

Evaluation of the inhibition of monoamine oxidase A by bioactive coffee compounds protecting serotonin degradation

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ABSTRACT

Monoamine oxidase A (MAO-A) is a major enzyme responsible for the deamination of neurotransmitters such as serotonin (5-HT) in the central nervous system. The decrease in 5-HT levels is accompanied by disorders at the affective and somatic levels, leading to depression and disorders of the satiety center. The aim of this study was to evaluate the degree of MAO-A inhibition by chlorogenic acids, as well as green, light-, and dark-roasted coffee extracts and bioactive compounds from beans of the species *Coffea canephora* and *Coffea arabica*. Data for analysis was obtained using isothermal titration calorimetry and molecular docking. The results showed that caffeine and ferulic acid, as well as green Robusta coffee, demonstrated the greatest inhibition of MAO-A activity, which may increase the bioavailability of serotonin. We believe that green coffee shows potential antidepressant activity by inhibiting MAO-A, and may be used for treating depression and potentially, type 2 diabetes.

1. Introduction

Monoamine oxidase A (MAO-A) is an amine oxidase isoenzyme found in neurons of the brain, catecholaminergic neurons, and fibroblasts. The highest concentration of MAO-A can be found in the nucleus subcoeruleus, locus coeruleus, striatum, and black matter (Tong et al., 2013, 2017). MAO-A is responsible for the dysfunction of the monoaminergic system, which occurs via cell apoptosis resulting from reactive oxygen species formed during oxidative stress. MAO-A contains a peptide with the sequence SER-GLY-GLY-CYS-TYR, which binds to the flavin adenine dinucleotide cofactor at residue CYS-406, forming a strong thioether bond (Mialet-Perez et al., 2018; Tong et al., 2013, 2017). The long-term disorder of the monoamine metabolism leads to the degradation of neurotransmitters and hormones such as serotonin (5-HT), noradrenaline, adrenaline, and dopamine. This contributes to low levels of neurotransmitters in the center of the nervous system, which disrupts behavior and promotes the development of depression and neurodegenerative diseases (Mialet-Perez et al., 2018; Naoi et al., 2018).

Depression is a disease widely prevalent in the 21st century, which an increasing number of people suffer by showing constant stress, rush, and a strive for goals at all costs and in loneliness. In several studies it has been hypothesized that the brain monoamine levels are low during the development of depression. The monoamine-related hypothesis of depression proposes that the cause of this illness is the underactivity of monoamines, especially a deficiency in the central noradrenergic and serotonergic systems, which occurs by the loss of monoamine-releasing neurons (Hillhouse and Porter, 2015; Hindmarch, 2001; 2002).

A high monoamine oxidase level, particularly isoenzyme MAO-A, leads to an excessive metabolism of 5-HT which plays an important role in the regulation of the mood. A decrease in 5-HT levels in the body disturbs motor perception, and causes drowsiness and dysregulation of the satiety center; this leads to an increased appetite for high-carbohydrate products, especially fast-absorbable monosaccharides, which in turn promotes the development of obesity and leads to metabolic diseases such as diabetes (de la Fuente et al., 2017; Kumar et al., 2019; Palacios et al., 2017). Additionally, the MAO-A-derived reactive oxygen species contribute to lower levels of 5-HT, which in turn cause

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neurogenic disorders and persistent hyperglycemia (Manjarrez et al., 2006; Umbarkar et al., 2015). Therefore, natural and specific ligands that inhibit MAO-A activity are sought after.

The specificity of MAO-A for an active center consisting of the amino acids ILE-335, TRY-397, TYR-407, TYR-444, and LYS-305 may be targeted for inhibiting enzyme activity. The interactions between a ligand and the active site of the enzyme are characterized by hydrophobic interactions involving aromatic, aliphatic, or amine residues (Geha et al., 2002; Ma et al., 2004).

The therapeutic usage of MAO-A inhibitors such as toloxatone, amiflamine, and moclobemide, protects 5-HT from degradation and increases its bioavailability in the nervous system. An increase in 5-HT levels allows for an increased control of carbohydrate intake, which is important in reducing diet-related diseases, improves well-being, prevents depression, and reduces aggression (López-Nicolás et al., 2013; Vina et al., 2012). Clinical and epidemiological studies have reported that coffee bean extracts show a promising antidepressant effect (Sato et al., 2011; Hall et al., 2015; Herraiz & Chaparro, 2006). Presumably, this effect includes the inhibition of MAO-A activity, and oxidative deamination of 5-HT by the coffee bean components. Since depression is caused by low levels of 5-HT, it should improve as the levels of 5-HT increase, and this can be achieved by using high-activity MAO-A inhibitors. Therefore, an important research problem involves the identification of the coffee species, degree of roasting, and specific bioactive compounds that have the highest, potential antidepressant effect which may be used to support the fight against depression (Kele & Ohmacht, 1996; Hall et al., 2015; Herraiz & Chaparro, 2006; Petzer et al., 2013).

In this study, we analyzed the inhibition of MAO-A activity by coffee extracts, the bioactive components isolated from coffee, and individual chlorogenic acids (CHAs) (Fig. 6) by performing isothermal titration calorimetry (ITC) and molecular docking (MD) simulation. The aim of this study was to identify the component or fraction among the selected coffee bean extracts that showed the highest affinity for the enzyme depending on the roasting level, and the most stable enzyme-ligand complexes based on thermodynamic analyses.

2. Materials and methods

2.1. Chemicals and reagents

Human MAO-A recombinant enzyme expressed in baculovirus-infected BTI insect cells, 5-HT ($\geq 98\%$), caffeic acid ($\geq 99\%$), 3-caffeoylquinic acid ($\geq 99\%$), 4-caffeoylquinic acid ($\geq 99\%$), 5-caffeoylquinic acid ($\geq 99\%$), caffeine ($\geq 99\%$), ferulic acid ($\geq 99\%$), 3,5-dicaffeoylquinic acid ($\geq 99\%$), 4,5-dicaffeoylquinic acid ($\geq 99\%$), and dihydrocaffeic acid ($\geq 99\%$) were purchased from Sigma Aldrich (St. Louis, MO, USA), and nylon filters were sourced from Chromacol (Herts, UK).

2.2. Materials and preparation of coffee extracts

The coffee beans used in the study included green Brazil Cerrado Arabica coffee (*Coffea arabica* L.) and green Indie Cherry Robusta coffee (*Coffea canephora* L.) purchased from Bero Polska (Neumann Kaffee Gruppe, Gdynia, Poland). The coffee beans were roasted at two temperatures: at 180 °C for 6 min to achieve a light roast, and at 230 °C for 6 min to achieve a dark roast by creating a convection in an air cyclone system using the Vertiflow® Roaster 102 (Selmi Group, Santa Vittoria d'Alba, Italy). The roasted coffee beans were cooled to room temperature (24 °C), then ground and sieved to obtain a fraction size of 480 to 680 μm for further extract preparation. The aqueous coffee extracts were then prepared from the green (non-roasted), light-, and dark-roasted coffees at a 1:5.75 (v/v) ratio of coffee to water, which were boiled at 110 °C for 10 min (PS-5682, Vienna, Austria). The prepared extracts were cooled and filtered using a vacuum pump (18035.3 N, KNF Neuberger, Trenton, NJ, USA). The separation of the extracts from spent coffee grounds was performed using a Büchner funnel and vacuum. A

filter paper was placed on top of the funnel to cover all of the holes in the filter. The paper was moistened with a small amount of the extract, which helped the paper adhere to the plate and prevented the coffee grounds and sediment from passing through during filtration. The coffee suspension was poured onto the filter paper and pressed through the funnel using the vacuum pump, and then collected in a bottle under the funnel. The extracts were then freeze-dried and the resulting powder was stored at $-25\text{ }^{\circ}\text{C}$ before performing fractionation and the calorimetric assay (Grzelczyk & Budryn, 2019).

2.3. Isolation of coffee components using ultra-high-performance liquid chromatography

Coffee components were separated using a gradient ultra-high-performance liquid chromatography-electrospray ionization-tandem mass spectrometry (UHPLC-ESI-MS) system comprising a mass spectrometer (LCMS-2020, Shimadzu, Tokyo, Japan), equipped with an electrospray ionization source. Separation was performed at 30 °C using the Kinetex EVO C18 Corre-Shell (150 mm $\times$ 3.0 mm $\times$ 2.6 μm) analytical column obtained from Phenomenex (Torrance, CA, USA). The mobile phase solvent A consisted of water:formic acid (99.9:0.1, v/v) and solvent B comprised acetonitrile:formic acid (99.9:0.1, v/v); a flow rate of 0.2 mL/min was used. The injection volume was 2 μL . The gradient elution started at 0% B, reaching 3% B at 2 min, and then an isocratic elution was maintained for 2 min; the next gradient elution was restored to achieve 6% B at 10 min, 8% B at 17 min, 10% B at 24 min, 15% B at 32 min, 18% B at 34 min, 24% B at 20 min, and 30% B at 28 min, and then back to 0% B at 38 min. Calibration curves were generated for standard compounds using six different concentration levels in a range of 0.01–1.0 mg/mL. MS spectra were obtained using the collision-induced dissociation mode using nitrogen. Instrument control, data acquisition, and evaluation were performed using the LabSolutions 5.60 SP2 (Shimadzu). The profile of the isolated CHAs is shown in Fig. 1. The freeze-dried extracts contained 4.21–26.3 g/100 g d.m. of CHAs in the Robusta coffee extract in the form of mono- and diesters of caffeic or ferulic acid with quinic acid; the Arabica coffee extract contained 2.9–19.52 g/100 g d.m. of CHAs. The caffeine content was approximately 6 g/100 g d.m. in Robusta, and 3 g/100 g d.m. in the Arabica coffee extract (Table 1).

2.4. Isolation of coffee fractions using centrifugal partition chromatography (CPC)

The freeze-dried extracts of green, light-, and dark-roasted coffee were fractionated by performing CPC using the Spot Prep II system (Armen, France). The two-phase solvent system used was prepared using three solvents: water, ethanol, and ethyl acetate (5:1:4, v/v/v). The CPC rotor was filled with a hydrophilic stationary lower phase within 30 min, at a flow rate of 8 mL/min, and a speed of $1400 \times g$. In the next step, 10 g of freeze-dried coffee extract was dissolved in 50 mL of the two-phase solvent system, yielding the solution used for fractionation. The coffee solution was automatically loaded using an external pump. Three fractions were collected: monoesters of caffeic or ferulic acid with quinic acid, further described as monochlorogenic acids; diesters of caffeic acid with quinic acid, described as dichlorogenic acids; and caffeine. Elution of the dichlorogenic acids occurred mostly from 28 to 50 min, after which the more polar monochlorogenic acids were eluted from 62 to 75 min, and both were detected by their UV absorbance at a wavelength of 320 nm; caffeine was eluted from 50 to 62 min and was detected at 280 nm (Fig. 5). The samples were collected in 50-mL tubes. The 50-mL subfractions of each group of compounds were combined, concentrated to remove organic solvents, re-lyophilized, and stored at $-25\text{ }^{\circ}\text{C}$ for calorimetric experiments (Budryn et al., 2014). The CHAs obtained from the fractionation process were as follows: monochlorogenic acids (3-caffeoylquinic acid, 3-CQA; 4-caffeoylquinic acid, 5-caffeoylquinic acid, 3-feruloylquinic acid, 4-feruloylquinic acid, and 5-feruloylquinic

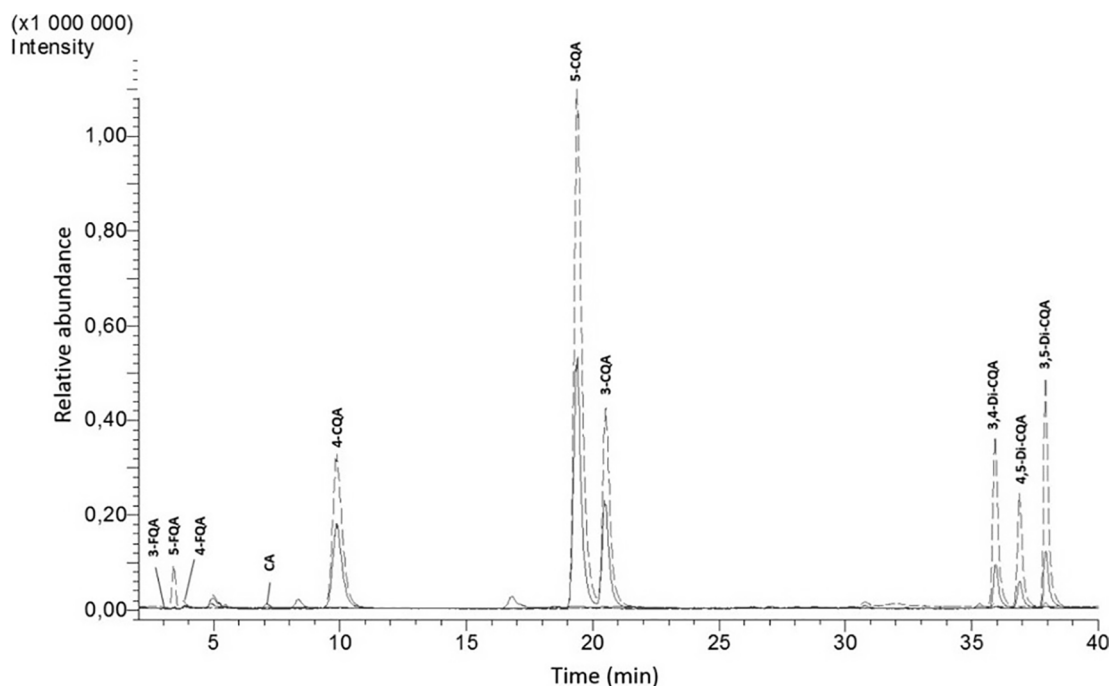


Fig. 1. LC-MS chromatograms of green (green dash line) and roasted Arabica coffee extracts (brown line). 3-FQA – 3-O-Feruloylquinic acid; 4-FQA – 4-O-Feruloylquinic acid; 5-FQA – 5-O-Feruloylquinic acid; CA- Caffeic acid; 3-CQA – 3-O-Caffeoylquinic acid; 4-CQA – 4-O-Caffeoylquinic acid; 5-CQA – 5-O-Caffeoylquinic acid; 3,4-Di-CQA – 3,4-Di-O-Caffeoylquinic acid; 4,5-Di-CQA – 4,5-Di-O-Caffeoylquinic acid; 3,5-Di-CQA – 3,5-Di-O-Caffeoylquinic acid.

Table 1

Concentration of chlorogenic acids and caffeine in green and roasted coffee extracts.

Compound (g/100 g d.b.)	Arabica green	Arabica light roasted	Arabica dark roasted	Robusta green	Robusta light roasted	Robusta dark roasted
Caffeic acid	0.03 ± 0.00 ^a	0.04 ± 0.00 ^a	0.19 ± 0.00 ^b	0.03 ± 0.01 ^a	0.03 ± 0.00 ^a	0.03 ± 0.00 ^a
Ferulic acid	0.24 ± 0.01 ^a	0.65 ± 0.00 ^c	0.14 ± 0.01 ^b	0.19 ± 0.13 ^b	0.23 ± 0.01 ^b	0.22 ± 0.00 ^b
3-O-Caffeoylquinic acid	2.82 ± 0.01 ^b	0.35 ± 0.02 ^a	0.35 ± 0.03 ^a	5.18 ± 0.08 ^c	3.24 ± 0.02 ^a	0.5 ± 0.00 ^a
5-O-Caffeoylquinic acid	6.15 ± 0.05 ^b	3.02 ± 0.03 ^c	1.51 ± 0.06 ^a	9.67 ± 0.26 ^d	4.54 ± 0.11 ^a	2.83 ± 0.02 ^a
4-O-Caffeoylquinic acid	2.07 ± 0.02 ^b	1.03 ± 0.00 ^c	0.4 ± 0.01 ^a	4.34 ± 0.03 ^a	3.04 ± 0.04 ^a	0.34 ± 0.01 ^a
3-O-Feruloylquinic acid	0.38 ± 0.01 ^b	0.13 ± 0.00 ^c	0.08 ± 0.00 ^a	0.80 ± 0.00 ^a	0.37 ± 0.00 ^a	0.23 ± 0.00 ^a
5-O-Feruloylquinic acid	1.19 ± 0.10 ^b	0.52 ± 0.00 ^c	0.20 ± 0.00 ^a	3.08 ± 0.00 ^a	1.23 ± 0.00 ^d	0.53 ± 0.02 ^a
4-O-Feruloylquinic acid	0.48 ± 0.00 ^b	0.21 ± 0.00 ^c	0.02 ± 0.00 ^a	1.07 ± 0.00 ^a	0.51 ± 0.00 ^a	0.17 ± 0.00 ^a
3,5-Di-O-Caffeoylquinic acid	2.04 ± 0.01 ^b	1.29 ± 0.03 ^c	0.34 ± 0.00 ^a	3.51 ± 0.00 ^a	2.82 ± 0.00 ^a	0.45 ± 0.00 ^a
4,5-Di-O-Caffeoylquinic acid	0.65 ± 0.03 ^b	0.37 ± 0.04 ^c	0.06 ± 0.00 ^a	1.29 ± 0.03 ^a	1.17 ± 0.00 ^a	0.09 ± 0.02 ^a
3,4-Di-O-Caffeoylquinic acid	1.55 ± 0.01 ^b	0.9 ± 0.04 ^c	0.22 ± 0.02 ^a	2.30 ± 0.02 ^a	2.02 ± 0.00 ^a	0.32 ± 0.00 ^a
Total chlorogenic acids	17.60 ± 1.02 ^b	8.60 ± 1.10 ^c	3.51 ± 0.88 ^d	31.46 ± 1.51 ^d	19.20 ± 1.18 ^e	5.71 ± 0.41 ^d
Caffeine	4.51 ± 0.35 ^a	4.35 ± 0.21 ^{a,b}	3.91 ± 0.14 ^d	6.11 ± 0.45 ^b	6.08 ± 0.20 ^e	6.07 ± 0.15 ^d

Values are expressed as mean value ± SD; n = 6; different letters in one row correspond to significant differences (P < 0.05).

acid) were isolated from green and roasted coffees in the range of 8.91–50.41 g/100 g db. of the fraction dry basis; dichlorogenic acids (3,5-dicaffeoylquinic acid, 3,5-Di-CQA; 4,5-dicaffeoylquinic acid, and 3,4-dicaffeoylquinic acid) were isolated from green and roasted coffees in the range of 3.09–22.35 g/100 g db. (Table 2). Caffeine comprised 10.00–21.90 g/100 g db. of its fractions isolated from green and roasted coffees.

2.5. Isothermal titration calorimetry

The inhibition of MAO-A enzyme activity was evaluated by performing ITC using the MicroCal PEAQ-ITC 200 calorimeter (Malvern, Worcestershire, UK). The analyses were carried out as described by Grzelczyk and Budryn (2019) with few modifications. A calorimeter cell with a capacity of 0.2 mL was filled with 0.1 mmol/L of MAO-A solution in ultrapure water (deionized, LC-MS grade); the syringe was subsequently filled with titrants which were degassed aqueous solutions of CHAs, green, light- or dark-roasted coffee extracts, or their fractions, at concentrations of 10 mmol/L calculated based on 5-caffeoylquinic acid

or/and 5-HT at 2 mmol/L. The measurements were carried out at 36.6 °C with continuous stirring (307 rpm), and a total of 19 injections were performed within 50 min. The generated plots show the heat of interactions between MAO-A and CHAs, the coffee extracts, or their isolated fractions (Fig. 2). The titration of a ligand with water was performed to subtract the energetic effects of dilution. During the experiments, enthalpy changes (ΔH), Gibbs free energy changes also described as affinity (ΔG), entropy changes (ΔS), the dissociation constant (K_D), binding constant (K_A), and the inhibition constant (K_i) were measured. The K_i values for the oxidation of 5-HT catalyzed by MAO-A were calculated by using the Michaelis-Menten equation. The data was calculated using the MicroCal PEAQ-ITC Analysis software. In order to calculate the inhibition activity, the signals of 5-HT oxidation in the presence of inhibitors was subtracted from the signals of 5-HT oxidation without inhibitors, while considering the enthalpy recorded during the interactions of the inhibitors with MAO-A. The inhibition activity was calculated as: $[(\Delta H - \Delta H_i) / \Delta H] \times 100\%$, where ΔH_i is the signal of 5-HT oxidation in the presence of an inhibitor, and ΔH is the signal of 5-HT oxidation without an inhibitor. The percentage of inhibition of 5-HT

Table 2

Concentration of chlorogenic acids and caffeine in fractions of coffee extracts (%).

Compound (g/100 g d.b.)	3-O-Caffeoylquinic acid	5-O-Caffeoylquinic acid	4-O-Caffeoylquinic acid	3-O-Feruloylquinic acid	5-O-Feruloylquinic acid	4-O-Feruloylquinic acid	3,5-Di-O-Caffeoylquinic acid	4,5-Di-O-Caffeoylquinic acid	3,4-Di-O-Caffeoylquinic acid	Total chlorogenic acids	Caffeine
Green Robusta											
Monochlorogenic acids	10.07 ± 0.02 ^a	16.1 ± 1.10 ^c	13.22 ± 0.10 ^c	3.29 ± 0.11 ^b	4.40 ± 0.00 ^b	3.33 ± 0.01 ^b	nd	nd	nd	50.41 ± 1.21 ^{e,f}	nd
Dichlorogenic acids	0.08 ± 0.02 ^b	0.12 ± 0.00 ^b	0.10 ± 0.00 ^b	0.01 ± 0.00 ^d	0.02 ± 0.00 ^d	0.01 ± 0.00 ^d	5.07 ± 0.1 ^a	8.38 ± 0.13 ^a	8.9 ± 1.2 ^f	22.69 ± 2.10 ^{e,f}	nd
Caffeine	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	21.9 ± 1.15 ^g
Light roasted Robusta											
Monochlorogenic acids	9.33 ± 0.12 ^a	12.6 ± 1.15	7.36 ± 0.10	2.19 ± 0.10 ^b	3.30 ± 0.02 ^b	2.13 ± 0.00 ^b	nd	nd	nd	36.91 ± 1.10 ^{e,f}	nd
Dichlorogenic acids	0.04 ± 0.04 ^b	0.06 ± 0.00 ^b	0.05 ± 0.01 ^b	0.004 ± 0.00 ^b	0.01 ± 0.00 ^b	0.008 ± 0.00 ^b	3.59 ± 0.01 ^b	4.18 ± 0.01 ^c	3.14 ± 0.01 ^c	11.08 ± 0.10 ^a	nd
Caffeine	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	21.00 ± 2.03 ^g
Dark roasted Robusta											
Monochlorogenic acids	3.69 ± 0.02 ^c	7.36 ± 0.04 ^a	4.57 ± 0.02 ^d	0.29 ± 0.00 ^b	1.19 ± 0.00 ^b	1.03 ± 0.00 ^b	nd	nd	nd		18.13
Dichlorogenic acids	0.002 ± 0.00 ^b	0.01 ± 0.01 ^b	0.001 ± 0.00 ^b	0.003 ± 0.00 ^b	0.008 ± 0.00 ^b	0.006 ± 0.01 ^b	2.03 ± 0.02 ^d	0.52 ± 0.01 ^d	2.66 ± 0.09 ^a	5.21 ± 0.1 ^f	nd
Caffeine	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	18.14 ± 1.11 ^g
Green Arabica											
Monochlorogenic acids	4.04 ± 0.02 ^a	12.50 ± 1.00 ^b	4.9 ± 0.45 ^c	2.21 ± 0.10 ^d	3.36 ± 0.10 ^d	2.26 ± 0.00 ^d	nd	nd	nd	29.27 ± 1.11 ^{e,f}	nd
Dichlorogenic acids	0.008 ± 0.00 ^b	0.01 ± 0.08 ^b	0.01 ± 0.00 ^b	0.004 ± 0.00 ^b	0.006 ± 0.00 ^b	0.001 ± 0.00 ^b	3.05 ± 0.00 ^b	4.26 ± 0.12 ^c	5.77 ± 0.45 ^a	13.12 ± 0.15 ^f	nd
Caffeine	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	12.95 ± 2.0 ^g
Light roasted Arabica											
Monochlorogenic acids	2.96 ± 0.40 ^c	8.33 ± 1.21 ^e	2.08 ± 0.04 ^d	1.11 ± 0.01 ^d	2.21 ± 0.01 ^d	1.14 ± 0.02 ^d	nd	nd	nd	17.56 ± 0.25 ^{e,f}	nd
Dichlorogenic acids	0.002 ± 0.00 ^b	0.004 ± 0.0 ^b	0.001 ± 0.00 ^b	0.001 ± 0.00 ^b	0.001 ± 0.00 ^b	nd	2.27 ± 0.00 ^d	3.47 ± 0.01 ^d	3.01 ± 0.4 ^f	8.76 ± 0.45 ^{e,f}	nd
Caffeine	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	12.15 ± 1.23 ^g
Dark roasted Arabica											
Monochlorogenic acids	0.67 ± 0.03 ^c	7.14 ± 1.0	0.83 ± 0.01 ^d	0.01 ± 0.00 ^b	0.14 ± 0.01 ^b	0.12 ± 0.01 ^b	nd	nd	nd	8.91 ± 0.25 ^{e,f}	nd
Dichlorogenic acids	nd	nd	nd	nd	nd	nd	0.44 ± 0.01 ^d	1.35 ± 0.02 ^c	1.3 ± 0.04 ^e	3.09 ± 0.15 ^{e,f}	nd
Caffeine	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	10.0 ± 1.01 ^g

Values are expressed as mean value ± SD; n = 6; different letters in one row correspond to significant differences (P < 0.05), nd – not detected.

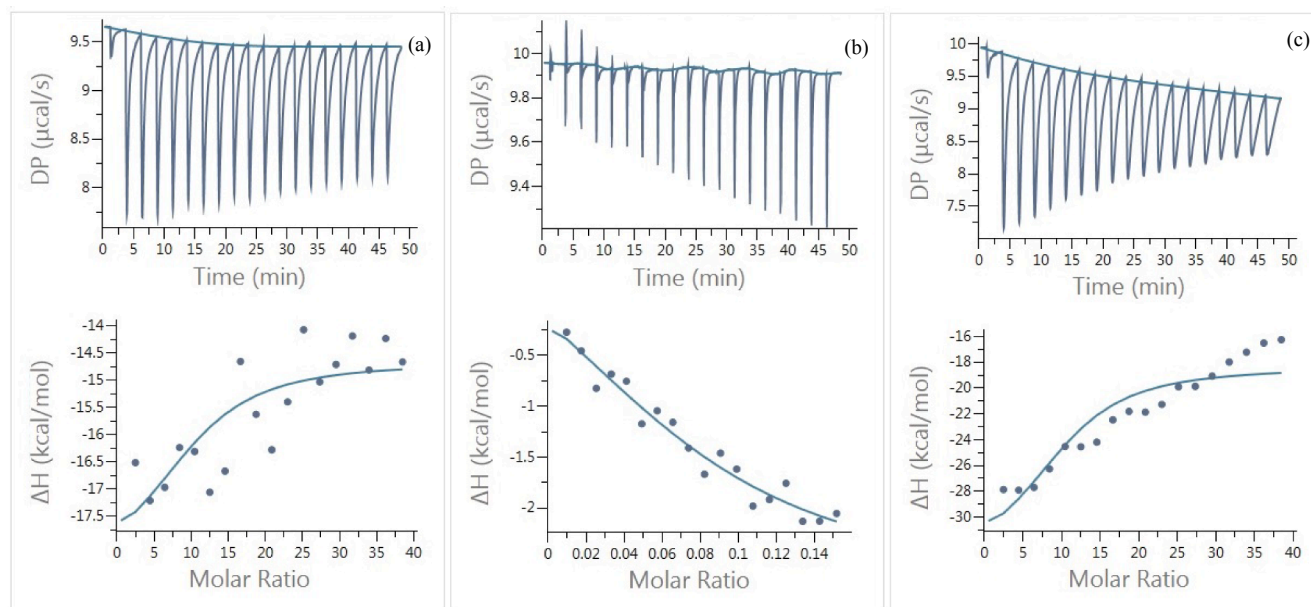


Fig. 2. ITC raw data of (a) 5-HT (serotonin) oxidation by MAO-A; Interactions of MAO-A with (b) 3-O-caffeoylquinic acid; Oxidation of 5-HT catalyzed by MAO-A at a presence of: (c) 3-O-caffeoylquinic acid. Curves in bottom figures show the fitting of data to a one-set model by the nonlinear Levenberg–Marquardt fitting algorithm supplied by the instrument.

oxidation at a given concentration of the ligand was calculated and it was used to calculate the concentration of ligands that inhibited 50% of the oxidation reaction (IC_{50}) (Pal et al., 2012; Budryn et al., 2018).

2.6. Docking simulation

The X-ray crystalline structure of human MAO-A (2BXR) was obtained from the Protein Data Bank database (<http://www.rcsb.org/pdb>). The modelling involved three steps. The chemical structures of all bioactive coffee compounds (CHAs and caffeine) were built-up manually, and the partial charges required for MD simulations were calculated as previously reported (Gasteiger & Marsili, 1980). The docking of bioactive coffee compounds to the prepared enzyme structure model was performed using the Autodock Vina docking software (Scripps Research, La Jolla, CA, USA), using the default configuration parameters (the scoring function in Vina uses the Lennard-Jones potential model). The interactions observed comprised hydrogen bonds (H-bonds),

hydrophobic stabilization, electrostatic interactions, entropic penalty due to the number of rotatable bonds, and the internal energy of the ligand (Ferrazzano et al., 2016; Jung et al., 2017).

2.7. Statistical analysis

Extraction of green, light-, and dark-roasted coffee was conducted independently in triplicate. The analyses of compounds in the extracts or isolated fractions were carried out in triplicate. The statistical analysis was based on the determination of the average values of nine measurements and their standard deviation, as well as one-way analysis of variation, using Statistica 10.0 software at a significance level of $p < 0.05$. The significant differences among the mean values were estimated using the Tukey's honestly significant difference test.

Table 3

Therodynamic parameters of interactions between MAO-A and hydroxycinnamic acids and their esters with quinic acid (chlorogenic acids) or caffeine.

Compound	N	K_D ($\mu\text{mol/L}$)	$K_A \cdot 10^3$ (L/mol)	ΔH (kJ/mol)	ΔG (kJ/mol)	ΔG (kJ/mol) Docking simulation	ΔS (J/mol $\cdot$ K)	IC_{50} (1 $\mu\text{mol/L}$ inhibitor:1 $\mu\text{mol/L}$ MAO-A)	K_i ($\mu\text{mol/L}$) K_M 5-HT 0.28 mmol/L
Caffeic acid	1	101.0 $\pm$ 9.4 ^a	9.9 $\pm$ 0.3	-0.2 $\pm$ 0.11 ^b	-23.7 $\pm$ 1.3	-28.89	75.8 $\pm$ 0.20	7.9 $\pm$ 0.1	103 $\pm$ 1.2 ^{a,d}
Ferulic acid	1	108.0 $\pm$ 2.0 ^a	9.3 $\pm$ 0.5	-9.7 $\pm$ 0.13 ^a	-23.5 $\pm$ 2.3 ^a	-30.14	44.7 $\pm$ 0.22	7.8 $\pm$ 0.1	<0.001
3-O-Caffeoylquinic acid	1	23.3 $\pm$ 0.2	42.9 $\pm$ 1.8 ^a	6.4 $\pm$ 0.08 ^c	-27.5 $\pm$ 1.5	-35.17	109.6 $\pm$ 0.28	7.9 $\pm$ 0.0	179 $\pm$ 2.2 ^{a,d}
5-O-Caffeoylquinic acid	1	12.0 $\pm$ 1.5 ^a	83.3 $\pm$ 0.8	2.3 $\pm$ 0.86 ^c	-29.1 $\pm$ 0.3	-36.01	101.6 $\pm$ 1.30	5.5 $\pm$ 0.1	0.01 $\pm$ 0.0
4-O-Caffeoylquinic acid	1	13.2 $\pm$ 1.2 ^b	75.8 $\pm$ 5.2 ^a	13.5 $\pm$ 2.3 ^c	-28.9 $\pm$ 1.8	-37.26	136.9 $\pm$ 1.41	6.0 $\pm$ 0.0	0.09 $\pm$ 0.0
3,5-Di-O-Caffeoylquinic acid	1	54.0 $\pm$ 0.1	18.5 $\pm$ 1.6	-0.27 $\pm$ 2.95 ^b	-25.3 $\pm$ 1.2	-52.33	80.8 $\pm$ 1.08 ^c	7.6 $\pm$ 0.0	70.5 $\pm$ 1.2
4,5-Di-O-Caffeoylquinic acid	1	38.0 $\pm$ 0.1	26.3 $\pm$ 1.5	-0.15 $\pm$ 5.27 ^b	-26.2 $\pm$ 1.1	-42.29	84.1 $\pm$ 1.52 ^c	7.2 $\pm$ 0.1	69.2 $\pm$ 1.0
Dihydrocaffeic acid	1	63.0 $\pm$ 0.1	15.8 $\pm$ 1.7	-0.29 $\pm$ 3.12 ^b	-24.9 $\pm$ 1.1	-41.86	79.4 $\pm$ 0.20 ^c	7.0 $\pm$ 0.0	71.5 $\pm$ 2.1
Caffeine	1	104.5 $\pm$ 7.4 ^b	9.6 $\pm$ 0.0 ^b	-3.47 $\pm$ 0.01	-23.6 $\pm$ 0.9	-27.21	65.0 $\pm$ 0.36	5.2 $\pm$ 0.1	130.0 $\pm$ 0.1

Values are expressed as mean value $\pm$ SD; n = 9; different letters in one column or no index correspond to significant differences ($P < 0.05$).

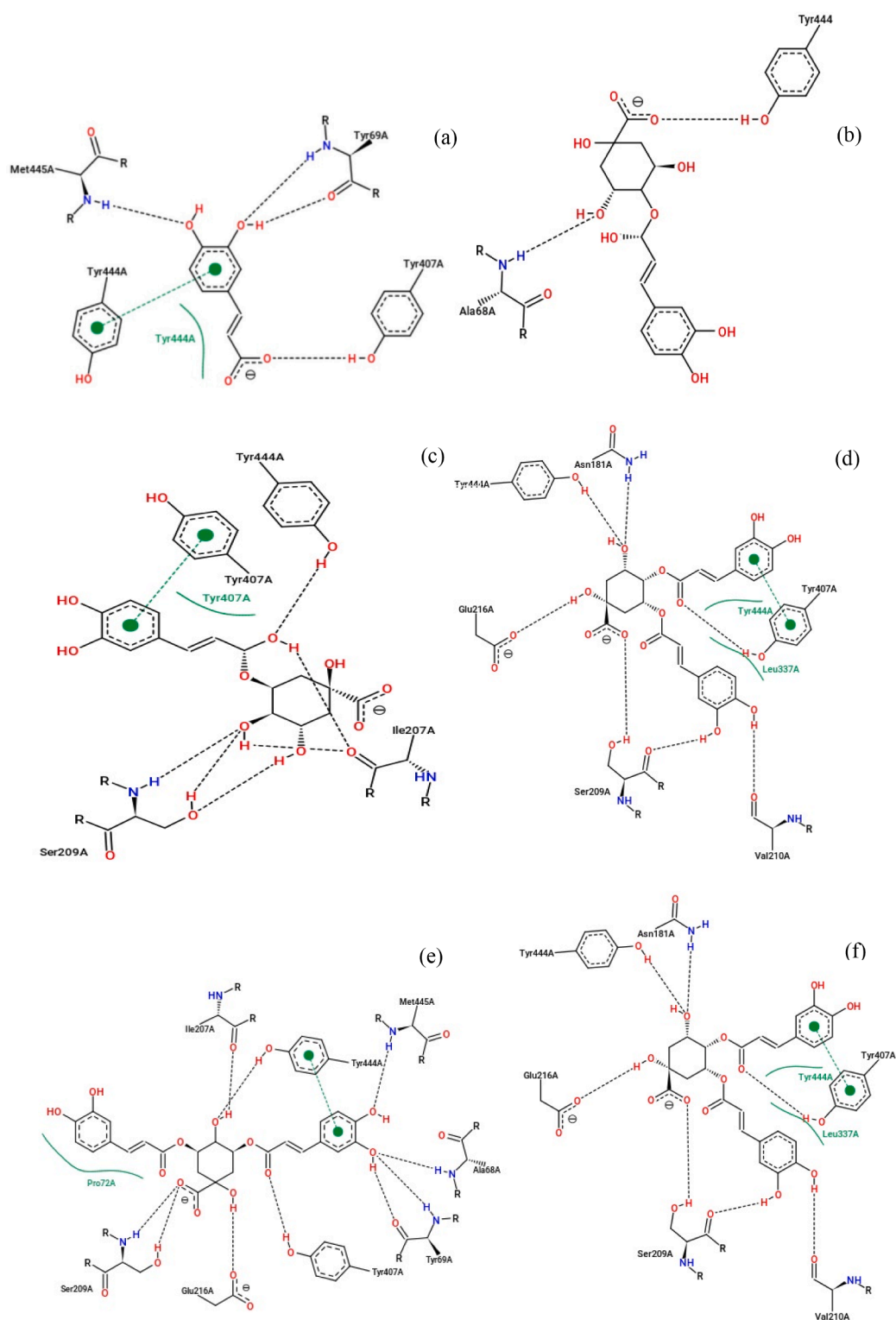


Fig. 3. Depiction (in 2D) of the main interactions established between active site of MAO-A and (a) Caffeic acid; (b) 4-O-caffeoylquinic acid; (c) 5-O-caffeoylquinic acid; (d) 3,4-di-O-caffeoylquinic acid; (e) 3,5-di-O-caffeoylquinic acid; (f) Ferulic acid; (g) 3-O-caffeoylquinic acid; (h) Caffeic acid; (i) Caffeine. Continuous green lines represent hydrophobic interactions, while black dashed lines show hydrogen bonds and green dashed lines pi-pi interactions. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

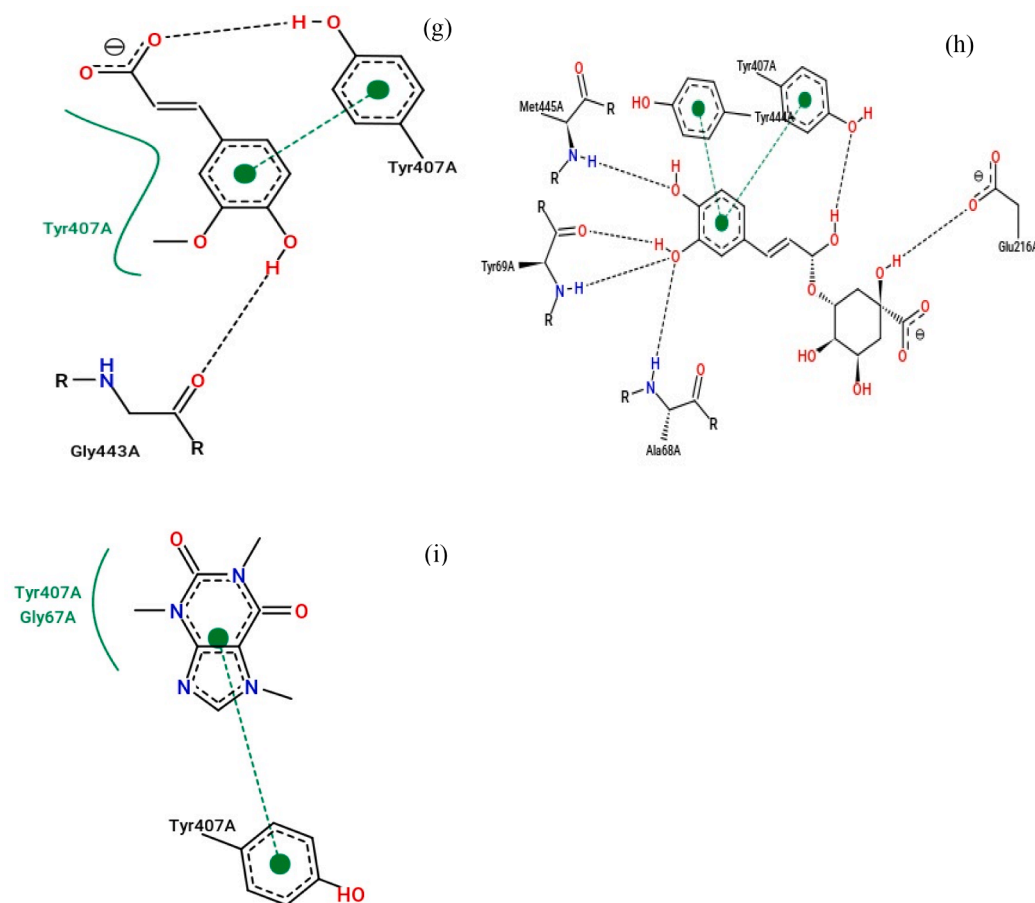


Fig. 3. (continued).

3. Results and discussion

3.1. Characterization of the complexes of CHAs and caffeine with MAO-A based on ITC

The inhibition of MAO-A activity by coffee components was first analyzed in a test using single bioactive substances by performing ITC. During an ITC analysis, an exothermic or endothermic reaction is recorded, which occurs from the binding of compounds in the active place, or from conformational transformation. The heat is generated as a consequence of ligand-to-protein binding, which is measured as an energy peak (kcal/s). A representative calorimetric titration profile of MAO-A with 5-HT, and 3-CQA at pH 7.4 is shown in Fig. 2. Each peak in the binding isotherm represents a single injection of CHAs or caffeine into the MAO-A solution. The endothermic character of the peaks in Fig. 2 is associated with the inhibitor-to-enzyme interaction. As the sites available on MAO-A became progressively occupied during titration, the energetic effect decreased. Table 3 presents the thermodynamic parameters of the ligand–protein interaction determined experimentally by performing ITC. The reaction between the CHAs and MAO-A was mostly an exothermic process, which was confirmed by the negative changes in ΔH ; only 3-CQA, 4-CQA, and 5-CQA showed a positive energy effect (endothermic reaction) (Fig. 2). The results revealed that the stoichiometry of the resulting complexes of CHAs or caffeine with MAO-A had an inhibitor-to-enzyme molar ratio of 1, indicating that the experimental data agreed with the 1:1 reaction model characterized by a single binding site, and the concentrations of reagents (protein and ligand) could be calculated with reasonable accuracy, and that the protein was folded properly (Dębowski et al., 2016; Freyer & Lewis, 2008; Robinson, 1985; Vignoli et al., 2011). The MAO-A-5-HT and

MAO-A-5-HT-3-CQA titration plots reached binding saturation, which was defined as no change in ΔH at the subsequent injection (Fig. 2a and c). The MAO-A-3-CQA plot suggested that apart from binding to a specific high-affinity site, the polyphenol possibly adsorbed to lower-affinity sites on the surface of the protein. The series of titrations showed a high negative ΔH value for ferulic acid (-9.7 ± 0.13 kJ/mol) and caffeine (-3.47 ± 0.01 kJ/mol), and the highest positive value (13.5 ± 2.3 kJ/mol) was recorded for 4-CQA; 5-CQA showed the highest ΔG value (-29.1 ± 0.3 kJ/mol) for MAO-A. The total energetic effects of the interactions of MAO-A with most of the investigated phenolics (caffeic, ferulic, dihydrocaffeic acids, 3,5-Di-CQA, 4,5-Di-CQA, and caffeine), were exothermic ($\Delta H < 0$). Endothermic interactions resulted from the binding of MAO-A to 3-CQA, 4-CQA, and 5-CQA. These endothermic interactions may be a result of accompanying rearrangement effects. The K_D of the complex ranged from 12 to 108 $\mu\text{mol/L}$. The significant difference in K_D values might have resulted from the use of a neutral pH condition, at which phenolic acids are deprotonated, and could contribute to the formation of electrostatic interactions. The most beneficial thermodynamic parameters (highly negative ΔH , ΔG , and high K_D) were found to be associated with 5-CQA and caffeic acids, as well as caffeine. This shows that these compounds exhibit a high activity-inhibition potential for the enzyme. In addition, the negative ΔS values of most coffee compounds interacting with MAO-A may indicate that the phenolic compounds/caffeine could bind to the hydrophobic sites of the proteins, including the active center (Hall et al., 2015; Martinez-Gonzalez et al., 2017; Petzer et al., 2013). From the ITC analysis, it may be concluded that the majority of interactions that formed ligand–protein complexes, excluding monochlorogenic acids, showed a non-covalent hydrophobic character, as the ΔH and ΔG values were negative and ΔS value was positive.

It is worth noting that most of the bioactive coffee compounds were competitive against the enzyme's basic substrate, 5-HT. This was observed in the present study as well as in the study by [Dhiman, et al. \(2018\)](#), who reported that caffeic acid can act as a competitive inhibitor of MAO-A at a concentration of 0.5–5.0 μM . This was recorded in the ITC model using the Michaelis-Menten equation as the K_i value decreased with increasing concentrations of the ligand. [Dhiman et al. \(2018\)](#) found caffeic acid to be an MAO-A inhibitor, showing IC_{50} values of 8.51 μM and 8.87 μM depending on the concentration of enzyme in the system. This was also confirmed by using the ITC approach in the present study, where a similar IC_{50} value was obtained ($7.9 \pm 0.01 \mu\text{M}$). The MAO-A inhibition can also be proved by the fact that the positively charged hydrogen atoms from the –OH group of the polyphenols interacted with the neighboring oxygen and nitrogen atoms of amino acid residues, and there were interactions in the active center dominated by the formation of H-bonds. Additionally, [Petzer et al., \(2013\)](#) reported that caffeine is highly competitive for the substrate of MAO-A as a result of reversible interactions. In their study, the K_i value measured was 0.70 mM, whereas it was found to be lower (0.13 mM) in our study, which shows that the use of higher concentrations of caffeine within a tested range may increase the competitiveness of the inhibitor. The *in vivo* study by [Pechlivanova et al. \(2012\)](#) evaluated the antidepressant effect of caffeine using an animal model. The authors reported that administering 8 mg/kg/day of caffeine to mice exhibiting chronic stress shows a positive reaction and increases the level of 5-HT in the hippocampus, which shows that caffeine increases the level of neurotransmitters owing to the synergistic action of caffeine with other bioactive compounds ([Kale & Addepalli, 2014](#)). Similarly in the present study, we observed that a concentration of 5.2 $\mu\text{mol/L}$ of caffeine reduced enzyme activity by 50% (Table 3).

Therefore, it may be considered that among the coffee components that inhibited MAO-A activity, caffeine as well as caffeic acid and its methoxy-derivative, ferulic acid, contributed the most towards inhibition. Caffeic and ferulic acids are present in relatively low concentrations in coffee, but they are metabolites of the esters of caffeic and ferulic acids with quinic acid, and are found both in the intestinal lumen and plasma in the human body. Dihydrocaffeic acid is another metabolite of CHAs. [Chen et al. \(2015\)](#) and [Xu et al. \(2013\)](#) showed that ferulic acid treatment at a dosage of 10–80 mg/kg significantly reduces depression-like effects, which was speculated in the present study from the high enthalpy changes in the interactions of ferulic acid with MAO-A, and the formation of strong complexes. Ferulic acid showed very interesting characteristics; it had the highest complex formation constant as a result of the interactions by hydrophobic bonds and highly negative reaction enthalpy.

3.2. Characterization of the complexes of CHAs and caffeine with MAO-A based on MD simulations

MD simulations based on the docking of coffee bioactive compounds with MAO-A were generated to better explain the ITC results. MD simulations of the interactions between MAO-A and the different bioactive compounds extracted from coffee beans allows for an accurate prediction of the interactions at the atomic level in the active center of the enzyme, such as H-bonds, hydrophobic, and van der Waals interactions, and the affinity of reagents, to indicate the most preferable and stable complexes of bioactive compounds with MAO-A, potentially protecting 5-HT against deamination and increasing its bioavailability ([Delpont et al., 2017](#)). Molecular modeling of the interactions of human MAO-A with caffeine and CHAs confirmed that the inhibitors docked at the MAO-binding site. The main interactions established between the active center of MAO-A and the coffee components were H-bonds as well as hydrophobic, van der Waals, and *pi-pi* interactions, depicted in Fig. 3. The affinity of ligands to the protein was analyzed, as the ΔG during MAO-A titrations determined using ITC, was compared with the ΔG calculated by performing MD ($\Delta G_{\text{predicted}}$) simulations and shown in

Table 3. Using both methods, the ΔG was calculated as negative, which indicated the spontaneous nature of the interactions. The ΔG determined using the ITC method was in the range from –32.5 to –29.1 kJ/mol for ferulic acid and 3-O-caffeoylquinic acid, respectively. When derived using the MD method, the values of ΔG were in the range from –52.23 to –27.21 kJ/mol for 3,5-Di-CQA and caffeine, respectively. The interaction of 3,5-Di-CQA showed a higher $\Delta G_{\text{predicted}}$ value to that of 5-HT (Fig. 4). However, the differences were observed in the levels of the particular energies making up the total ΔG . The 3,5-Di-CQA interaction showed higher solvation and hydrogen bond energy than those of 5-HT interactions. The repulsion interactions weakened the affinity of 3,5-Di-CQA owing to its complex structure of three basic molecules that contained two phenolic rings, which led to the generation of intermolecular stresses. The 5-HT interaction showing hydrophobic forces was stronger. Certain differences in the free enthalpy values determined by using the dissused techniques may be due to the fact that the MD observations mainly reflect the effects resulting from the docking of ligands, in only the energetically most preferable site which is the active center, whereas the ITC parameters may be affected by additional interactions at residues located outside the catalytic site ([Strydom et al., 2011](#)).

The active center in MAO-A comprises five amino acid residues: ILE-335, TYR-397, TYR-407, TYR-444, and LYS-305, and the binding of the substrate at the active site of the enzyme is characterized mostly by hydrophobic interactions ([Geha et al., 2002](#)). From the graphs presented in Fig. 3, it can be observed that the CHAs and caffeine showed hydrophobic interactions and bound with the protein via H-bonds. The highest number of H-bonds were observed in the interactions of MAO-A with 3,5-di-O-caffeoylquinic acid (ten H-bonds) at residues TYR-444A, TYR-407A, TYR-69A (two H-bonds), SER-209A (two H-bonds), ALA-68A, GLU-216A, ILE-207A, and MET-445A, along with one additional hydrophobic and *pi-pi* interaction (Fig. 3e). Additionally, the H-bonds strongly influenced the type of MAO-A interactions with 4,5-di-O-caffeoylquinic acid; they occurred at ASN-181A, TYR-444A, GLU-216A, LEU-337A, SER-209A (two H-bonds), and VAL-210A residues (Fig. 2f). Interactions with 3,4-di-O-caffeoylquinic acid occurred at ASN-181A, TYR-444A, GLU-216A, LEU-337A, SER-209A (two H-bonds), and VAL-210A residues (Fig. 3d). The MD shown in Fig. 3 revealed that caffeine formed *pi-pi* interaction with MAO-A at the TYR-407A residue despite having a hydrophobic structure (Fig. 3i). From studying the caffeic acid interactions (Fig. 3a), it was observed that caffeic acid formed three H-bonds in the active site via hydroxyl moieties and hydrophobic forces, as well as *pi-pi* interactions with the phenolic ring of the TYR-444A residue. Although the hydrophobic interactions with the TYR-407A (Fig. 3c,d,f,g,h,i), TYR-444A (Fig. 3e,h) and GLY-67A (Fig. 3i) residues played an important role in complexing at the MAO-binding pocket with the concerned CHA, the H-bonds appeared to have stabilized the bond formation in the concerned solutions. This confirmed the pH dependence of the complex formation ability. The reported pK_a values of 5-CQA phenolic hydrogens were in the range of 8.21–8.42 ([Wildermuth et al., 2016](#)). An increase in pH above the pK_a value causes the phenolic hydrogens to dissociate and yield phenolate anions. At a pH lower than the above mentioned pK_a value, the CHAs used in the ITC and MD tests occur in an undissociated form. The above interactions provided by MD simulations confirmed the results of the ITC tests, suggesting that coffee components are able to form complexes with the enzyme at the active site, and block the oxidative deamination of 5-HT.

3.3. Characterization of the interactions between MAO-A and coffee extracts and their fractions

The tests performed on individual bioactive coffee compounds provided a lot of information on the potential effects of coffee as an MAO-A inhibitor. A mixture of compounds, however, can act differently compared to a single substance. Therefore, in the next stage of the study, the coffee extracts and fractions of coffee extracts characterized by high concentrations of monochlorogenic acids, dichlorogenic acids, or

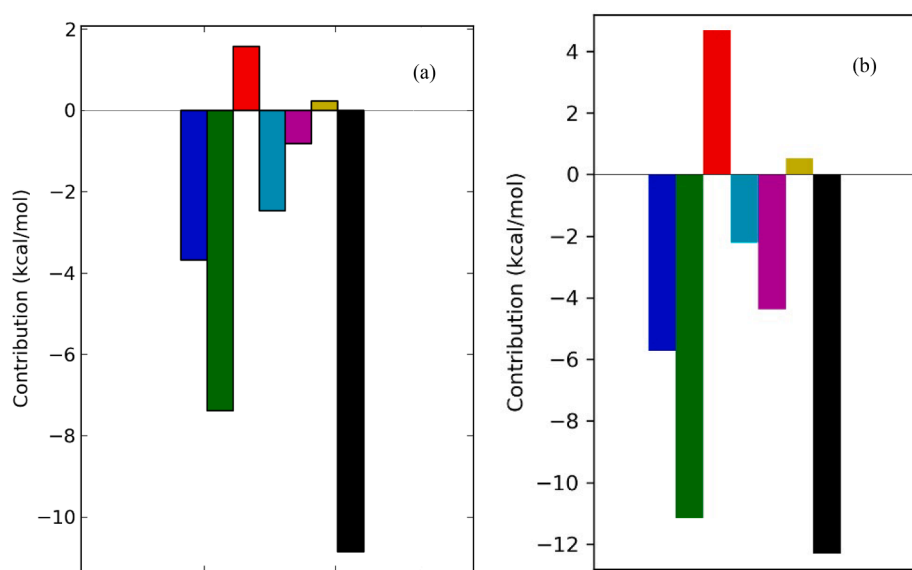


Fig. 4. Energetic contributions (kcal/mol) to binding energy for ligands: (a) 5-HT; (b) 3,5-di-O-caffeoyl-quinic acid; Contributions (from left to right) depicted are: van der Waals interactions (navy blue), solvation energy (green), repulsion (red), hydrophobic interactions, (light blue), hydrogen bonds (purple), energy of entropic losses associated with ligand's rotatable bonds (yellow) and total predicted binding energy (black). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

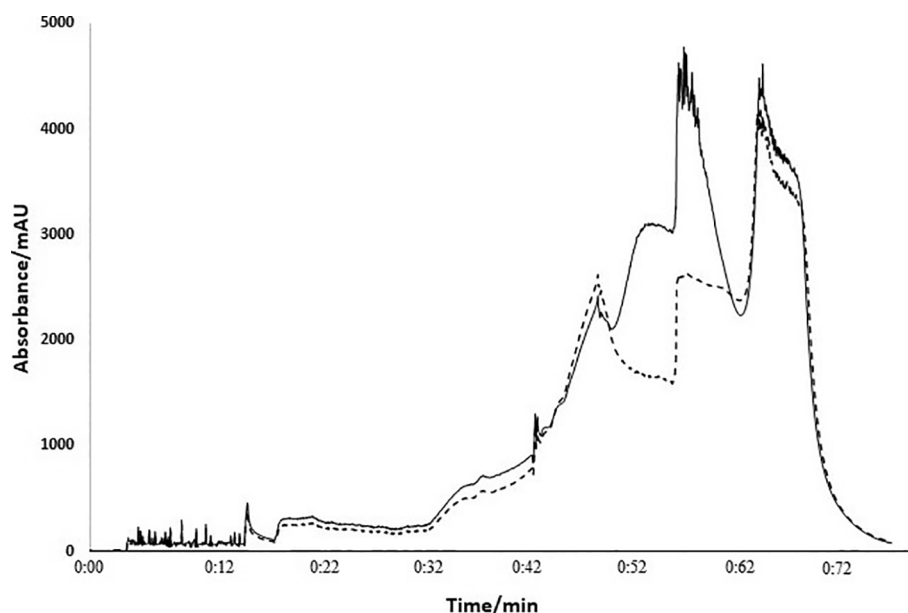


Fig. 5. Centrifugal partition chromatogram (CPC) of crude aqueous green (GCE) coffee extracts at 320 nm (---) and 280 nm (-----), fractions: 28–50 min - dichlorogenic acids, 50–62 min - caffeine, 62–75 min monochlorogenic acids.

caffeine were analyzed using ITC. The fractions were obtained from green, light-, and dark-roasted coffee extracts of the two species. There are no reports on the interactions between MAO-A and fractions of coffee extracts, which are a mixture of coffee compounds from a single group. The fractions which show high enzyme-inhibition activity can be used in food production, or in pharmacology as a drug for stabilizing 5-HT levels. Therefore, it was investigated whether the inhibitory effect of coffee could be increased by isolating monochlorogenic, dichlorogenic, and caffeine fractions (Table 4). The fractions were characterized by the content of their main component: CHAs, Di-CHAs, or caffeine; however, they also contained small amounts of other coffee compounds (Table 2).

The K_A values were obtained for complexes between MAO-A and isolated fractions of coffee extracts in the range of 4.0 to 19.7 L/mol for dichlorogenic acids from dark-roasted Robusta, and monochlorogenic acid fractions from green Robusta coffee extracts. It was observed that all caffeine fractions were characterized by positive ΔH and negative ΔG values as well as a negative ΔS value. In this case, the course of the

reaction was determined by the factor ΔS , which at low values indicated a limited mobility of reagents, caused by the formation of complexes dominated by hydrophobic interactions. Dichlorogenic acids showed the greatest tendency to form non-covalent and hydrophobic interactions, characterized by negative ΔH and ΔS values of the reactions (Martinez-Gonzalez et al., 2017). The compounds responsible for the high affinity for MAO-A were found in the monochlorogenic acid fraction isolated from green Robusta extracts ($\Delta H = -175.8$ kJ/mol; $\Delta G = -50.7$ kJ/mol). Roasting the coffee led to a change in the thermodynamic parameters depending on the coffee fraction. Caffeine is characterized by a high thermal stability; however, it changes into a non-hydrophobic form during roasting which was observed as a difference in the affinity for the enzyme.

Non-fractionated coffee extracts obtained from different degrees of roasting were tested in the next stage, to evaluate whether the observations on the single substances and fractions were related to the activity of the extracts. The effects of the enzyme interactions with the

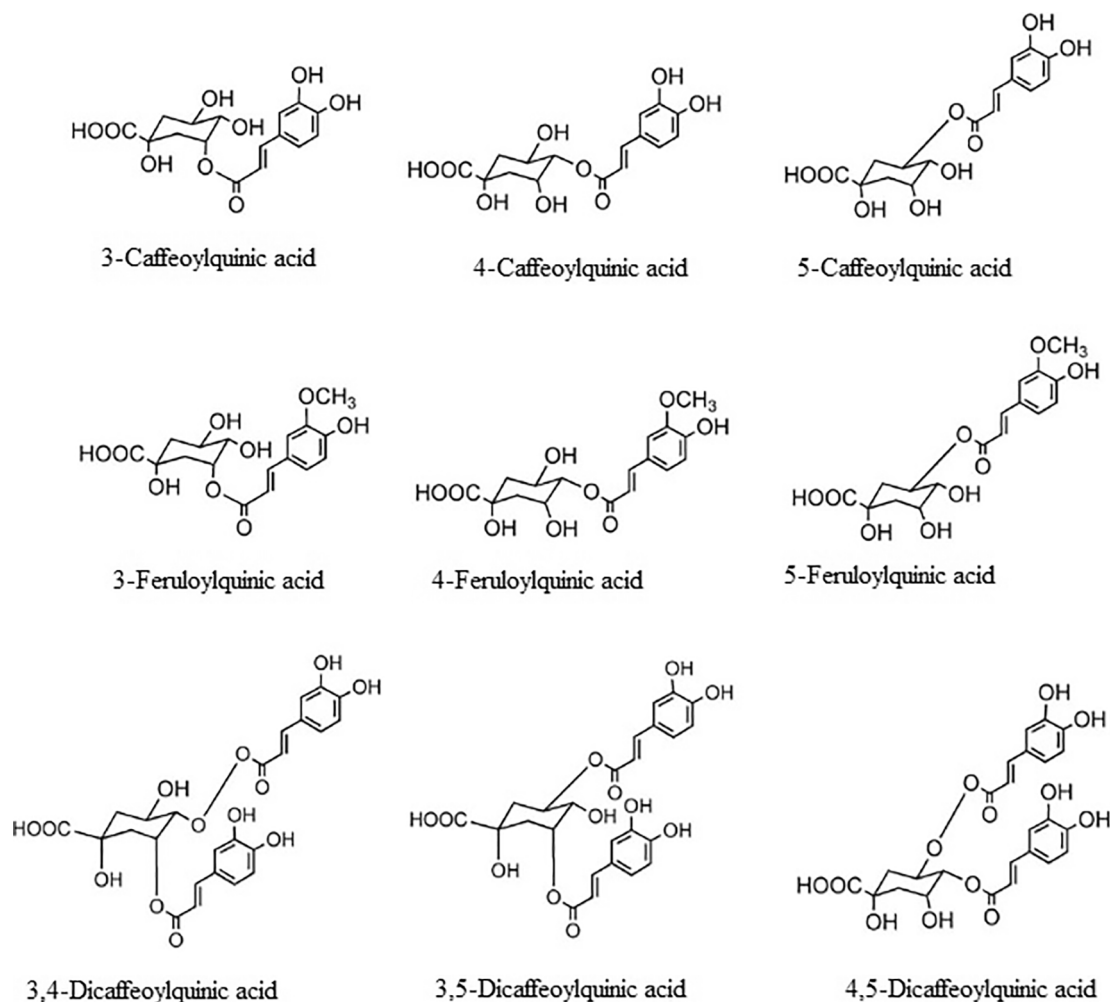


Fig. 6. Chemical structures of chlorogenic acid (Stalmach, Mullen, Nagai, & Crozier, 2006).

compounds contained in green Arabica and Robusta extracts are shown in Table 5. The negative ΔH caused by binding, and the positive entropy component of the thermodynamic effects that occurred during interactions, suggest an important contribution of H-bonds and hydrophobic interactions to inhibitor-enzyme total affinity. This was also confirmed in the MD simulations, as it was observed that mainly H-bonds occurred between the ligands and the protein (Fig. 3). The ΔH of the coffee extract interactions with MAO-A did not differ significantly among species or the degree of roasting. The ΔH values ranged from -0.16 to -0.50 kJ/mol and were related to a high content of hydrophobic regions in the coffee components which were capable of performing hydrophobic, van der Waals, and electrostatic interactions, as well as the formation of hydrogen bonds (López-Nicolás et al., 2013) (Table 4). The complex-formation constant decreased with the degree of roasting, from green to dark-roasted beans, although the decrease was insignificant ($p > 0.05$). The highest affinity (negative ΔG) was found to be -23.7 kJ/mol for green Robusta and -20.1 kJ/mol for green Arabica extract, and the ΔS was the highest at 75.3 and 63.4 J/mol $\cdot$ K for green Robusta and green Arabica extracts, respectively. Interactions of all coffee extracts with MAO-A were slightly exothermic (Table 5). The coffee extracts exhibited inhibitory properties in relation to the deamination of 5-HT by MAO-A enzyme, and therefore they may contain specific compounds that can act as MAO-A inhibitors when absorbed and temporarily accumulated after consumption of coffee.

It was observed that the affinity of green coffee for the enzyme was higher than that of the roasted coffees. This may be due to the higher concentration of CHAs present in non-roasted coffees. The reduction in CHA

concentration in dark-roasted bean extracts versus green beans was 84% and 85% for Robusta and Arabica, respectively. Polyphenols are degraded during roasting, and they partially form covalent bonds with carbohydrates and can be linked to melanoidins through both amino acids and sugars, which decreases the number of CHAs available for interactions resulting in a low enzyme affinity (Martínez-González et al., 2017). Particularly, it was observed that the for MAO-activity inhibition by coffee extracts was significantly lower than that of individual compounds, which suggests that the extract which comprises a mixture of more than a dozen compounds inhibits the enzyme with an efficiency higher than that of individual compounds. Dark-roasted Robusta coffee extracts exhibited a high affinity for the enzyme, which may be increased in this case via the binding of several different compounds exhibiting a wide range of affinities to different residues of MAO-A at the active site (Fig. 3) and at other regions. In dark-roasted coffee, up to 30% of the CHAs can be bound to Maillard reaction products, such as melanoidins. In their bound form, they may be able to interact with MAO-A, especially if the phenolic moiety remains available.

4. Conclusions

This study used the ITC technique and MD simulations to provide relevant information at the molecular level on the MAO-A inhibition activity of coffee in relation to its species, the degree of roasting, and properties of the individual bioactive compounds. The analysis has shown that coffee is a good candidate for studying the inhibition of enzyme activity *in vivo*, and protects 5-HT from deamination, while increasing its bioavailability for a number of nervous system centers.

Table 4

Parameters of interactions between MAO-A and coffee isolates.

Isolates after fractionation 10 mmol/L as chlorogenic acid	K_D ($\mu\text{mol/L}$)	$K_A \cdot 10^3$ (L/mol)	ΔH (kJ/mol)	ΔG (kJ/mol)	ΔS (J/mol $\cdot$ K)	IC_{50} ($\mu\text{mol/L}$) inhibitor concentrations: 1 $\mu\text{mol/L}$ MAO-A)	K_i ($\mu\text{mol/L}$) K_M 5-HT 0.28 mmol/L
<i>Green Robusta</i>							
Monochlorogenic acids	51.0 $\pm$ 0.03	19.7 $\pm$ 1.4	-175.8 $\pm$ 0.1 ^a	-50.7 $\pm$ 0.1	-404.1 $\pm$ 0.1	3.9 $\pm$ 0.0	12.7 $\pm$ 0.0
Dichlorogenic acids	73.1 $\pm$ 0.02	13.6 $\pm$ 1.2 ^b	-17.7 $\pm$ 0.0	-35.2 $\pm$ 0.1	56.6 $\pm$ 0.0	3.3 $\pm$ 0.0	75.7 $\pm$ 0.0
Caffeine	148.1 $\pm$ 1.45	6.8 $\pm$ 1.1 ^b	-0.7 $\pm$ 0.0	-17.4 $\pm$ 0.1	53.8 $\pm$ 0.0	3.0 $\pm$ 0.0	4.22 $\pm$ 0.0
<i>Light roasted Robusta</i>							
Monochlorogenic acids	119.2 $\pm$ 0.02 ^a	8.3 $\pm$ 2.1	-2.5 $\pm$ 0.1	-21.6 $\pm$ 0.1	61.6 $\pm$ 0.0	60.9 $\pm$ 0.5	<0.001
Dichlorogenic acids	116.0 $\pm$ 0.04	8.6 $\pm$ 1.1 ^b	-0.1 $\pm$ 0.0	-22.2 $\pm$ 0.0	71.3 $\pm$ 0.0	11.2 $\pm$ 0.1	63.0 $\pm$ 0.1
Caffeine	216 $\pm$ 0.03	4.6 $\pm$ 1.1 ^b	7.2 $\pm$ 0.1	-11.9 $\pm$ 0.0	-61.7 $\pm$ 0.0	29.9 $\pm$ 0.3	54.1 $\pm$ 0.0
<i>Dark roasted Robusta</i>							
Monochlorogenic acids	<0.0001	–	-110.9 $\pm$ 0.2 ^a	-17.8 $\pm$ 0.0	13.1 $\pm$ 0.1	7.4 $\pm$ 0.0	139.0 $\pm$ 0.1
Dichlorogenic acids	249 $\pm$ 0.01	4.0 $\pm$ 1.3 ^b	-19.6 $\pm$ 0.1	-10.3 $\pm$ 0.1	-29.8 $\pm$ 0.0	5.7 $\pm$ 0.0	134.0 $\pm$ 0.1
Caffeine	115.5 $\pm$ 0.02 ^a	8.6 $\pm$ 1.1 ^c	11.1 $\pm$ 0.0	-22.3 $\pm$ 0.1	107.8 $\pm$ 0.0	6.0 $\pm$ 0.0	29.2 $\pm$ 0.0
<i>Green Arabica</i>							
Monochlorogenic acids	103.0 $\pm$ 0.02	9.7 $\pm$ 1.0 ^b	1.1 $\pm$ 0.1	-25.0 $\pm$ 0.1	84.2 $\pm$ 0.0	27.2 $\pm$ 0.1	12.7 $\pm$ 0.0
Dichlorogenic acids	101.0 $\pm$ 0.02	9.9 $\pm$ 1.2 ^b	-0.9 $\pm$ 0.3	-33.2 $\pm$ 0.1	79.4 $\pm$ 0.0	11.5 $\pm$ 0.1	49.9 $\pm$ 0.0
Caffeine	77.0 $\pm$ 0.01	12.8 $\pm$ 1.0 ^c	1.3 $\pm$ 0.0	-17.38 $\pm$ 0.1	-112.1 $\pm$ 0.0	9.1 $\pm$ 0.0	60.7 $\pm$ 0.1
<i>Light roasted Arabica</i>							
Monochlorogenic acids	148.2 $\pm$ 0.04	6.7 $\pm$ 1.0	2.1 $\pm$ 0.04	-17.3 $\pm$ 0.0	62.8 $\pm$ 0.0	68.7 $\pm$ 0.2	117.0 $\pm$ 0.1
Dichlorogenic acids	153.0 $\pm$ 0.02	6.5 $\pm$ 1.0	0.2 $\pm$ 0.03	-16.8 $\pm$ 0.06	54.9 $\pm$ 0.1	7.7 $\pm$ 0.0	206.0 $\pm$ 0.1
Caffeine	112 $\pm$ 0.01 ^a	8.19 $\pm$ 1.1	2.1 $\pm$ 0.02	-21.1 $\pm$ 0.06	74.9 $\pm$ 0.0	10.9 $\pm$ 0.1	94.6 $\pm$ 0.0
<i>Dark roasted Arabica</i>							
Monochlorogenic acids	135.6 $\pm$ 0.03	7.3 $\pm$ 1.0	0.3 $\pm$ 0.0	-18.9 $\pm$ 0.08	62.2 $\pm$ 0.0	40.6 $\pm$ 0.3	10.1 $\pm$ 0.0
Dichlorogenic acids	170.2 $\pm$ 0.01 ^a	5.8 $\pm$ 1.1	0.9 $\pm$ 0.0	-15.1 $\pm$ 0.09	51.8 $\pm$ 0.0	8.7 $\pm$ 0.1	169 $\pm$ 0.1
Caffeine	192.0 $\pm$ 0.02 ^a	5.2 $\pm$ 1.2	0.2 $\pm$ 0.1	-13.4 $\pm$ 0.05	43.9 $\pm$ 0.1	13.5 $\pm$ 0.0	10.1 $\pm$ 0.0

Values are expressed as mean value $\pm$ SD; n = 9; different letters in one row correspond to significant differences (P < 0.05).**Table 5**

Parameters of interactions between MAO-A and coffee extracts.

Compound	K_D (mmol/L)	$K_A \cdot 10^3$ (L/mol)	ΔH (kJ/mol)	ΔG (kJ/mol)	ΔS (J/mol $\cdot$ K)	IC_{50} (1 $\mu\text{mol/L}$ inhibitor: 1 $\mu\text{mol/L}$ MAO-A)	K_i ($\mu\text{mol/L}$) K_M 5-HT 0.28 mmol/L
Green Arabica	0.4 $\pm$ 0.0	2.8 $\pm$ 0.1 ^b	-0.50 $\pm$ 0.0	-20.1 $\pm$ 0.1	63.4 $\pm$ 0.0	1.4 $\pm$ 0.0	11.8 $\pm$ 0.2
Light roasted Arabica	4.6 $\pm$ 0.3 ^a	0.2 $\pm$ 0.0	-0.24 $\pm$ 0.0	-13.8 $\pm$ 0.4	43.9 $\pm$ 0.0	1.2 $\pm$ 0.0	12.3 $\pm$ 0.5
Dark roasted Arabica	2.1 $\pm$ 0.0	0.4 $\pm$ 0.4 ^a	-0.16 $\pm$ 0.0	-15.8 $\pm$ 0.4 ^a	50.7 $\pm$ 0.1	1.2 $\pm$ 0.0	18.1 $\pm$ 0.4
Green Robusta	0.1 $\pm$ 0.3 ^a	11.1 $\pm$ 0.0	-0.39 $\pm$ 0.0	-23.7 $\pm$ 0.1 ^a	75.3 $\pm$ 0.0	1.5 $\pm$ 0.0	5.1 $\pm$ 0.2 ^c
Light roasted Robusta	0.8 $\pm$ 0.1	1.2 $\pm$ 0.1 ^b	-0.28 $\pm$ 0.0	-18.3 $\pm$ 0.5	58.4 $\pm$ 0.1	1.4 $\pm$ 0.0	12.8 $\pm$ 0.1
Dark roasted Robusta	1.6 $\pm$ 0.2 ^a	0.6 $\pm$ 0.0	-0.24 $\pm$ 0.0	-16.6 $\pm$ 0.4	52.7 $\pm$ 0.0	2.2 $\pm$ 0.1	15.3 $\pm$ 0.4 ^c

Values are expressed as mean value $\pm$ SD; n = 6; different letters or no index in one column correspond to significant differences (P < 0.05).

The green Robusta coffee extract showed the highest affinity for the enzyme, and a high inhibition activity resulting from a high content of polyphenols such as CHAs, which bind to the enzyme at the active site. Roasting the coffee lowered the concentrations of free polyphenols available, which lowered the affinity for the enzyme. Our study has shown that coffee, especially in the green form, is a potential antidepressant capable of inhibiting MAO-A and increasing the concentration

of 5-HT, which is also beneficial for decreasing sugar intake and prevention of type 2 diabetes development.

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CRediT authorship contribution statement

Joanna Grzelczyk: Conceptualization, Methodology, Formal analysis, Formal analysis, Writing - original draft, Project administration, Funding acquisition. **Grażyna Budryn:** Conceptualization, Methodology, Formal analysis, Writing - original draft, Funding acquisition. **Jorge Peña-García:** Formal analysis. **Dominik Sz wajgier:** Writing - review & editing. **Ilona Gałązka-Czarnecka:** Writing - review & editing. **Joanna Oracz:** Writing - review & editing. **Horacio Pérez-Sánchez:** Methodology, Formal analysis, Formal analysis.

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.

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