the 30 min protocol at reflux was chosen for this purpose. The results show that the wax-extraction efficiency of the DCM method was approximately 5% and 24% lower than that of the 2-MeOx dry processes at RT and reflux (both for 30 min), respectively.

This experimental proof is consistent with literature data in which 2-MeOx dry was described as an efficient green solvent for lipid extraction



Scheme 1. Schematic representation of the C_n-5HT extraction procedures.

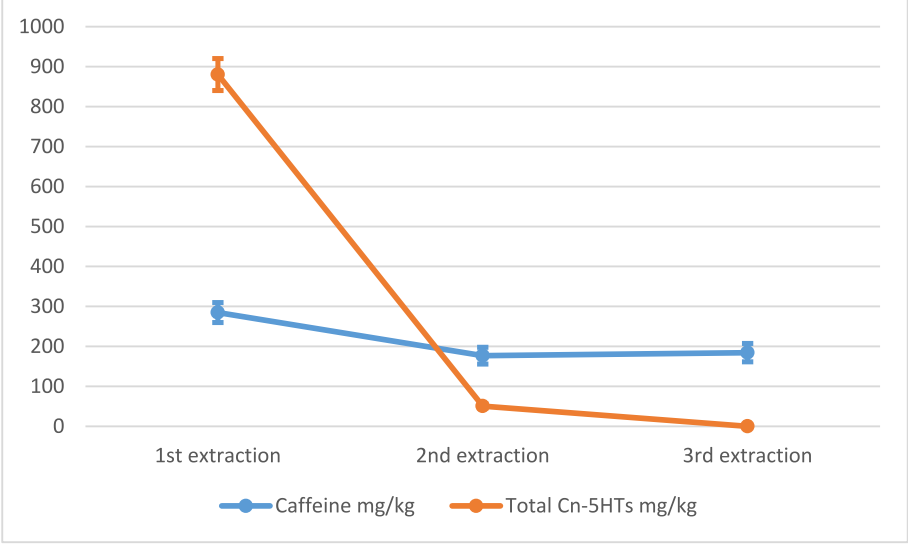


Fig. 2. Determination of total wax (in terms of C_n-5HT content) and caffeine in Arabica Brazil (natural) green coffee beans in three successive Soxhlet extractions (each lasting 2 h, siphoning 50 times) with 2-MeOx dry. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 2
Comparison of the effect of different conditions (time, temperature and solvent) on C_n-5HT and caffeine extraction from green coffee beans (Arabica Brazil, natural).

Compound	Extraction time (min)	2-MeOx dry RT	2-MeOx dry 40 °C	2-MeOx dry 50 °C	2-MeOx dry 60 °C	2-MeOx dry Reflux	DCM Reflux
Caffeine mg/kg±SD[1]	15	65.4 ± 4.1	73.0 ± 6.5	79.4 ± 9.6	91.5 ± 6.9	102.5 ± 5.5	//
	30	64.5 ± 7.4	83.8 ± 4.5	84.6 ± 5.7	107.9 ± 16.4	131.6 ± 19.4	73.5 ± 0.7
	60	61.7 ± 10.0	96.3 ± 6.2	159.5 ± 5.0	168.4 ± 7.9	180.7 ± 8.3	//
C_{20}-5HT mg/kg±SD[1]	15	129.4 ± 16.9	137.4 ± 7.4	176.1 ± 7.6	178.2 ± 4.6	183.9 ± 4.8	//
	30	154.3 ± 15.1	178.6 ± 23.8	190.4 ± 17.6	182.7 ± 1.6	193.1 ± 12.5	131.5 ± 21.9
	60	176.5 ± 12.1	186.1 ± 16.8	200.9 ± 5.4	196.5 ± 6.3	204.7 ± 10.0	//
C_{22}-5HT mg/kg±SD[1]	15	382.7 ± 28.1	426.5 ± 23.3	485.1 ± 4.3	505.0 ± 13.6	527.9 ± 11.8	//
	30	442.0 ± 14.1	500.2 ± 1.3	497.6 ± 6.6	565.4 ± 53.7	547.6 ± 20.8	396.0 ± 14.1
	60	446.5 ± 10.0	492.9 ± 12.2	538.9 ± 16.3	524.1 ± 40.6	554.6 ± 28.1	//
C_{24}-5HT mg/kg±SD[1]	15	47.2 ± 16.6	53.2 ± 8.2	54.7 ± 0.8	62.4 ± 9.9	65.7 ± 9.0	//
	30	49.2 ± 11.5	59.9 ± 8.1	69.6 ± 7.2	64.6 ± 13.9	67.0 ± 2.8	43.5 ± 0.7
	60	69.4 ± 2.9	69.8 ± 4.0	76.5 ± 6.3	82.9 ± 5.4	88.1 ± 5.0	//
Total	15	593.2 ± 17.4	662.9 ± 21.1	775.7 ± 13.5	787.7 ± 16.1	827.6 ± 19.7	//
C_n-5HTs mg/kg±SD[1,2]	30	691.8 ± 8.6	795.2 ± 32.8	810.6 ± 33.2	868.5 ± 20.9	870.4 ± 14.7	643.1 ± 14.6
	60	742.5 ± 21.2	797.9 ± 20.8	868.3 ± 22.6	859.3 ± 12.1	908.0 ± 14.0	//
C_n-5HTs % in relation to total content	15	61.9–65.6	69.0–73.5	81.9–84.8	82.9–86.3	86.8–91.0	//
	30	73.4–75.2	81.9–88.9	83.5–90.6	91.1–95.5	91.9–95.0	67.5–70.6
	60	77.5–82.0	83.5–87.9	90.8–95.7	91.0–93.6	96.0–99.0	//

[1] SD values calculated over three repetitions of the experiment.
[2] Quantity calculated also considering minority C_n-5HTs identified by UV spectrum (λ_{max} = 279.4 nm) and quantified on the basis of the C_{22}-5HT calibration curve.

and thus a potential replacement for hexane in vegetable-oil extraction and meal-defatting processes (Claux et al., 2021).

3.3. Evaluation of 2-MeOx re-use

Industrial solvent-recycling processes are crucial, and when 2-MeOx dry is used, the recovered organic phase, after distillation and separation in a decanter at 55 °C, was observed to be saturated with water (2-MeOx/H_2O 95.5/4.5). In order to re-obtain 2-MeOx dry, this mixture must undergo a further distillation step, a process that requires considerable energy input (Sicaire et al., 2014). For this reason, the extraction efficiency of aqueous 2-MeOx as a selective dewaxing agent was also investigated. Seeing as the percentage composition of the binary 2-MeOx/H_2O mixture does not essentially vary at 40 °C and 50 °C, as the solubility of water in 2-MeOx is reported to change from 4.3% at 39.6 °C to 4.4% at 50.1 °C, the protocol at 50 °C for 30 min was applied. The C_n-5HT extraction yield did not change (Fig. 3) compared that of the dry solvent, while the amount of caffeine removed increased from 84.6 ± 5.7 to 712.4 ± 25.8 ppm (i.e. from 0.5% to 4.5% of the total caffeine content).

Since traces of lipid hydrolysis have been observed following oil extraction from spent ground coffee when 2-MeOx is used as an alternative to hexane (Chemat et al., 2022), serotonin was sought in the extracts obtained from both dry and aqueous 2-MeOx. Its non-detection in all samples shows that the conditions applied in this work do not result in C_n-5HT hydrolysis.

3.4. Coffee wax content and post-harvest treatments

Coffee origin and the different post-harvest operations applied, including the processes named dry (natural), semi-dry (semi-wet or honey), and wet (washed), can strongly influence the chemical composition of the beans (Cortés-Macías, Fuentes López, Gentile, Girón-Hernández, & Fuentes López, 2022; Das, 2021; Farah, 2019; Wu et al., 2022). C_n-5HT content in semi-wet and wet Arabica Brazil samples was therefore monitored. Using the exhaustive Soxhlet procedure with 2-

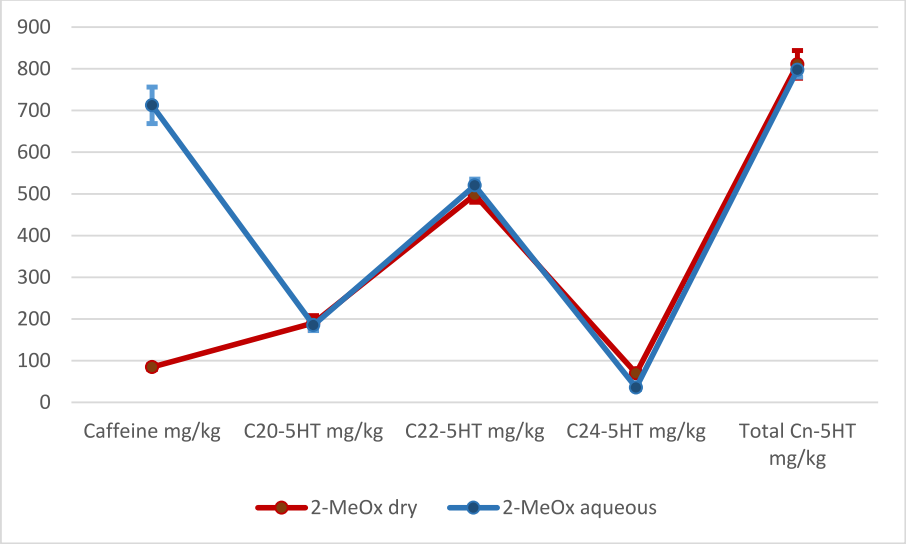


Fig. 3. C_n-5HT and caffeine extraction yields using 2-MeOx dry and aqueous 2-MeOx at 50 °C for 30 min from green Arabica Brazil (natural) beans. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

MeOx dry, a comparison was made with a previously analysed natural sample in order to assess any possible differences in impact on the digestibility of the commodity. All of the examined coffee beans were found to have similar ratios (around 10%) of moisture content to dry matter (paragraph 2.3.).

The data reported in Table 3 clearly show how post-harvest treatments can affect the total wax content of green beans. In particular, C_n-5HT content was reduced by about 36% by wet treatment, compared to natural beans, thus showing the important effect of the characteristic fermentation step related to this process. This is consistent with Tinoco et al. (2019), who observed that enhanced C_n-5HT reduction was induced by yeast supplementation during coffee post-harvest wet treatment. As far as semi-wet treatment is concerned, a slight decrease in C_n-5HT content can be observed with respect to the more wax-rich natural sample.

The relative amounts of the three C_n-5HTs monitored remained constant across all samples analysed, with C_{22}-5HT always being the most abundant, followed by C_{20}-5HT and then C_{24}-5HT. These values are in line with those reported for Arabica coffee (Folstar et al., 1979; Tinoco et al., 2019), although the minority C_{24}-5HT is not among those commonly considered. On the other hand, it can be seen that, while the C_{22}-5HT/C_{20}-5HT ratio remains almost constant for the three treatments, the semi-wet and, especially, wet processes lead to a reduction in the relative content of C_{24}-5HT. The PCA biplot shown in Fig. 4 further highlights the effect of the wet treatment on the wax content in Arabica Brazil green coffee beans. In addition, the close correlation between the variables C_{20}-5HT and Total C_n-5HTs is highlighted by the small amplitude of the angle between the two directions identified by the vector loadings.

It should be noted, however, that not all coffee origins undergo all types of post-harvest treatment, as these processes are strictly related to the country in which the coffee is grown (Das, 2021; Tassew, Yadessa, Bote, & Obso, 2021; Widodo, Yulianto, Ariyanto, & Paramita, 2023).

Table 3
Comparison of C_n-5HT contents in Arabica Brazil green coffee samples with different post-harvest treatments (natural, semi-wet and wet) via exhaustive Soxhlet extraction with 2-MeOx dry.

Sample	C_{20}-5HT mg/kg ±SD[1]	C_{22}-5HT mg/kg ±SD[1]	C_{24}-5HT mg/kg ±SD[1]	Total C_n-5HTs mg/kg[a]±SD[1,2]
Arabica Brazil natural	229.8 ± 2.6	600.2 ± 27.9	57.3 ± 4.2	930.9 ± 27.0
Arabica Brazil semi-wet	216.3 ± 34.6	536.5 ± 28.6	35.8 ± 9.3	852.1 ± 19.8
Arabica Brazil wet	185.8 ± 8.8	363.5 ± 13.3	9.3 ± 1.6	598.5 ± 11.7

[1] SD values calculated over three experiment repetitions.
[2] Quantity calculated by also considering minority C_n-5HTs identified in UV spectrum (λ_{max} = 279.4 nm) and quantified on the basis of the C_{22}-5HT calibration curve.

4. Conclusion

C_n-5HTs (analytical parameter for green-coffee-bean wax content) may be responsible for an increase in gastric disorders in predisposed consumers of the beverage. In order to obtain a 'stomach-friendly' brew, some commercial products, referred to as dewaxed, undergo a C_n-5HT removal processes that mainly employ DCM as the solvent.

In pursuit of the goal of developing sustainable processes that can be applied to the food sector, this work demonstrates the efficiency of 2-MeOx dry, a bio-based solvent, in the extraction of C_n-5HTs from green Arabica Brazil coffee beans without any matrix pre-treatment.

In particular, the three most abundant C_n-5HTs, C_{20}-5HT, C_{22}-5HT, and C_{24}-5HT, were synthesised as standards, and then quantified in the extracts obtained under the different experimental conditions. Taking into consideration the possibility of solvent re-use, aqueous 2-MeOx was also evaluated.

The obtained results show that simple procedures, involving extraction for 30 min at 40–50 °C, yield beans with a C_n-5HT content of <250 ppm, i.e. within the value reported as a good industrial standard for defining a coffee as dewaxed. Moreover, under the same extraction conditions, 2-MeOx performs better than DCM, allowing about 24% more wax to be removed.

The selectivity of 2-MeOx for C_n-5HTs over caffeine has also been demonstrated by quantifying the latter in the extracts obtained. A maximum removal of 4.5% was found when using aqueous 2-MeOx, demonstrating the poor solubility of 1,3,7-trimethyxanthine in this solvent system.

Given the USA and EU regulatory agencies' (FDA and EFSA) increasing focus on the use of solvents in food processing and their potential impact on human health, the results of this preliminary study lay the groundwork for future evaluations into the use of 2-MeOx as a safer replacement for DCM, as has already been carried out for hexane. The origin and post-harvest treatments of green coffee can strongly influence

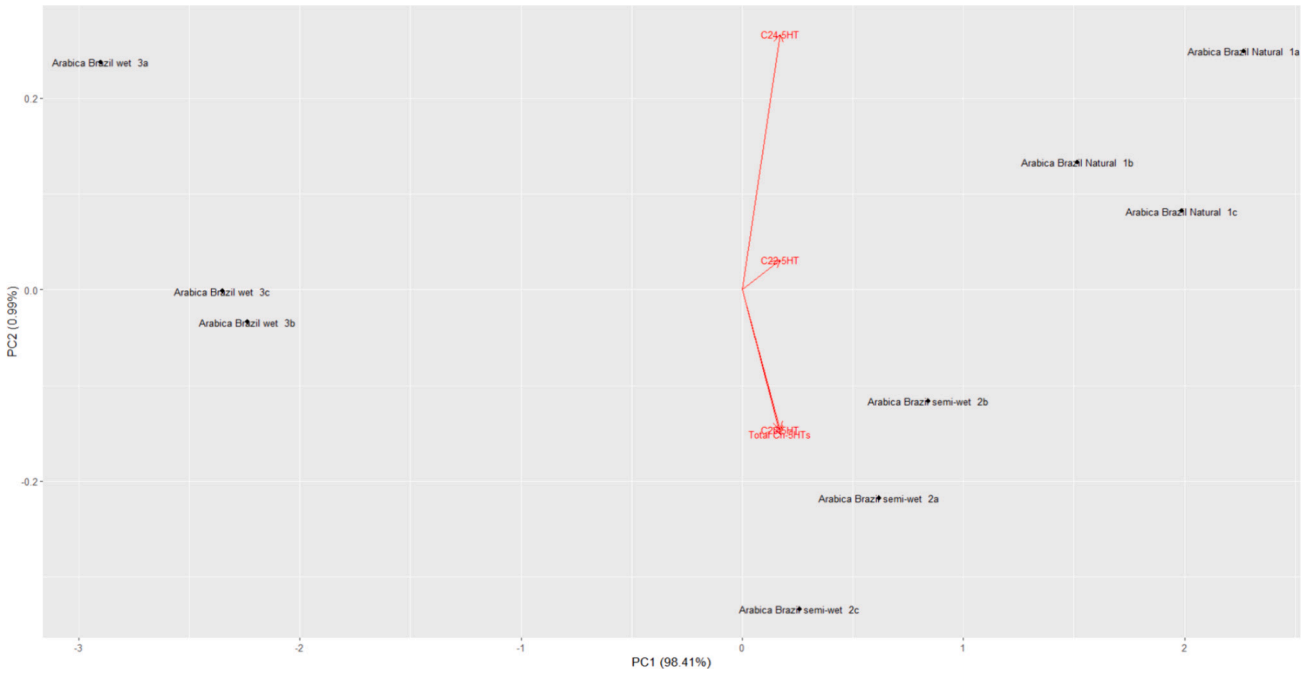


Fig. 4. Principal Component Analysis (PCA) scores biplot of green coffee beans submitted to different post-harvest treatments. C_n-5HT content was considered for three replicates of the Arabica Brazil natural (1a, 1b, 1c), semi-wet (2a, 2b, 2c) and wet (3a, 3b, 3c) extraction protocols. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

the chemical composition of the final products. Based on sample availability, the wax content of the Arabica Brazil raw beans named natural, semi-wet and wet was evaluated. As a result, the fermentation process, characteristic of the wet treatment, appears to be effective in reducing the total amount of C_n-5HTs by about 36%, while the relative ratio between the different $^{\beta}$*N*-alkanoyl-5-hydroxytryptamines remains almost constant in the three different samples analysed.

CRediT authorship contribution statement

Fabio Beccari: Methodology, Investigation. **Arianna Binello:** Writing – original draft, Supervision, Data curation. **Silvia Tagliapietra:** Writing – original draft, Validation, Data curation, Conceptualization. **Patrizia Bovolin:** Supervision, Methodology, Conceptualization. **Giancarlo Cravotto:** Writing – review & editing, Validation, Supervision.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

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References

Amorim, J. L., Lima, F. A., Brand, A. L. M., Cunha, S., Rezende, C. M., & Fernandes, P. D. (2021). Two new $^{\beta}$*N*-Alkanoyl-5-Hydroxytryptamides with relevant Antinociceptive activity. *Biomedicines, 9*(5), 455. https://doi.org/10.3390/biomedicines9050455

Baldino, L., Scognamiglio, M., & Reverchon, E. (2021). Elimination of tryptamines from green coffee by supercritical CO2 extraction. *The Canadian Journal of Chemical Engineering, 99*, 1345–1351. https://doi.org/10.1002/cjce.23928

Brand, A. L. M., Lima, F. A., Tinoco, N. A. B., Mota, J. C., Moreira, I. G. S., Novaes, F. J. M., … Rezende, C. M. (2023). $^{\beta}$*N*-Alkanoyl-5-Hydroxytryptamines (C_n-5HTs) in coffee: A review. *Food Reviews International, 39*(7), 4761–4780. https://doi.org/10.1080/87559129.2022.2059807

Brand, A. L. M., Silva, A. C. R., Garrett, R., & Rezende, C. M. (2024). Quantitative lipidomics in green robusta coffees from the Brazilian Amazon by LC-HRMS. *Food Bioscience, 57*, Article 103472. https://doi.org/10.1016/j.fbio.2023.103472

Calvo-Flores, F. G., Monteagudo-Arrebola, M. J., Dobado, J. A., & Isac-García, J. (2018). Green and bio-based solvents. *Topics in current chemistry (Cham), 376*(3), 18. https://doi.org/10.1007/s41061-018-0191-6

Cascant, M. M., Breil, C., Garrigues, S., de la Guardia, M., Fabiano-Tixier, A. S., & Chemat, F. (2017). A green analytical chemistry approach for lipid extraction: Computation methods in the selection of green solvents as alternative to hexane. *Analytical and Bioanalytical Chemistry, 409*(14), 3527–3539. https://doi.org/10.1007/s00216-017-0323-9

Castaldo, L., Narváez, A., Izzo, L., Graziani, G., & Ritieni, A. (2020). In vitro bioaccessibility and antioxidant activity of coffee Silverskin polyphenolic extract and characterization of bioactive compounds using UHPLC-Q-Orbitrap HRMS. *Molecules (Basel, Switzerland), 25*(9), 2132. https://doi.org/10.3390/molecules25092132

Chemat, A., Ravi, H. K., Hostequin, A. C., Burney, H., Tomao, V., & Fabiano-Tixier, A. S. (2022). Valorization of spent coffee grounds by 2-methyloxolane as bio-based solvent extraction. Viable pathway towards bioeconomy for lipids and biomaterials. *Oilseeds & fats Crops and Lipids, 29*, 7. https://doi.org/10.1051/ocl/2021052

Claux, O., Rapinel, V., Goupy, P., Patouillard, N., Vian, M. A., Jacques, L., & Chemat, F. (2021). Dry and aqueous 2-Methyloxolane as green solvents for simultaneous production of soybean oil and defatted meal. *ACS Sustainable Chemistry & Engineering, 9*(21), 7211–7223. https://doi.org/10.1021/acssuschemeng.0c09252

Commission Directive (EU) 2023/175. Official Journal of the European Union https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32023L0175, (assessed 08/01/2024).

Cortés-Macías, E. T., Fuentes López, C., Gentile, P., Girón-Hernández, J., & Fuentes López, A. (2022). Impact of post-harvest treatments on physicochemical and sensory characteristics of coffee beans in Huila, Colombia. *Postharvest Biology and Technology, 187*(9), Article 111852. https://doi.org/10.1016/j.postharvbio.2022.111852

Das, S. (2021). Review post-harvest processing of coffee: An overview. *Coffee Science, 16*, Article e161976. https://doi.org/10.25186/.v16i.1976

European Directive 2009/32/EC (Annex I, Part II). https://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2009:141:0003:0011:EN:PDF, (2009). assessed 25/01/24.

European Food Safety Authority. (2022). Safety assessment of 2-methyloxolane as a food extraction solvent. *EFSA Journal, 20*(3), 7138. https://doi.org/10.2903/j.efsa.2022.7138

Farah, A. (2019). *Coffee: Production, quality and chemistry*. London: Royal Society of Chemistry.

FDA Code of Federal Regulations 2023 (Title 21, Chapter 1, Subch. B, Part 173, Sec. 173.255). https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/cfrsearch.cfm?fr=173.255, (2023). assessed 08/01/24.

Folstar, P., van der Plas, H. C., Pilnik, W., Schols, H. A., & Melger, W. C. (1979). Liquid chromatographic analysis of N-Alkanoyl-5-hydroxytryptamine (C-5-HT) in green coffee beans. *Journal of Agricultural and Food Chemistry, 27*(2). https://doi.org/10.1021/JF60221A031

Lang, R., Bardelmeier, I., Weiss, C., Rubach, M., Somoza, V., & Hofmann, T. (2010). Quantitation of (beta)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. *Journal of Agricultural and Food Chemistry, 58*(3), 1593–1602. https://doi.org/10.1021/jf903612h

Loader, T. B., Taylor, C. G., Zahradka, P., & Jones, P. J. (2017). Chlorogenic acid from coffee beans: Evaluating the evidence for a blood pressure-regulating health claim. *Nutrition Reviews, 75*(2), 114–133. https://doi.org/10.1093/nutrit/nuw057

Menzio, J., Binello, A., Barge, A., & Cravotto, G. (2020). Highly-efficient caffeine recovery from green coffee beans under ultrasound-assisted SC–CO2 extraction. *Processes, 8*, 1062. https://doi.org/10.3390/pr8091062

Mkhonto, B., & Chetty, M. (2021). 2-Methyltetrahydrofuran as a potential green solvent for lipids extraction from spent coffee grounds for fuel grade hydrocarbons production. *Chemical Engineering Transactions, 89*, 409–414. https://doi.org/10.3303/CET2189069

Nebesny, E., & Budryn, G. (2002). Effect of the roasting method on the content of 5-hydroxytryptamides of carboxylic acids in roasted coffee beans. *Die Nahrung, 46*(4), 279–282. https://doi.org/10.1002/1521-3803(20020701)46:4<279::AID-FOOD279>3.0.CO;2-R

Nehlig, A. (2022). Effects of coffee on the gastro-intestinal tract: A narrative review and literature update. *Nutrients, 14*(2), 399. https://doi.org/10.3390/nu14020399

Nieber, K. (2017). The impact of coffee on health. *Planta Medica, 83*(16), 1256–1263. https://doi.org/10.1055/s-0043-115007

Pace, V., Hoyos, P., Castoldi, L., Domínguez de María, P., & Alcántara, A. R. (2012). 2-Methyltetrahydrofuran (2-MeTHF): A biomass-derived solvent with broad application in organic chemistry. *ChemSusChem, 5*(8), 1369–1379. https://hdl.handle.net/2434/886950.

Pietsch, A. (2017). Chapter 10 - decaffeination—Process and quality. In B. Folmer (Ed.), *The craft and science of coffee* (pp. 225–243). Academic Press. https://doi.org/10.1016/B978-0-12-803520-7.00010-4.

Polese, B., Izzo, L., Mancino, N., Pesce, M., Rurgo, S., Tricarico, M. C., … Ritieni, A. (2022). Effect of dewaxed coffee on gastroesophageal symptoms in patients with GERD: A randomized pilot study. *Nutrients, 14*(12), 2510. https://doi.org/10.3390/nu14122510

Preedy, V. R. (2015). *Coffee in Health and Disease Prevention*. Academic Press Elsevier. https://doi.org/10.1016/C2012-0-06959-1

Rapinel, V., Claux, O., Abert-Vian, M., McAlinden, C., Bartier, M., Patouillard, N., … Chemat, F. (2020). 2-Methyloxolane (2-MeOx) as sustainable lipophilic solvent to substitute hexane for green extraction of natural products. Properties, applications, and perspectives. *Molecules (Basel, Switzerland)* (Vol. 25,(15), 3417. https://doi.org/10.3390/molecules25153417

Ravi, H. K., Vian, M. A., Tao, Y., Degrou, A., Costil, J., Trespeuch, C., & Chemat, F. (2019). Alternative solvents for lipid extraction and their effect on protein quality in black soldier Fly (*Hermetia illucens*) larvae. *Journal of Cleaner Production, 238*, Article 117861. https://doi.org/10.1016/j.jclepro.2019.117861

da Rosa, J. S., Freitas-Silva, O., Rouws, J. R. C., da Silva Moreira, I. G., Novaes, F. J. M., de Almeida Azevedo, D., … de Rezende, C. M. (2016). Mass spectrometry screening of Arabica coffee roasting: A non-target and non-volatile approach by EASI-MS and ESI-MS. *Food Research International, 89*, 967–975. https://doi.org/10.1016/j.foodres.2016.03.021

Roselius, L., Kurzhals, H., Sylla, K. F., & Hubert, P. (1979). https://www.freepatentsonline.com/4168324.html.

Sicaire, A. G., Vian, M., Fine, F., Joffre, F., Carré, P., Tostain, S., & Chemat, F. (2015). Alternative bio-based solvents for extraction of fat and oils: Solubility prediction, global yield, extraction kinetics, chemical composition and cost of manufacturing. *International Journal of Molecular Sciences, 16*(4), 8430–8453. https://doi.org/10.3390/ijms16048430

Sicaire, A. G., Vian, M. A., Filly, A., Li, Y., Bily, A., & Chemat, F. (2014). 2-methyltetrahydrofuran: main properties, production processes, and application in extraction of natural products. In F. Chemat, & M. Vian (Eds.), *Alternative solvents for natural products extraction*. Berlin, Heidelberg: Green Chemistry and Sustainable Technology. Springer. https://doi.org/10.1007/978-3-662-43628-8_12.

Speer, K. I., & Kölling-Speer, I. (2006). The lipid fraction of the coffee bean. *Brazilian Journal of Plant Physiology, 18*(1), 201–216. https://doi.org/10.1590/S1677-04202006000100014

van den Stegen, G. D. H. (1979). The effect of dewaxing of green coffee on the coffee brew. *Food Chemistry, 4*(1), 23–29. https://doi.org/10.1016/0308-8146(79)90027-X

Tassew, A. A., Yadessa, G. B., Bote, A. D., & Obso, T. K. (2021). Influence of location, elevation gradients, processing methods, and soil quality on the physical and cup quality of coffee in the Kafa biosphere reserve of SW Ethiopia. *Heliyon, 7*(8), Article e07790. https://doi.org/10.1016/j.heliyon.2021.e07790

Tinoco, N. A. B., Pacheco, S., Godoy, R. L. O., Bizzo, H. R., de Aguiar, P. F., Leite, S. G. F., & Rezende, C. M. (2019). Reduction of $^{\beta}$N-alkanoyl-5-hydroxytryptamides and diterpenes by yeast supplementation to green coffee during wet processing. *Food Research International (Ottawa Ont.), 115*, 487–492. https://doi.org/10.1016/j.foodres.2018.10.007

Widodo, P. B., Yulianto, M. E., Ariyanto, H. D., & Paramita, V. (2023). Efficacy of natural and full washed post-harvest processing variations on arabica coffee characteristics. *Materials Today Proceedings, 87*(2), 79–85. https://doi.org/10.1016/j.matpr.2023.02.376

Wu, H., Gu, J., Amrit, B. K., Nawaz, M. A., Barrow, C. J., Dunshea, F. R., & Suleria, H. A. R. (2022). Effect of processing on bioaccessibility and bioavailability of bioactive compounds in coffee beans. *Food Bioscience, 46*, Article 101373. https://doi.org/10.1016/j.fbio.2021.101373