Supramolecular solvent extraction of bioactives from coffee cherry pulp

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Abstract

The potential of supramolecular solvents (SUPRAS) is investigated for the extraction of bioactive compounds from coffee cherry pulp, one of the major by-products generated in the coffee industry. SUPRAS made up of hexagonal inverted aggregates of octanoic acid in ethanol:water mixtures provided good extraction yields for bioactives (3.6±0.3 mg caffeine g^{-1} and 0.9±0.1 mg protocatechuic acid g^{-1}) at a low solvent:sample ratio of 4:1 v/w and under mild operations conditions (5 min extraction at room temperature). SUPRAS-based extraction was optimized and extracts were analyzed to identify the main phenolic and alkaloid compounds. A variety of bioactives were present and extracts showed high antioxidant capacity by different assays (45% for DPPH and 91% for ABTS). Extraction efficiencies with SUPRAS were clearly superior than those obtained with organic solvents commonly used for valorization of coffee residues.

Keywords: supramolecular solvents; coffee cherry pulp; bioactive compounds; valorization

1. Introduction

Coffee is the most popular beverage with a production of over 9 billion kg of beans per year and it is cultivated in around 70 countries (International Coffee Organization, 2017). Coffee berries contain the beans surrounded by different layers: first the silverskin, then the parchment, the mucilage, the pulp and finally the skin (Esquivel and Jiménez, 2012). During the dry process, coffee cherries are sun-dried and then they are dehusked to remove the skin, the pulp, the mucilage, the parchment and part of the silverskin (Esquivel and Jiménez, 2012). These by-products are known as coffee husks. In the wet and semi-wet processes, after separating ripened from unripe berries with water, fruits are de-pulped to remove the skin and the pulp. The by-product or waste that is generated at this step is known as coffee cherry pulp or coffee pulp (Murthy and Madhava Naidu, 2012; Pandey et al., 2000). In the wet process, the beans are further fermented to remove the mucilage and the remaining pulp, then dehusked and finally dried. After any of these three processes, the beans are roasted and generate as by-product the silverskin. Considering that each 100 kg of mature fruits are composed by around 39 kg of pulp, 22 kg of mucilage and 39 kg of parchment coffee, it is easily concluded that the amount of residues generated is extremely high (Alves et al. 2017). Finally, spent-coffee grounds are generated during the production of instant coffee and coffee brewing (Kovalcik et al. 2018).

In coffee producing countries, the unsafe disposal of the corresponding wastes has a negative impact on the environment due to their high concentration in caffeine, polyphenols and tannins and high acidity (Murthy and Naidu, 2012). The large-scale management of coffee waste is still challenging. A very attractive strategy is its valorization to obtain benefits as compost, fuel, animal feed, bio-solvents or bioactive compounds, among others. Bioactive compounds obtained from coffee by-products are mainly alkaloids, melanoidins and polyphenolic compounds that exert beneficial antioxidant, anti-bacterial or anti-fungal effects of interest for the food, pharmaceutical and cosmetic industries (Belščak-Cvitanović

and Komes, 2017; Esquivel and Jiménez, 2012; Galanakis, 2015; Janissen and Huynh, 2018; Rodrigues et al., 2017).

Extraction of bioactives from coffee by-products has been investigated using different solvents and techniques. Moderate polar solvents are usually employed, such as methanol, ethanol or isopropanol, sometimes mixed with water (up to 40% v/v) under typical sample to solvent ratios in the range 1:10-1:100 v/w. Supercritical fluids (Andrade et al., 2012), subcritical water (Getachew and Chun, 2017), and deep eutectic solvents (Yoo et al., 2018) have been also used. Techniques include conventional solid-liquid extraction (Mussatto et al., 2011; Zuorro and Lavecchia, 2012), Soxhlet extraction (Murthy et al., 2012), supercritical fluid extraction (SFE) with and without co-solvent (Andrade et al., 2012), ultrasound (USAE) (Andrade et al., 2012; Getachew and Chun, 2017; Yoo et al., 2018) and microwave assisted extraction (MAE) (Getachew and Chun, 2017; Pavlović et al., 2013). Spent coffee grounds have been the most investigated coffee waste for the extraction of bioactives (Kovalcik et al., 2018) and in a lesser extent coffee husks (Andrade et al., 2012) and coffee silverskin (Narita and Inouye, 2014). As mentioned above, coffee cherry pulp is one of the main by-products of the wet processing of coffee (~40% of the coffee is wet processed, Garde et al. 2017). However, it is still hardly investigated for the extraction of bioactives despite its good antioxidant properties (Heeger et al., 2017, Murthy et al., 2012).

In this study, we investigate the suitability of supramolecular solvents (SUPRAS) for the extraction of bioactives from coffee cherry pulp that was obtained by a wet process. SUPRAS are nanostructured liquids produced spontaneously from colloidal suspensions of amphiphiles by self-assembly processes (Ballesteros-Gómez et al., 2018; Caballo et al., 2017). SUPRAS production involves the application of an external stimuli (pH or temperature change, addition of salt or addition of a poor solvent for the amphiphile) to the colloidal suspension where the amphiphiles arrange as three-dimensional aggregates, which are usually normal or inverted micelles or vesicles (Ballesteros-Gómez et al., 2010). The application of an external stimulus diminishes the repulsion among the polar groups of the amphiphilic

molecules, which causes the growth of the aggregates that finally separate as a new liquid phase named coacervate or SUPRAS (Ballesteros-Gómez et al., 2018). The organized structures in the supramolecular phase are held together by intermolecular interactions, such as ion–ion, ion–dipole, dipole–dipole, hydrogen bonding, π–π and cation–π. Although these interactions are weaker than covalent bonds they can produce very stable assemblies and provide multiple biding forces for extraction, which makes them very efficient extractants (Caballo et al., 2017; Steed et al., 2007).

SUPRAS are tunable solvents since by changing the environmental conditions and/or the amphiphile functional group/s is possible to tailor their composition and structure (Ballesteros-Gómez et al., 2018). Thus, SUPRAS have been designed to exclude proteins and carbohydrates from extraction by chemical and physical mechanisms, respectively (Ballesteros-Gómez and Rubio, 2012). These versatile and efficient extraction materials have proved successful for the recovery of a variety of compounds for analytical purposes (e.g. PAHs, mycotoxins, perfluorinated compounds, drugs, dyes, etc.) (Ballesteros-Gómez et al., 2010; Caballo et al., 2017). However, their application to the extraction of bioactives from biomass or waste is still limited (Salatti-Dorado et al., 2019; Torres-Valenzuela et al, 2019).

Here, we investigate the suitability of SUPRAS produced by the addition of water to colloidal suspensions of decanoic or octanoic acid in ethanol (Ruiz et al., 2007) for the extraction of bioactives from coffee cherry pulp. SUPRAS components were selected from *Generally Recognized As Safe* (GRAS) chemicals in order to produce a green and biocompatible solvent for further application in the development of cosmetics, nutraceuticals or functional foods. SUPRAS extraction was optimized on the basis of the extraction yields for caffeine and protocatechuic acid, the two most abundant bioactives in this by-product (Heeger et al., 2017). SUPRAS extracts were further screened to elucidate the profile of phenolic and alkaloid compounds and to measure their antioxidant activity.

Materials and methods

2.1. Chemicals and solutions

The list of chemicals and solutions is provided in the Supplementary Material (SI).

2.2. Coffee cherry pulp

Coffee cherry pulp was obtained using the wet method by the mechanical peeling of ripe coffee fruits freshly harvested from an experimental lot located in Armenia City, Colombia (latitude 4°32'54'' north, longitude 75°39'54'' west and altitude 1500 MAS). Coffee cheery pulp was dried to reduce water activity and extend its shelf-life. The process was carried out at 60 ºC during 8 hours, up to reach around 9.5% of water content. Finally, sample size was reduced by using a coffee mill until obtaining a homogeneous powder (particle size <2 mm).

2.3. SUPRAS production and composition

A variety of SUPRAS were produced by dissolving decanoic or octanoic acid in ethanol and then adding water (pH ~3) to induce the growth of the aggregates of the amphiphiles. The volume of the ternary mixture, consisting of variable percentages of ethanol and water and 5% v/v of amphiphile, was 50 mL. The mixture was shaken on a vortex (Vorterex, Heathrow Scientific, Vernon Hills, IL, USA) for 1 min at 500 rpm and then centrifuged (Mixtasel BLT, Selecta, Cham, Suiza) for 5 min at 3,000 rpm. The SUPRAS separated as a new liquid top phase in equilibrium with the bulk solution. Then both phases (SUPRAS and equilibrium solution, EqS) were independently collected and stored in closed glass containers at room temperature until use (~20-25 °C, within one week). Figure 1.1 shows a schematic for SUPRAS production. The composition of each SUPRAS, which is dependent on the ethanol:water ratio in the synthetic solution, was determined. The concentration of amphiphile, water and ethanol in the SUPRAS was calculated as weight percent (w/w, %). Water content was determined by coloumetric Karl Fischer titration (KF 831 model, Methrom, Herisau, Switzerland) after proper dilution with methanol. The

amphiphile content was determined by weighting SUPRAS aliquots (~200 µL) before and after the evaporation of water and ethanol. Finally, the ethanol content in the SUPRAS was calculated by weight difference.

2.4. SUPRAS extraction

Figure 1.2 shows a schematic of the SUPRAS extraction procedure. Extractions were done in 2 mL-microtubes Safe-Lock (Eppendorf Iberica, Madrid, Spain) by mixing 200 mg of coffee cherry pulp and variable volumes of the different types of SUPRAS (and corresponding EqS) that were previously produced as explained in section 2.3. The mixtures were vortex-shaken at 2,990 rpm for 5 minutes and then centrifuged for 10 minutes at 10,000 rpm. A sequential design was used to determine significant variables affecting the extraction yields of caffeine and protocatechuic acid, the two bioactives chosen as models for valorization of coffee cherry pulp .Variables were optimized by varying each factor at a time because of the different composition of the SUPRAS that were investigated, which can be considered as different solvents. First, we investigated the effect of the amphiphile (decanoic acid or octanoic acid) and of the ethanol concentration employed for SUPRAS formation. Conditions giving the maximum yield for both target compounds were selected as optimal. Then we evaluted the effect of the ratios EqS:SUPRAS v/v and sample:extraction solvent w/v. All the experiments were carried out at room temperature. For each experiment, results were presented as mean±standard deviation for three individual extractions. Statistical comparisons were performed with Minitab software Ver. 18 (Minitab Inc, State College, Pennsylvania, USA) using one-way analysis of variance (ANOVA) and Tukey's tests (p-value < 0.05).

2.5. Analysis of bioactive compounds by LC-MS/MS

Caffeine, protocatechuic acid, 5-chlorogenic acid, gallic acid and caffeic acid were quantified by LC-MS/MS in diluted SUPRAS extracts (dilution with methanol 1:5-1:100 v/v). Calibration curves were prepared in methanol (0.1-20 mg L^{-1}). Other common bioactives found in the extracts were tentatively identified based on characteristic transitions (Table S1) and semi-quantified against 5-chlorogenic acid

(due to the lack of authentic standards): rutin, 5-O-feruloylquinic acid, 3-O-coumaroylquinic acid, p-coumaric acid, caffeic acid,n-O-dicaffeoyl quinic acids (three isomers), 4-chlorogenic acid , 3-chlorogenic acid and 4-O-feruloylquinic acid. Quantification and target screening of bioactives was carried out by LC-MS/MS (conditions specified in SI).

2.6. Antioxidant activity assays

SUPRAS extracts obtained under optimal conditions (200 mg of coffee peel, 670 µL of SUPRAS and 130 µL of EqS) were tested for the evaluation of the antioxidant activity by the DPPH and ABTS assays. The inhibition percentage (or antioxidant capacity) was calculated (Régnier et al., 2015). For the DPPH assay, SUPRAS (blanks or extracts diluted 1:0 to 1:1000 v/v in methanol) were mixed with 250 µL of DPPH solution. The absorbance of the mixture was measured at 517 nm at 20 second-intervals during 20 min in a multilabel plate reader Victor 3 1420 (Perkin Elmer, Waltham, Massachusetts, USA). For the Trolox equivalent (TE) antioxidant capacity assay, the $ABTS^{\bullet+}$ radical cation was produced as described in SI. The assays were run by mixing 66 µL of Trolox (0, 10, 30, 50 and 70 µM diluted in methanol) or of SUPRAS (blanks or extracts diluted 1:0 at 1:1000 v/v in methanol) with 154 µL of $ABTS^{\bullet+}$ solution. The absorbance of the mixture was measured at 732 nm at 40 second-intervals for 60 min and the percentage of inhibition expressed as TEAC (Apak et al., 2013).

2. Results and discussion

3.1. SUPRAS production and composition

SUPRAS of different composition were prepared from ternary mixtures of octanoic or decanoic acid, ethanol and water. Alkyl carboxylic acids have been previously reported to produce SUPRAS in hydro-organic media (Ruiz et al., 2007), being tetrahydrofuran:water the mixture more used for analytical purposes (Ballesteros-Gómez et al., 2018). These amphiphiles give inverted micelles in water-miscible organic solvents (e.g. THF, acetone, dioxane, propanol, butanol, acetonitrile, etc.) and the addition of

200 water (a “poor solvent” for the amphiphile) triggers the assembly of the aggregates into the SUPRAS, a
201 new highly packed phase with an inverted hexagonal arrangement (Figure 1.1). In this structure, the
202 carboxylic groups surround aqueous cavities while the hydrocarbon chains are dispersed in the organic
203 solvent. The SUPRAS is in equilibrium with a hydro-organic solution (EqS) containing the amphiphile at
204 the critical aggregation concentration. Both the SUPRAS and the EqS are immiscible. In this paper, a
205 mixture of ethanol:water was selected for the production of SUPRAS. Ethanol was selected against THF
206 because of its lower toxicity. Indeed, ethanol, decanoic and octanoic acid are authorized food ingredients.
207 These carboxylic acids were selected because they usually provide better extraction yields and they also
208 form SUPRAS in a wider range of conditions than acids with higher and lower hydrocarbon chain length
209 (Ballesteros-Gómez et al., 2018).
210
211 Table 1 shows the composition of the SUPRAS that were produced by mixing octanoic or decanoic acid
212 (5% v/v) with variable volume ratios of ethanol and water. These ratios were determined by the minimal
213 and the maximal percentage of organic solvent required to form decanoic and octanic acid-based
214 SUPRAS (Ruiz et al., 2007). The volume percentages of ethanol varied in the intervals 9.5-33 % and 19-
215 38% for octanoic and decanoic acid, respectively. As shown in Table 1, the concentration of amphiphile
216 in the SUPRAS was very high. So, SUPRAS contained a huge number of binding sites for bioactives.
217 This should enable the efficient extraction of the target compounds even at low SUPRAS/coffee pulp
218 ratios.
219
220 The concentration of water and ethanol in the SUPRAS increased as the percentage of ethanol did in the
221 synthetic solution (r-Pearson = 0.95-0.98 and 0.990-0.995, for water and ethanol with octanoic and
222 decanoic acid, respectively), while the concentration of amphiphile decreased accordingly (r-Pearson = -
223 0.95-(-0.98) and -0.986-(-0.995) with octanoic and decanoic acid, respectively). So, these SUPRAS can
224 be considered as environment-responsive (Ballesteros-Gómez and Rubio, 2012). Accordingly, SUPRAS
225 composition can be tailored by adequate selection of the composition of the synthetic solution (Table 1).

Likewise, as water is progressively incorporated into the SUPRAS, the size of the aqueous cavities of the hexagonal aggregates increases and this behavior opens the door to the use of these SUPRAS as restricted access liquids (Ballesteros-Gómez and Rubio, 2012). Thus, exclusion of polar macromolecules (e.g. polysaccharides), takes place by size-exclusion mechanisms due to the small pores of the SUPRAS network. On the other hand, proteins precipitate owing to the decrease of the dielectric constant and the formation of macromolecular complexes with carboxylic acids. In this way, SUPRAS will extract bioactives and simultaneously exclude major matrix components in coffee cherry pulp.

3.2. SUPRAS-based extraction of bioactives from coffee pulp

Optimization was carried out following the procedure specified in section 2.4 (see also Figure 1.2) and considering the extraction efficiency obtained for caffeine and protocatechuic acid. These bioactives were expected to be solubilized in the SUPRAS by mixed-mode mechanisms, namely dispersion interactions with the hydrocarbon chains and hydrogen bonds with the carboxylic groups.

First, we investigated the influence of the composition of the eight synthesized SUPRAS (Table 1) on the extraction yields of the target bioactives. For this purpose, both the SUPRAS and EqS, which are immiscible, were added to the sample at a ratio of 1:1:2 sample:SUPRAS:EqS, g:mL:mL. The role of the EqS was to favor the wetting and dispersibility of the sample. Figure 2 shows the results expressed as a function of the percentage of ethanol used in the synthesis of the SUPRAS. Statistical operations including ANOVA table and results of Tukey's tests are shown in Tables S2 and S3 of Supplementary Information.The maximal extraction yields (i.e. around 1.6 mg/g caffeine and 0.39 mg/g protocatechuic acid) were not significantly different for the octanoic and decanoic acid. However, the optimal conditions were achieved at different synthetic conditions (at 33-38% and at 24% of ethanol for decanoic and octanoic acid, respectively).

Regarding the composition of these optimal SUPRAS, the decanoic acid-based SUPRAS had a higher ethanol (25.6-32.7%) and water (14.4-19%) contents compared to the octanoic acid-based SUPRAS (i.e. 21% of ethanol and 12.3% of water) (see Table 1). This different behavior can be qualitatively interpreted as follows: the two binding forces driving the extraction of the target bioactives (hydrogen bonding and dispersion) decrease and increase, respectively, with increasing length of the hydrocarbon chain (Burke, 1984). Short-chain carboxylic acids are better proton donors than longer carboxylic acids. So, considering the high polarity of the target bioactives (log K_{ow} -0.07 for caffeine and 0.86 for protocatechuic acid, source: DrugBank) and the number of hydrogen bonds acceptors (3 for caffeine and 4 for protocatechuic acid), it is reasonable to assume that extraction will be favored with SUPRAS made up of octanoic acid, because stronger hydrogen bonds can be established. In the case of SUPRAS synthesized from decanoic acid, more water and ethanol were necessary, particularly for caffeine, to increase hydrogen bonding. In fact, recoveries for the target bioactives were highly correlated with SUPRAS water content (r-Pearson 0.96 and 0.97 for caffeine and protocatechuic acid, respectively for decanoic acid-based SUPRAS). Overall, maximum extraction of bioactives at the lowest consumption of ethanol (cost-benefit ratio taking into account that similar prices are expected for food grade natural octanoic and decanoic acids), was found with SUPRAS made up from 5% v/v of octanoic acid, 24% v/v of ethanol and 71 % v/v of water. These conditions were selected as optimal for further studies.

Secondly, we optimized the influence of the ratio SUPRAS:EqS. For this aim, extractions were carried out with a total volume of 1.2 mL of SUPRAS+EqS and the content of the SUPRAS phase varied from 33 to 100%. Results are shown in Figure 3 and the corresponding statistical operations including ANOVA table and results of Tukey's tests are shown in Tables S4 and S5. The results clearly show that extraction yields increased as the SUPRAS phase did, especially for caffeine, a highly polar compound that partitioned between the SUPRAS and the EqS. Lower losses of protocatechuic acid were observed in the EqS due to its lower polarity. Maximum extraction efficiencies for both bioactives were

reached for extractant phases containing 83% and 100% of SUPRAS, so these phases were selected for further studies.

Next, we studied the sample:extractant phase ratios (mg:mL) in the range 1:3 to 1:6. For this purpose, the amount of sample was kept constant (i.e. 200 mg) and the volume of the extractant phase varied from 0.6 to 1.2 mL. As can be seen in Figure 4 (see also Tables S6 and S7 for Tukey tests), not significant differences were found in the extraction efficiencies for both bioactives in the whole interval for SUPRAS:EqS and in the interval 1:4.5-1:6 for SUPRAS. Since absolute values were slightly higher for SUPRAS:EqS in the interval 1:4-1:6, a ratio 1:4 was selected as optimal. Significantly higher solvent to sample ratios have been reported for the extraction of bioactives from solid coffee waste, many times using pretreatment, high temperature or energy-assisted techniques. For example standard solvent extraction procedures based on aqueous methanol or ethanol have been reported at ratios of 30-40 mL/g and carried out at room temperature or 50 °C (Mussato et al., 2011; Zuorro and Lavechia, 2011). Lower ratios (9-10 mL/g) were reported by Murthy et al. (2012) with Soxhlet extraction using aqueous isopropanol at 50 °C and pretreatment with viscozyme and by Pavlović et al. (2013) by microwave-assisted extraction. The use of deep eutectic solvents also required high ratios of 17-100 mL/g under ultrasonic assisted extraction at room temperature or at 80 °C (Mouratoglou et al, 2016; Yoo and Lee, 2018).

Extraction yields of 3.6±0.3 mg caffeine g^{-1} and 0.9±0.1 mg protocatechuic acid g^{-1} were obtained under optimal conditions, which are specified in Figure 1. These values were in agreement with those previously reported by Heeger et al. (2017). The content of caffeine and protocatechuic acid in coffee cherry pulp obtained by wet or semi-dry processes from six varieties varied from 3.4 to 6.8 mg g^{-1} and from ~0.2 to 3.1 mg g^{-1}, respectively.

3.3. *Comparison of the extraction efficiency of SUPRAS and conventional organic solvents for bioactives in coffee pulp*

The extraction efficiency of SUPRAS for caffeine and protocatechuic acid was compared with that obtained by organic solvents commonly used for extraction of bioactives from coffee residues (e.g. methanol, ethanol, acetone, and acetonitrile). Aqueous mixtures with polar solvents are also commonly employed for this aim.. However, since these mixtures limit the extraction of less polar bioactives (e.g. flavonoids), we preferred to do a comparison with pure polar solvents for wide scope extraction purposes. We employed the same extraction procedure for polar solvents than that optimized for SUPRAS (i.e. solvent:sample ratio of 4:1 v/w and experimental conditions as reported in Figure 1.2). SUPRAS extracted ~7-fold more caffeine (3.6±0.3 mg g^{-1}) than methanol (0.31 ± 0.05 mg g^{-1}). Values for ethanol (0.187 ± 0.004 mg g^{-1}), acetone (0.205 ± 0.009 mg g^{-1}) and acetonitrile (0.22 ± 0.02 mg g^{-1}) were even lower. Results for protocatechuic acid were also better for SUPRAS that extracted this bioactive ~11-fold more efficiently (0.9±0.1 mg g^{-1}) than methanol (0.12± 0.07 mg g^{-1}). Ethanol (0.055 ± 0.009 mg g^{-1}), acetone (0.041± 0.006 mg g^{-1}) and acetonitrile (0.0272± 0.0007 mg g^{-1}) were even less efficient. Results for other phenolic compounds identified in the following section are shown in Figure S1. The multiple binding sites and adequate balance of hydrogen bonds and dispersion interactions provided by SUPRAS could be the reason for this higher extraction efficiency.

3.4. *Phenolic compounds and alkaloids profile in SUPRAS extracts*

SUPRAS extracts obtained under optimal conditions were analyzed by LC-MS/MS to investigate their content in phenolic compounds and alkaloids. The most abundant phenolic compounds were protocatechuic acid (0.9±0.1 mg g^{-1}), gallic acid (0.25±0.02 mg g^{-1}) and 5-chlorogenic acid (0.13±0.01 mg g^{-1}). Other phenolic compounds were present at significantly lower levels: rutin (30.5±2 µg g^{-1}), 5-O-feruloylquinic acid (17.3±0.5 µg g^{-1}), 3-O-coumaroylquinic acid (13.4±0.4 µg g^{-1}), p-coumaric acid (4.5±0.1 µg g^{-1}), caffeic acid (2±0.2 µg g^{-1}), n-O-dicaffeoyl quinic acids (1.6±0.1 µg g^{-1}, sum of three

isomers), 4-chlorogenic acid (1.1±0.05 µg g^{-1}), 3-chlorogenic acid (0.34±0.04 µg g-1) and 3-O-feruloylquinic acid (0.4±0.03 µg g^{-1}). The isomeric form of each class of compound was assigned on the basis of relative retention times and main fragments according to (Angelino et al., 2018). Figure 5 (LC-MS/MS chromatogram) shows the most abundant phenolic compounds in SUPRAS extracts. Regarding alkaloids, caffeine (3.6±0.3 mg g^{-1}) was the major compound followed by trigonelline which was detected with about 15-fold lower abundance.

Samples were also extracted with conventional solvents (i.e. methanol, ethanol, acetone and acetonitrile) under the same experimental conditions that those used for SUPRAS and the extracts analyzed by LC-MS/MS to quantify the most abundant phenolic compounds (protocatechuic acid, gallic acid, 5-CGA and rutin). Figure S1 shows the results obtained, which prove the higher efficiency of SUPRAS compared to the most used organic solvents.

Levels of 5-chlorogenic acid in *Castillo* variety were lower than those reported from other varieties cultivated in South America (*Caturra*, *Catuai*, *Maragogype* from Honduras and *Bourbon* from El Salvador, 0.7-0.9 mg g^{-1}, Heeger et al., 2017). The same study reported gallic acid and rutin at levels below 100 µg g^{-1} in these varieties. A higher value of 0.73 mg g^{-1} of gallic acid was reported for a *Bourbon* variety cultivated in Congo.

3.5 Antioxidant activity of SUPRAS extracts

Phenolic compounds are the main contributors to the antioxidant activity of coffee and by-products (Belščak-Cvitanović et al., 2017). The antioxidant activity of SUPRAS blanks was negligible under any dilution or experimental condition. The antioxidant activity of SUPRAS extracts was measured by the DPPH and ABTS assays. For this purpose diluted SUPRAS with concentrations in the ranges 0.14-36 g L^{-1} and 0.3-287 g L^{-1} for DPPH and ABTS assays, respectively, were tested. Linearity for antioxidant activity versus SUPRAS concentration was observed up to 15 g SUPRAS L^{-1} and 25 g SUPRAS L^{-1} in the

DPPH and ABTS assays, respectively. At these concentrations, the DPPH and ABTS antioxidant capacity were 45±1% and 91±4%. The Trolox equivalent (TE) antioxidant capacity at 25 g SUPRAS L^{-1} was 334 µM.

The DPPH antioxidant capacity of coffee pulp extracts obtained under different extraction conditions, (which are hardly comparable) has been previously reported (Murthy and Naidu, 2012; Silva et al., 2013). Thus, DPPH activity of 65% has been found for extracts (0.5 g L^{-1}) obtained by Soxhlet extraction of 100 g sample with isopropanol:water 60:40 v/v at a solvent to sample ratio 10:1 v/w followed by evaporation to dryness (total yield 18.1%) (Murthy and Naidu, 2012). A similar DPPH antioxidant capacity (65.3%) was measured in coffee pulp extracts (20 g L^{-1}) obtained by extracting the samples by three consecutive times with ethanol (sample:solvent ratio 1:10 w/v) and evaporating the extracts to dryness (Silva et al., 2013). On the other hand, the values here obtained by the ABTS assay (corresponding to 40 µmol TE g^{-1} coffee pulp) were in accordance with those reported reported by Heeger et al. (2017) in the range 51-64.9 µmol TE g^{-1} coffee pulp (in weight, ~10% humidity) with different varieties of coffee cultivated in South America.

4. Conclusions

SUPRAS provides an efficient alternative extraction approach for the isolation of bioactive compounds from coffee cherry pulp, a less investigated coffee by-product than coffee husks, coffee silverskin or spent coffee grounds, but of major importance in the wet processing of coffee. SUPRAS extracts, rich in caffeine and polyphenols, can be of interest for the development of nutraceuticals, functional food or cosmetics. The extraction approach is simple (it is carried out at room temperature and atmospheric pressure in a single step and without external energy, such as ultrasound- or microwave-assisted extraction). While drying and grinding are common industrial steps, vortexing and centrifugation can be replaced by a more gentle mixing approach during longer extraction times (to keep the extraction efficiency rate) followed by decantation and filtration. Further purification steps for

concentration/separation of bioactives and for recovery and reuse of SUPRAS components are also probably required for industrial applicability. For this aim, different strategies could be employed, such as evaporation and/or freeze-drying for water and ethanol removal, back-extraction of bioactives with a poor solvent for the amphiphile (aqueous polar solvent mixture), dry fractionation for removal of octanoic acid and lipids or anionic exchange resins to retain octanoate after changing the pH to ~7. Nevertheless, the presence of octanoic acid in the final commercialized products should not be problematic since it is a food authorized ingredient and also can benefit the stability of bioactives For further implementation at industrial scale, the benefits of the amphiphile-rich extracts for enhancing the stability and bio-availability of bioactives will be addressed in the future.

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Figure captions

Figure 1. Schematic picture of the SUPRAS production and the extraction of coffee cherry pulp.

Figure 2. Extraction yields of caffeine and protocatechuic acid as mean ± standard deviation (*n*=3). Significant differences are indicated by different letters on the top of the bars (Tukey tests). Extraction of coffee cherry pulp (200 mg) with SUPRAS (400 µL) and corresponding EqS (800 µL) produced by mixing decanoic or octanoic acid in ethanol:water mixtures according to the conditions specified in Table 1. Percentages of ethanol on the X-axis represent the concentrations of this solvent in the synthetic solution.

Figure 3. Extraction yields of caffeine and protocatechuic acid as mean ± standard deviation (*n*=3). Significant differences are indicated by different letters on the top of the bars (Tukey tests). Extraction of coffee cherry pulp (200 mg) using an extractant phase consisting in SUPRAS + EqS. The content of the SUPRAS phase varied from 33 to 100% and the volume of the extractant phase was 1.2 mL. The ratio solvent to sample was 6:1 v/w. SUPRAS synthesis conditions: octanoic acid 5% v/v, ethanol 24 % v/v and water 71% v/v.

Figure 4. Extraction yields of caffeine and protocatechuic acid as mean ± standard deviation (*n*=3). Significant differences are indicated by different letters on the top of the bars (Tukey tests). Extraction of

coffee cherry pulp (200 mg) with SUPRAS or SUPRAS:EqS 88:17 v/v and different solvent to sample ratios 3:1-6:1 v/w. SUPRAS synthesis conditions: octanoic acid 5% v/v, ethanol 24 % v/v and water 71% v/v.

Figure 5. LC-(ESI-)MS/MS extracted ion chromatograms for the most abundant phenolic compounds in optimal SUPRAS extracts. Abbreviations: 5-CQA (5-O-Caffeoylquinic acid); 3-CouQA (3-O-Coumaroylquinic acid); 5-FQA (5-O-Feruloylquinic acid); n-DQA (n-O-Dicaffeoylquinic acids).

Figure 1

Fig.1

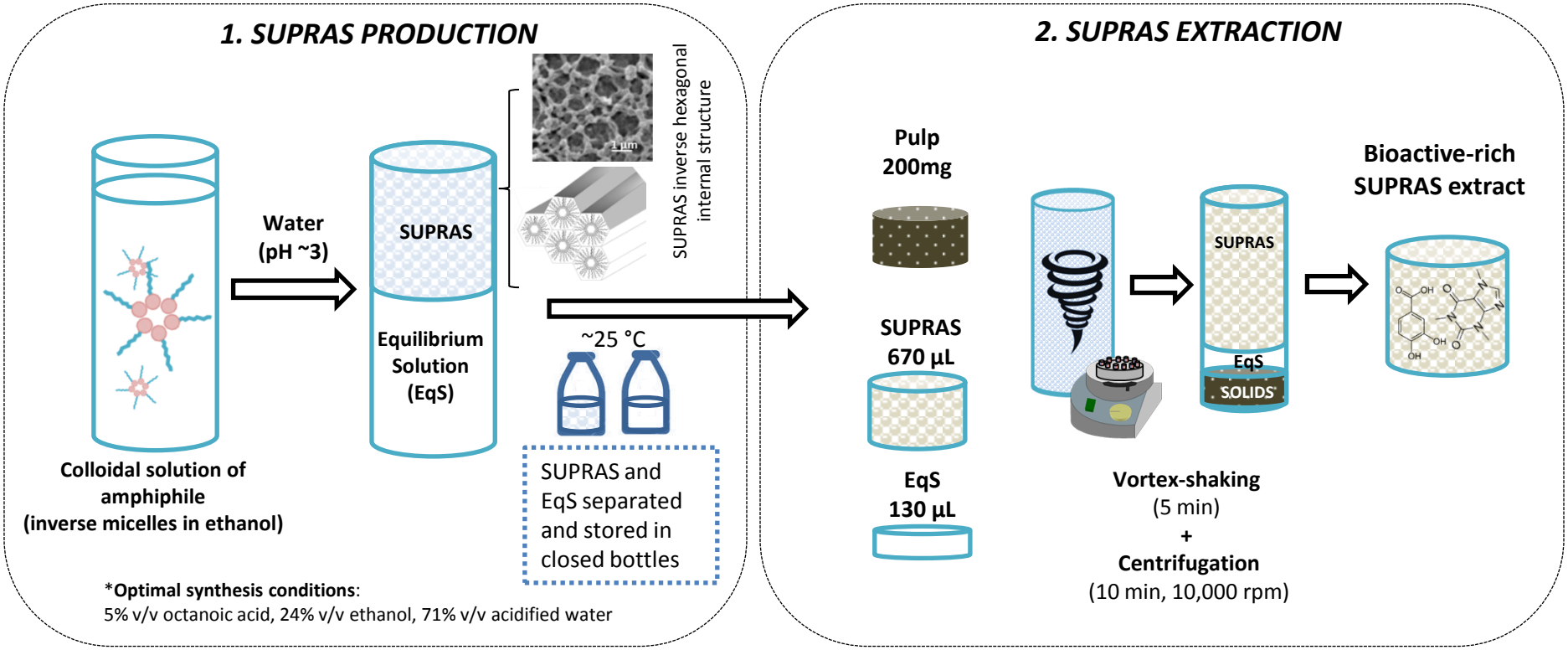
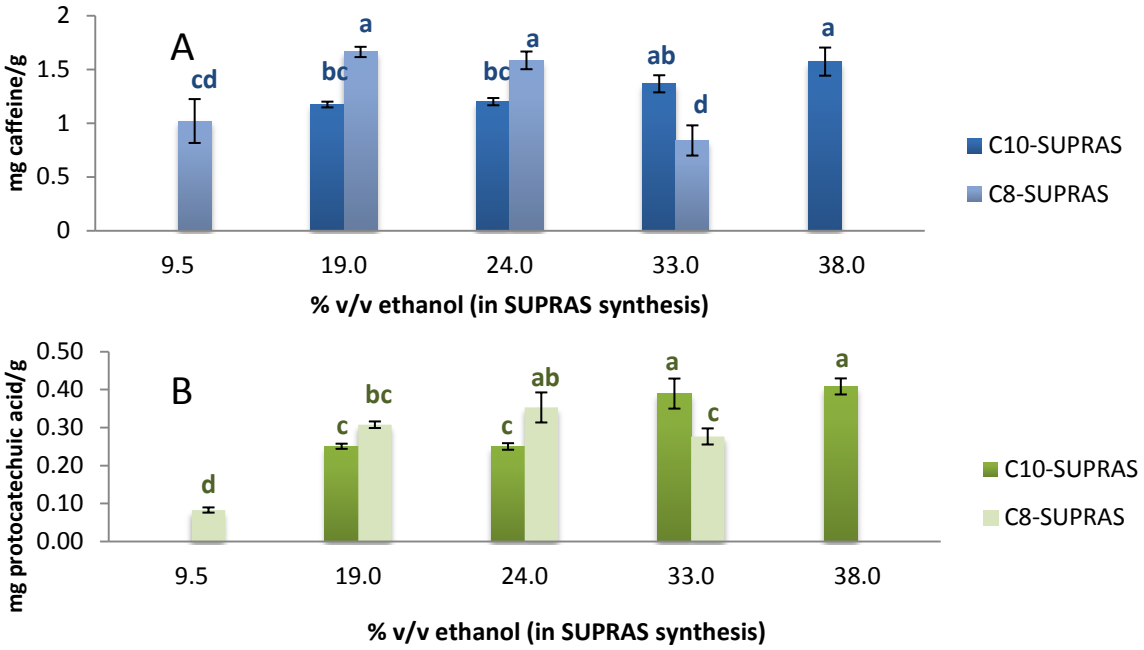


Figure 2-4



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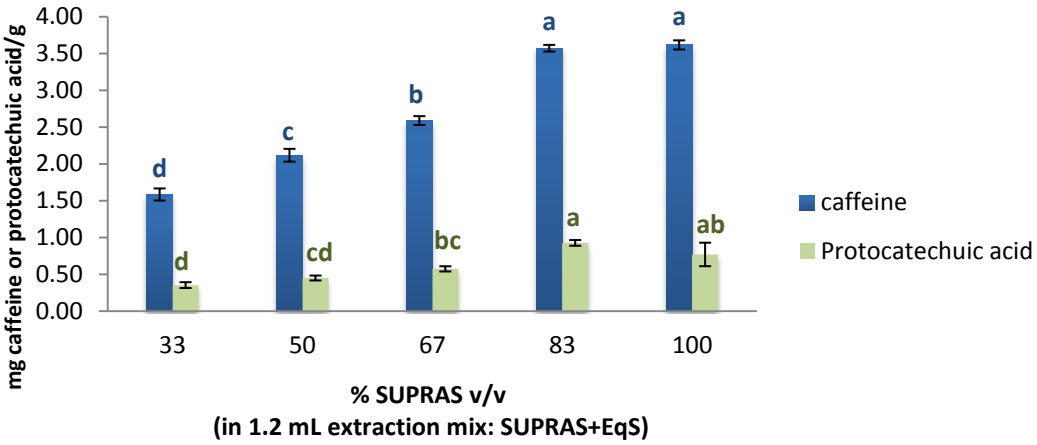
4.00
3.50
3.00
2.50
2.00
1.50
1.00
0.50
0.00
mg caffeine or protocatechuic acid/g
d
d
c
cd
b
bc
a
a
a
ab
33
50
67
83
100
% SUPRAS v/v
(in 1.2 mL extraction mix: SUPRAS+EqS)
caffeine
Protocatechuic acid

Figure 3

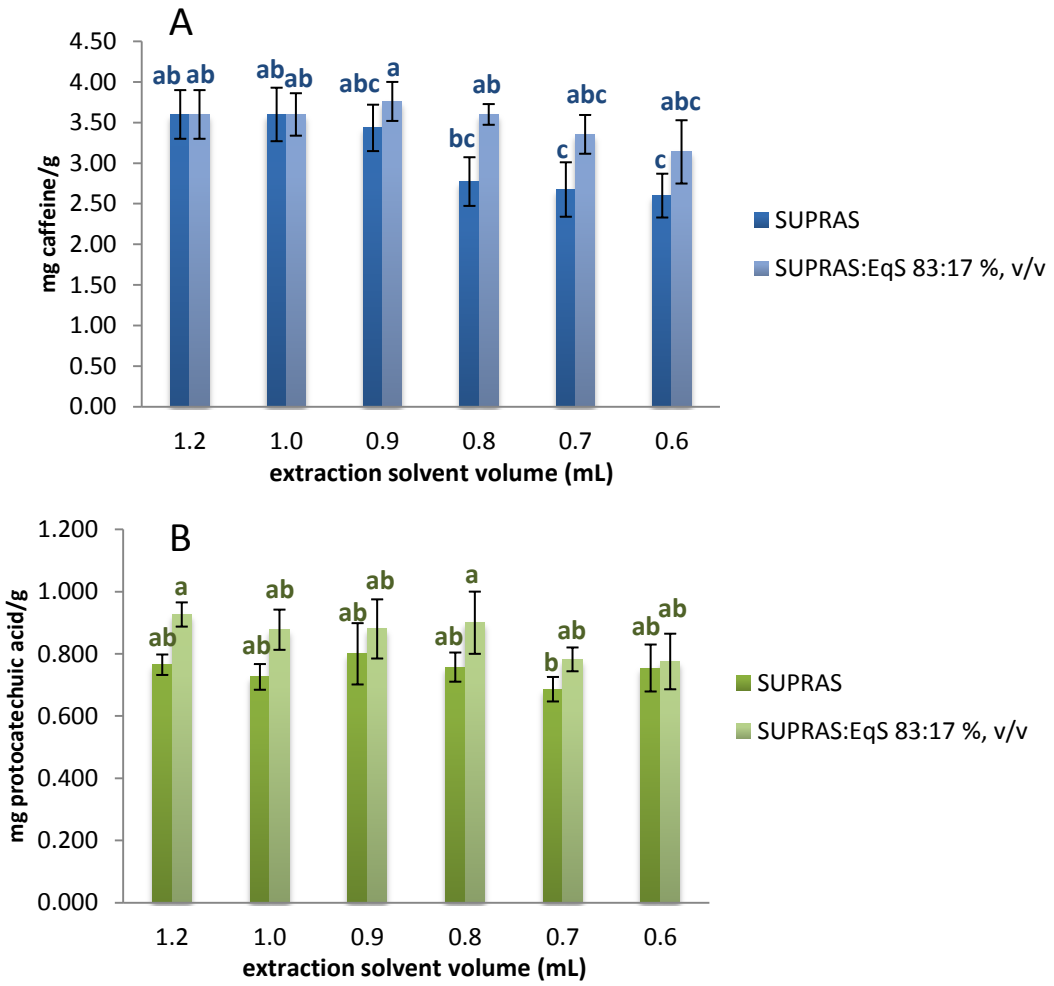
A
4.50
4.00
3.50
3.00
2.50
2.00
1.50
1.00
0.50
0.00
mg caffeine/g
ab ab
ab ab
abc a
bc ab
c abc
c abc
1.2
1.0
0.9
0.8
0.7
0.6
extraction solvent volume (mL)
SUPRAS
SUPRAS:EqS 83:17 %, v/v
B
1.200
1.000
0.800
0.600
0.400
0.200
0.000
mg protocatechuic acid/g
ab a
ab ab
ab ab
ab a
b ab
ab ab
1.2
1.0
0.9
0.8
0.7
0.6
extraction solvent volume (mL)
SUPRAS
SUPRAS:EqS 83:17 %, v/v

Figure 4

Figure 5

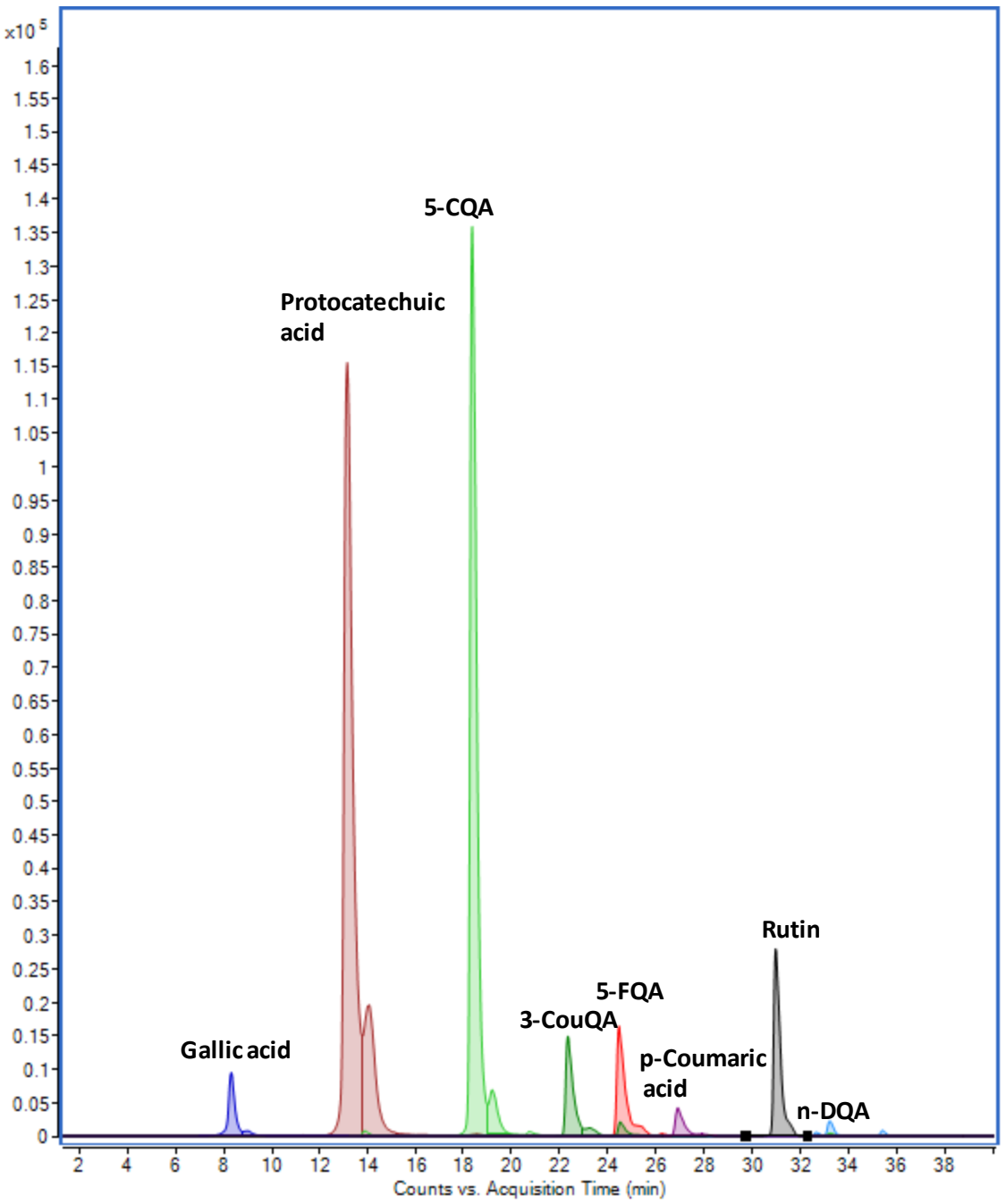

Figure 5

Table 1

Chemical composition of SUPRAS obtained from different ternary mixtures of amphiphile:ethanol:water (%, v/v). The optimal SUPRAS is shown in bold.

	Chemical composition of the synthetic solution (%, v/v)			**SUPRAS**		
				Chemical composition (%, v/v)		
	Amphiphile	**Ethanol**	**Water**	**Amphiphile**	**Ethanol**	**Water**
Octanoic acid	5	9.5	85.5	89 ± 1	5 ± 1	5.5 ± 0.3
	5	19	76	76 ± 1	14.7 ± 0.2	9.7 ± 0.4
	5	**24**	**71**	**67 ± 1**	**21 ± 1**	**12.3 ± 0.3**
	5	33	62	45 ± 2	32 ± 2	23.2 ± 0.4
Decanoic acid	5	19	76	83 ± 1	11 ± 1	6.05 ± 0.07
	5	24	71	75.7± 0.2	16.1 ± 0.1	8.1 ± 0.1
	5	33	62	59.9 ± 0.5	25.6 ± 0.5	14.4 ± 0.2
	5	38	57	48.2 ± 0.6	32.7 ± 0.7	19 ±1

***Credit Author Statement**

Laura Sofía Torres-Valenzuela: Investigation, Resources, Writing - Original Draft. **Ana Ballesteros-Gómez**: Conceptualization, Formal Analysis, Writing - Review & Editing, Supervision. **Soledad Rubio**: Writing - Review & Editing, Supervision, Funding acquisition.