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Supercritical anti-solvent fractionation for improving antioxidant and anti-inflammatory activities of an *Achillea millefolium* L. extract

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ABSTRACT

Achillea millefolium L. is a plant widely used in traditional medicine. Nowadays, there is a growing concern about the study of its bioactive properties in order to develop food and nutraceutical formulations. Supercritical anti-solvent fractionation (SAF) of an *A. millefollium* extract was carried out to improve its antioxidant and anti-inflammatory activities. A selective precipitation of phenolic compounds was achieved in the precipitation vessel fractions, which presented an antioxidant activity twice than original extract, especially when fractionation was carried out at 10 MPa. The main phenolic components identified in this fraction were luteolin-7-*O*-glucoside, 3,5-dicaffeoylquinic acid, 6-hidroxyluteolin-7-*O*-glucoside and apigenin-7-*O*-glucoside. However, separator fractions presented higher anti-inflammatory activity than precipitation vessel ones, particularly at 15 MPa. This fact could be related to separator fractions enrichment in anti-inflammatory compounds, mainly camphor, artemisia ketone and borneol.

Therefore, SAF produced a concentration of antioxidant and anti-inflammatory compounds that could be used as high-added valued ingredients.

1. Introduction

Achillea millefolium L. (yarrow) is a flowering plant widely used in folk medicine in Europe. Aqueous and alcoholic extracts from dried upper parts of yarrow have been employed in the treatment of digestive problems, hepato-biliary disorders and externally, for the treatment of skin and mucous membrane inflammation (Dias et al., 2013). The study of this plant, both its composition and biological activities, has awakened a growing interest in order to develop pharmaceutical, food and nutraceutical products. Vitas, Cvetanović, MašKović, Švarc-Gajić, and Malbaša (2018) produced kombucha beverages from a yarrow infusion and extracts. In addition, nowadays there are on the market several herbal tea mixtures (containing yarrow), mainly indicated for digestive problems.

Certain naturally occurring bioactive compounds present in *A. millefolium*, such as phenolic compounds, particularly chlorogenic and dicaffeoylquinic acids (DCQA) and flavonoids, as well as those belonging to the volatile oil fraction have been associated with health benefits (Mohammadhosseini, Sarker, & Akbarzadeh, 2017). Moreover, recent reports indicated that *Achillea* genus presents an important antioxidant activity, related to its flavonoids and total phenolic content (Giorgi, Mingozzi, Madeo, Speranza, & Cocucci, 2009; Mohammadhosseini et al., 2017). In addition, Trumbeckaite et al. (2011) reported that the radical-scavenging properties of a hydroalcoholic extract of *A. millefolium* were related to the presence of luteolin and chlorogenic acid in the extract, an in a lesser extent, to the presence of rutin and luteolin-7-*O*-glucoside. *A. millefolium* extracts have also been reported to present anti-inflammatory activity (Tadíc et al., 2017). Moreover, Kazemi (2015) showed that an *A. millefolium* essential oil, with high quantities of thymol and borneol, was able to inhibit nitric oxide production in macrophages stimulated with LPS (lipopolysaccharide).

Different approaches have been carried out in order to obtain fractions with high concentrations of phenolic compounds or essential oils components than original plants extracts; such as applying anion exchange resins (Kammerer, Boschet, Kammerer, & Carle, 2011), high pressure techniques (Fernández-Ponce, Casas, Mantell, & de la Ossa, 2015), membrane separation (Cissé, Vaillant, Pallet, & Dornier, 2011), supercritical fluid extraction with fractionation (Reverchon & de Marco, 2006) or chromatography methods (Pedan, Fischer, & Rohn, 2016;

Abbreviations: SAF, Supercritical anti-solvent fractionation; DCQA, Dicaffeoylquinic acid; SC-CO_2, Supercritical CO_2; UAE, Ultrasound-Assisted extraction; GAE, Gallic acid equivalents; THP-1/M, Human THP-1 monocytes differentiated to macrophages)

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Shaheen, Lu, Geng, Shao, & Wei, 2017). Recently, supercritical antisolvent fractionation (SAF) has been proposed for the fractionation of complex plants extracts. In addition, the use of carbon dioxide as a supercritical fluid offers many advantages, such as the low critical temperature of CO_2 and the absence of oxygen during extraction, which allows minimize or avoid the degradation of solutes, as well as the possibility of recovering a free-solvent fraction (Wijngaard, Hossain, Rai, & Brunton, 2012). In the SAF process, a polar liquid solution of a plant extract, containing several families of compounds, is sprayed continuously in a co-current with supercritical CO_2 (SC-CO_2), which acts as antisolvent. This contact allows the precipitation of more polar components from the liquid solution, insoluble in SC-CO_2, whereas the remaining compounds, that are mainly less polar components, remained dissolved and are recovered by downstream pressure reduction (Meneses, Caputo, Scognamiglio, Revenchon, & Adami, 2015).

For that matter, SAF technique has recently used to fractionate phenolic compounds from plants extracts. Therefore, Natolino, Da Porto, Rodríguez-Rojo, Moreno, and Cocero (2016) used this technique to obtain fractions enriched in polyphenols from a grape marc extract. Operating at 12 MPa, 45 °C and 0.99 CO_2 molar fraction, they obtained fractions with a relative enrichment of 350% of total polyphenols and a proanthocyanidins enrichment between 300 and 450%. Visentín, Cismondi, and Maestri (2011) also applied the SAF to improve carnosic acid (CA) recovery from an ethanolic extract of rosemary leaves, obtaining two different fractions, one insoluble with low concentration in CA (< 5%) and another resinous extract with 33% of CA. Moreover, Villanueva et al. (2015) carried out the fractionation of green tea extracts obtaining decaffeinated fractions with high concentration in catechins.

Nevertheless, there are only few studies relating the enrichment in phenolic compounds or essential oil components of the fractions obtained by this technique, with their biological activities. Chinnarasu et al. (2015) obtained precipitates with high antioxidant activity from the fractionation of olive leaves extracts and Marqués, Porta, Reverchon, Renuncio, and Mainar (2013) concentrated antioxidants from a defatted grape seed waste extract, achieving concentrations up to 2.7 higher than those in the starting solution. Sánchez-Camargo et al. (2016) also enhanced the antiproliferative activity of a rosemary fraction enriched in carnosic acid and carnosol.

In this context, and based on a previous work (Villanueva-Bermejo et al., 2017), the aim of the present study was to improve the antioxidant and anti-inflammatory activities of an *A. millefollium* extract using SAF technology. Besides, the original extract and fractions obtained were analyzed in order to relate its chemical composition with the biological activities found.

2. Materials and methods

2.1. Reagents and chemicals

Ethanol (99.5% purity) and Folin-Ciocalteu's reagent were obtained from Panreac (Barcelona, Spain). (±)-6-Hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox), 2,2′-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), gallic acid for titration (> 97.5%), potassium persulfate (99.9%) and thiazolyl blue tetrazolium bromide (MTT) were purchased from Sigma Aldrich (Madrid, Spain). Formic acid (99%) was obtained from Acros Organics (Madrid, Spain) and acetonitrile HPLC grade from Macron Fine Chemicals (Madrid, Spain). Reference standards for phenolic compounds, such as chlorogenic acid, cryptochlorogenic acid, diosmetin, ferulic acid, neochlorogenic acid, rosmarinic acid and vitexin (all analytical standard or HPLC purity ≥95%) were purchased from Sigma Aldrich (Madrid, Spain). 1,5-Dicaffeoylquinic acid (DCQA), 3,4-DCQA, 3,5-DCQA, 4,5-DCQA, apigenin, caftaric acid, casticin, luteolin, orientin, schaftoside and vicenin II were obtained from Phytolab (Madrid, Spain). Finally, amentoflavone, apigenin-7-*O*-glucoside, caffeic acid, homoorientin, luteolin-7-*O*-β-D-glucoside, quercetin and rutin were obtained from Extrasynthese S.A. (Genay, France) and luteolin-7-*O*-β-D-glucuronide from HWI Analytic GmbH (Rülzheim, Germany). The water used in this study was ultrapure type 1 (Millipore, Madrid, Spain). CO_2 (N38) was purchased from Carburos Metalicos (Madrid, Spain).

2.2. Yarrow samples and ultrasound-assisted extraction (UAE)

A. millefolium from Bulgaria was supplied by a local herbalist (Murcia, Spain). According to supplier specifications, the sample included inflorescences and upper dried leaves of the plant (harvested in spring) and sun-dried (water content < than 5% wt). The plant was ground using a Premill 250 hammer mill (Leal S.A., Granollers, Spain) and sieved (particle size < 500 μm). UAE plant extraction was carried out by using an ultrasonic device (Branson Digital Sonifier 250, Danbury, USA) with a power of 200 W and frequencies of 60 kHz. Extraction conditions employed were ethanol as extraction solvent (1:10 plant/solvent ratio), 30 min time, 40 °C temperature and an output of 70% with respect to the nominal amplitude. Finally, the extract was concentrated, until the final volume contained 17.9 mg/mL of total solid concentration (2.3% wt.) by rotary evaporation and stored at −20 °C until its use in the SAF process.

2.3. SAF process

A detailed explanation of the device and the process design employed can be found elsewhere (Villanueva-Bermejo et al., 2017). Briefly, fractionation of UAE yarrow solution (concentration of 17.9 mg/mL) was carried out at two different pressures (10 and 15 MPa), 40 °C and CO_2/extract flow ratio of 31.3 g/g (50 g/min for CO_2 and 1.6 g/min for UAE extract). The experiment started by pumping SC-CO_2 into the precipitation vessel until the pressure and temperature conditions were attained. Then, the UAE yarrow solution was pumped into the precipitator. After mixing, the yarrow extract components that were not soluble in SC-CO_2 + ethanol mixture precipitated in the precipitation vessel and were collected (precipitation vessel fraction). The fraction soluble in SC-CO_2 + ethanol went to separators where reduced pressure turned CO_2 into a gas and this fraction together with ethanol was also collected. The samples obtained in both separators were combined in a single fraction and ethanol removed by rotary evaporation under vacuum (separator fraction). Fractions were kept at −20 °C under darkness until analysis.

2.4. HPLC-PAD-ESI-QTOF-MS analysis

Phenolic compounds were analyzed by HPLC following the chromatographic method developed by Villalva et al. (2018). An Agilent HPLC 1260 Infinity series system (Agilent Technologies Inc., Santa Clara, CA, USA) was used for that purpose. Chromatographic separation was carried out by using a reverse phase ACE Excell 3 Super C18 column (150 mm × 4.6 mm, 3 μm particle size) from Advanced Chrmatography Technologies (Aberdeen, Scotland) protected by an ACE 3 C18-AR (10 mm × 3 mm) guard column. Dry samples were dissolved in ethanol to reach a 5 mg/mL concentration and filtered by 0.45 μm polyvinylidene fluoride (PVDF) filter before injection (20 μL). Retention time and UV–Vis spectrum of each chromatographic peak was compared with analytical standards for identification purpose; moreover, accurate mass from HPLC-ESI-QTOF-MS in negative mode analysis was used for compounds assignment.

Compounds quantification was carried out by using calibration curves from analytical standard, as previously described in Villalva et al. (2018). In addition, luteolin-6,8-di-*C*-glucoside, 6-hydroxyluteolin-7-*O*-glucoside and non-identified flavones were quantified by the calibration curve of orientin, luteolin-7-*O*-glucoside and luteolin respectively. Likewise, schaftoside and vicenin II calibration curves

were used for schaftoside isomer and apigenin-*C*-hexoside-*C*-pentoside quantification.

2.5. GC–MS analysis of the separator fractions

The analysis of the UAE extract and SAF fractions collected from the separator was carried out in an Agilent 7890A system (Agilent Technologies, Santa Clara, CA, USA). The unit comprised a split/splitless injector, a FID detector and a mass spectrometer detector (5975C triple-axis). The analysis was performed using an Agilent HP-5MS capillary column (30 m × 0.25 mm i.d., 0.25 μm phase thickness) and the following chromatographic method: 40 °C initial temperature, from 40 °C to 150 °C at 3 °C min^{-1}, isothermal at 150 °C for 10 min, then increased from 150 to 300 °C at 6 °C min^{-1} and finally isothermal at 300 °C for 1 min. Samples were dissolved in ethanol (at 5 mg/mL), filtered by 0.45 μm filters and inyected (1 μL) in splitless mode. Helium (99.99%) was employed as carrier gas (1 mL/min flow rate). The temperatures used were 250 °C for the injector and 230, 280 and 150 °C for the mass spectrometer ion source, interface and quadrupole, respectively. The mass spectrometer operated under electron impact mode (70 eV) and it was used in total ion current (TIC) mode (mass range from 40 to 500 *m/z*). The identification of compounds was performed by matching the mass spectral fragmentation patterns with the Wiley 229 mass spectral library, as well as comparing their corresponding retention index to those reported in the literature. Analyses were done in triplicate.

2.6. Determination of total phenolic content (TPC) and antioxidant activity

TPC determination was carried out according to Folin-Ciocalteau reagent method as described by Singleton, Orthofer, and Lamuela-Reventos (1999) using gallic acid as standard. In brief, 10 μL of samples (5 mg/mL for all samples, except for P10 and P15 which working solution was 3 mg/mL) were mixed with 50 μL of Folin-Ciocalteau reagent and 790 μL of deionized water. After 3 min, 150 μL of sodium carbonate solution (20% *w/v*) were added and mixed. After 2 h, the absorbance at 760 nm was recorded. The results were expressed as mg of gallic acid equivalents (GAE)/g dry sample. Analyses were performed at least in triplicate.

The antioxidant activity was measured using the $ABTS^{\bullet+}$ radical scavenging assay as described in Re et al. (1999). The reaction was placed with 990 μL of the diluted $ABTS^{\bullet+}$ radical solution and 10 μL of plant extract dilutions in order to achieve a 20% to 80% of radical inhibition (sample concentration varying from 5 mg/mL to 20 mg/mL). The reaction was allowed to stand until the absorbance reached a plateau, and the absorbance was recorded at 734 nm. Results were expressed as mmol Trolox equivalent/g dry sample (TEAC value). Analyses were done at least in triplicate.

2.7. Anti-inflammatory activity

Human THP-1 monocytes (ATCC, Manassas, VA, USA) were plated at a density of 5×10^5 cells/mL in 24 wells plates. Culture medium consisted in RPMI 1640 supplemented with 10% FBS, 100 U/mL penicillin, 100 mg/mL streptomycin, 2 mM L-glutamine (Gibco, Paisley, UK) and 0.05 mM β-mercaptoethanol (Sigma-Aldrich, Madrid, Spain) at 37 °C in 95% humidified air containing 5% CO_2. Monocytes differentiation to macrophages (THP-1/M cells) was induced by maintaining the cells with 100 ng/mL of phorbol 12-myristate 13-acetate (PMA) (Sigma-Aldrich, Madrid, Spain) for 48 h. THP-1/M cells viability, in presence of yarrow extract or fractions, was tested by MTT assay following Mosmann (1983) method. The assays were performed in triplicate.

After differentiation, THP-1/M cells were washed with PBS and incubated with 0.05 μg/mL of LPS from *E. coli* O55:B5 (Sigma-Aldrich, Spain) in presence of yarrow extract or fractions for 24 h. Then, the supernatants were collected and frozen at −20 °C. An anti-inflammatory drug, indomethacin (5 μg/mL), was used as a reference.

ELISA kits (BD Biosciences, Aalst, Belgium), according to manufacturer's instructions, were used to measure the release of TNF-α, IL-1β and IL-6 in the supernatants of THP-1/M cells. The quantification was carried out at 450 nm with substrate correction at 570 nm using a multiscanner autoreader (InfiniteM200 Tecan, Barcelona, Spain). The results were expressed as the mean of three determinations ± standard deviation.

2.8. Statistical analysis

Statistical analysis was performed using Statgraphics v. Centurion XVI package for Windows (Statpoint Inc., Warrenton, VA, USA). Statistical differences between samples were analyzed by one-way analysis of variance (ANOVA) and Fisher's least significant difference (LSD) procedure was applied to determine significant differences between means at $p \leq .05$.

3. Results and discussion

3.1. TPC content and antioxidant activity of yarrow extract and SAF fractions

UAE extracts from yarrow were carried out using ethanol as extraction solvent, at 40 °C and 30 min. Previous studies carried out in the research group (data not shown) showed that those conditions were the most appropriated to obtain extracts with a high content of TPC and an important antioxidant activity. Thus, this UAE extract (called original extract) presented a yield of 5.7 ± 0.9% (% dry wt.), a TPC of 54.30 ± 1.07 mg GAE/g extract and a TEAC value of 0.173 ± 0.004 mmol Trolox/g extract.

SAF process was carried out in order to enhance the antioxidant activity of this extract. Consequently, two different experiments, at 10 and 15 MPa of pressure, 40 °C and CO_2/extract flow ratio of 31.3 g/g, were developed. These conditions were based in a previous work (Villanueva-Bermejo et al., 2017).

Fractions obtained at 10 and 15 MPa from the precipitation vessel (called P10 and P15) and separators (called S10 and S15) were collected and its yield value, TPC content and antioxidant activity were determined (Table 1). Results indicated that SAF process achieved an important phenolic compounds enrichment in the precipitation vessel fractions. It is worth to mention that this enrichment was significantly higher when used pressure was 10 MPa, where P10 fraction presented an antioxidant activity 2-fold superior than the original extract. Regarding separator fractions, S10 and S15 showed a similar antioxidant activity and near 5-fold lower than original extract. This result was related to the lower quantity of TPC presented in these fractions.

Therefore, phenolic composition of the original extract and fractions

Table 1
Yield values (mass precipitated/mass of solid pumped), TPC content and antioxidant activity (TEAC value) in original extract and SAF fractions: P10 (precipitation vessel fraction at 10 MPa), P15 (precipitation vessel fraction at 15 MPa, S10 (separator fraction at 10 MPa) and S15 (separator fraction at 15 MPa). Data shown represents mean ± S.D. ($n = 3$).

Sample	Yield (%)	TPC (mg GAE/g)	TEAC value (mmol Trolox/g)
Original extract	5.7 ± 0.9	54.30 ± 1.07 ^{c}	0.173 ± 0.004 ^{c}
P-10	6.6 ± 1.1	136.44 ± 1.28 ^{a}	0.345 ± 0.002 ^{a}
S-10	52.1 ± 5.3	30.30 ± 0.25 ^{d}	0.035 ± 0.002 ^{d}
P-15	13.3 ± 2.4	119.20 ± 2.23 ^{b}	0.317 ± 0.003 ^{b}
S-15	42.6 ± 4.2	23.83 ± 0.85 ^{e}	0.033 ± 0.002 ^{d}

^{a,b,c,d,e}Different superscript letters denote statistical differences between samples within the same column. Significance level at $p \leq .05$ with Fisher's Least Significant Difference (LSD) test.

Table 2
Phenolic composition of original extract and SAF fractions (mg compound/g dry fraction). P10 (precipitation vessel fraction at 10 MPa), P15 (precipitation vessel fraction at 15 MPa), S10 (separator fraction at 10 MPa) and S15 (separator fraction at 15 MPa). Data shown represents mean ± S.D. (n = 3).

Compound	Original	P-10	S-10	P-15	S-15
Neochlorogenic acid	0.06 ± 0.00	0.18 ± 0.00$^{*}_{a}$	–	0.14 ± 0.00$^{}_{b}$	–
Caftaric acid	< L.Q.	0.18 ± 0.00$^{}_{b}$	–	0.22 ± 0.00$^{}_{a}$	–
Chlorogenic acid	0.62 ± 0.00	2.32 ± 0.0 $^{*}_{a}$	0.10 ± 0.00*	1.91 ± 0.04$^{}_{b}$	–
Cryptochlorogenic acid	0.01 ± 0.01	0.03 ± 0.00 $_{a}$	–	0.04 ± 0.01 $_{a}$	–
Vicenin II	0.37 ± 0.01	1.61 ± 0.00$^{*}_{a}$	–	1.12 ± 0.01$^{}_{b}$	–
Caffeic acid	0.17 ± 0.00	–	0.19 ± 0.00$^{*}_{A}$	–	0.18 ± 0.00$^{}_{B}$
Schaftoside isomer	0.26 ± 0.00	1.49 ± 0.05$^{*}_{a}$	–	0.90 ± 0.00$^{}_{b}$	–
Schaftoside	0.27 ± 0.00	1.36 ± 0.00$^{*}_{a}$	–	0.89 ± 0.00$^{}_{b}$	–
Homoorientin	0.02 ± 0.01	0.32 ± 0.00$^{*}_{a}$	–	0.16 ± 0.00$^{}_{b}$	–
Apigenin-*C*-hexoside –*C*-pentoside	0.30 ± 0.00	1.43 ± 0.01$^{*}_{a}$	–	0.85 ± 0.00$^{}_{b}$	–
Luteolin-6,8-di-*C*-glucoside	0.47 ± 0.00	2.39 ± 0.01$^{*}_{a}$	–	1.51 ± 0.00$^{}_{b}$	–
6-hidroxyluteolin-7-*O*-glucoside	1.45 ± 0.01	7.65 ± 0.02$^{*}_{a}$	0. 02 ± 0.00*	4.66 ± 0.00$^{}_{b}$	–
Rutin	0.51 ± 0.01	1.44 ± 0.00$^{*}_{a}$	–	1.34 ± 0.02$^{}_{b}$	–
Vitexin	0.13 ± 0.01	0.15 ± 0.00$^{*}_{b}$	–	0.25 ± 0.01$^{*}_{a}$	–
Luteolin-7-*O*-glucoside	7.69 ± 0.08	33.2 ± 0.07$^{*}_{a}$	0.39 ± 0.00*	23.9 ± 0.97$^{}_{b}$	–
Luteolin-7-*B*-glucuronide	0.20 ± 0.01	0.56 ± 0.00$^{*}_{a}$	–	0.59 ± 0.03$^{*}_{a}$	–
Ferulic acid	0.08 ± 0.02	0.09 ± 0.00$^{*}_{a}$	–	0.04 ± 0.00$^{}_{b}$	–
3,4-DCQA	0.38 ± 0.05	1.23 ± 0.00$^{*}_{a}$	–	0.69 ± 0.00$^{}_{b}$	–
1,5-DCQA	0.69 ± 0.01	2.86 ± 0.01$^{*}_{a}$	–	1.80 ± 0.09$^{}_{b}$	–
3,5-DCQA	3.62 ± 0.02	17.8 ± 0.03$^{*}_{a}$	0.28 ± 0.01$^{*}_{A}$	11.6 + 0.10$^{*}_{b}$	0.10 ± 0.00$^{}_{B}$
Apigenin-7-*O*-glucoside	1.79 ± 0.01	6.89 ± 0.03$^{*}_{a}$	0.32 ± 0.00*	5.88 ± 0.01$^{}_{b}$	–
4,5-DCQA	0.97 ± 0.01	4.30 ± 0.01$^{*}_{a}$	0.05 ± 0.00*	3.19 ± 0.00$^{}_{b}$	–
Rosmarinic acid	0.18 ± 0.00	–	–	–	–
Luteolin	4.47 ± 0.01	4.58 ± 0.01$^{}_{b}$	3.18 ± 0.00$^{}_{A}$	13.0 ± 0.00$^{*}_{a}$	0.95 ± 0.01$^{}_{B}$
Quercetin	0.47 ± 0.00	0.68 ± 0.00$^{}_{b}$	0.32 ± 0.01*	1.44 ± 0.01$^{*}_{a}$	–
Flavone n.i.	1.66 ± 0.00	1.15 ± 0.00$^{*}_{b}$	1.42 ± 0.00$^{*}_{A}$	3.33 ± 0.00$^{*}_{a}$	1.16 ± 0.00$^{}_{B}$
Apigenin	1.96 ± 0.00	1.00 ± 0.00$^{}_{b}$	1.83 ± 0.00$^{}_{A}$	4.74 ± 0.00$^{*}_{a}$	0.93 ± 0.00$^{}_{B}$
Diosmetin	0.50 ± 0.00	0.31 ± 0.00$^{}_{b}$	0.53 ± 0.00$^{}_{A}$	0.73 ± 0.00$^{*}_{a}$	0.50 ± 0.00$^{}_{B}$
Amentoflavone	0.42 ± 0.00	0.16 ± 0.00$^{*}_{b}$	0.52 ± 0.00$^{*}_{B}$	0.41 ± 0.00$^{*}_{a}$	0.62 ± 0.00$^{}_{A}$
Flavone n.i.	2.14 ± 0.00	0.57 ± 0.00$^{*}_{a}$	2.68 ± 0.00$^{*}_{B}$	0.57 ± 0.00$^{*}_{a}$	3.68 ± 0.00$^{}_{A}$
Casticin	0.29 ± 0.00	–	0.44 ± 0.01$^{}_{B}$	–	0.62 ± 0.01$^{}_{A}$

< L.Q.: below limit of quantification. *An asterisk indicates statistical differences between original extract and fractions. [a,b] Different lowercase letters denote statistical differences between P10 and P15 fractions. [A,B] Different capital letters denote statistical differences between S10 and S15 fractions. Significance level at $p \leq .05$ with Fisher's Least Significant Difference (LSD) test.

were analyzed in order to establish a relationship between its composition and the antioxidant activity found.

3.2. Phenolic characterization of yarrow extract and SAF fractions

The HPLC analysis of phenolic compounds in the original extract and SAF fractions are shown in Table 2. The main compounds identified in the original extract were flavonoids, either in glycosylated or in aglycone form, and phenolic acids. Therefore, the compounds presented in higher concentration corresponded to the flavonoids luteolin and its glycosylated form (luteolin-7-*O*-glucoside), as well as the dicaffeoylquinic acids, where the 3,5-DCQA standed out. These results are in agreement with others previously reported, where the main flavonoids found in several yarrow extracts were luteolin-7-*O*-glucoside, luteolin, apigenin-7-*O*-glucoside and apigenin, while within the dicaffeoylquinic acids, 3,5-DCQA was the one in a greater extent (Benedek, Gjoncaj, Saukei, & Kopp, 2007; Vitalini et al., 2011). Related to precipitation vessel fractions, its principal components were luteolin-7-*O*-glucoside, 3,5-DCQA, luteolin, apigenin-7-*O*-glucoside and 6-hidroxyluteolin-7-*O*-glucoside, representing between 73% (P10) and 60% (P15) of all phenolic compounds identified. Meanwhile, separator fractions contained a reduced quantity of phenolic compounds, where flavonoids aglycones standed out.

Precipitation vessel fractions and the original sample presented a similar phenolic composition, although the compounds concentration in the fractions was, in general, much higher, highlighting luteolin-7-*O*-glucoside and 3,5-DCQA. These results could be related to the low solubility of glycosylated flavonoids and phenolic acids in the SC-CO_2 + ethanol mixture, since these compounds are poorly soluble in low-polar solvents (Chebil et al., 2007). On the other hand, the flavonoids aglycones, as less polar compounds, would be more soluble in the mixture SC-CO_2 + ethanol and therefore they would be dragged to the separator fraction.

However, it should be noted that there were some differences between fractions P10 and P15, since P10 fraction presented a greater amount of luteolin-7-*O*-glucoside and 3,5-DCQA than P15 fraction, whereas P15 contained a higher quantity of luteolin and apigenin than P10. These results could indicate that an increase in pressure during the fractionation process, would increment the presence of aglycones in the precipitation vessel fraction. In order to explain these results, it must be taken into account the complex multicomponent structure of extracts, comprising substances in a wide polarity range and with different solubility in SC-CO_2, which could exert a strong effect regarding the partial solubility of compounds involved and their precipitation behavior. Nevertheless, observing the solubility behavior of pure compounds in SC-CO_2, it can be established that in the case of more polar compounds (e.g. hydroxycinnamic acids) an increase of pressure from 10 to 15 MPa resulted in 4.4–5.4 solubility increase (Murga, Sanz, Beltran, & Cabeza, 2003), while for less polar compounds (e.g. quercetin) this ratio was about 3.0–3.5 (Chafer, Fornari, Berna, & Stateva, 2004). Thus, according to the pure component solubility behavior, an increase of the precipitation pressure could increase the recovery of aglycones in precipitation vessel.

Thus, the higher antioxidant activity found in P10 and P15, compared to original extract, could be related with the significant increase in several phenolic compounds found in these fractions, mainly luteolin-7-*O*-glucoside, 3,5-DCQA, luteolin (only in P15), apigenin-7-*O*-glucoside and 6-hidroxyluteolin-7-*O*-glucoside. Besides, when comparing P10 and P15, it can be observed that P10, with the higher antioxidant activity, also presented the higher quantity of luteolin-7-*O*-

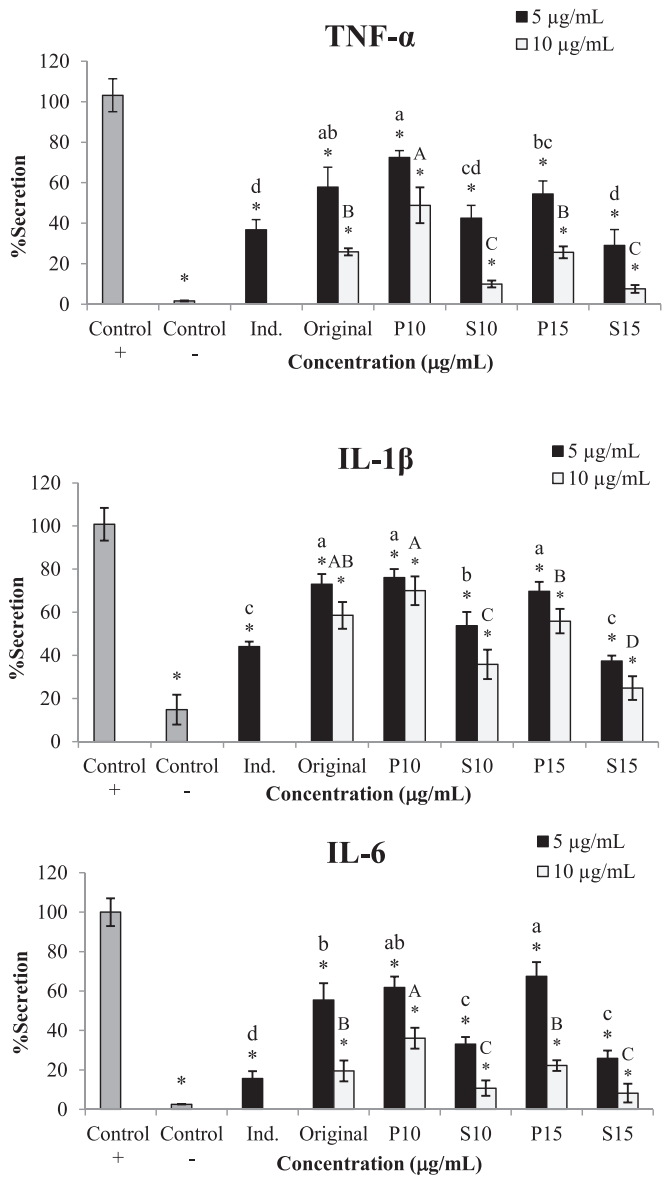

Fig. 1. Levels of TNF-α, IL-1β and IL6 secreted by THP-1/M activated with LPS in presence of original extract and SAF fractions. Positive control: cells stimulated with LPS but in absence of extract. Negative control: cells in contact just with RPMI media. Ind.: Indomethacin. Original: original yarrow extract. P10: precipitation vessel fraction at 10 MPa. P15: precipitation vessel fraction at 15 MPa. S10: separator fraction at 10 MPa. S15: separator fraction at 15 MPa. Each bar is the mean of three determinations ± S.D. *Denotes statistical differences when compares with positive control. ^{a,b,c,d,} Different lowercase letters indicate statistical differences between samples at 5 µg/mL. ^{A,B,C,D} Different capital letters indicate statistical differences between samples at 10 µg/mL. Significance level at $p \leq .05$ with Fisher's Least Significant Difference (LSD) test.

glucoside and 3,5-DCQA (representing almost 55% of the fraction). Kim et al. (2011) indicated that 3,5-DCQA presented an important antioxidant activity, significantly higher than chlorogenic acid. Besides, the antioxidant activity of luteolin-7-*O*-glucoside have also been reported (Antonisamy et al., 2016; Song & Park, 2014). However, it must be taken into account that this fact could be also due to synergies among all the phenolic compounds found in P10 fraction.

3.3. Anti-inflammatory activity of yarrow extract and SAF fractions

First, original extract and SAF fractions were evaluated for cytotoxicity on THP-1/M cells by MTT method. Results showed that, at the higher concentration used in the anti-inflammatory assays, 10 µg/mL, neither original extract nor SAF fractions presented cytotoxicity (cell viability ≥95%). Indomethacin at 5 µg/mL also presented no cytotoxicity.

The activation of THP-1/M was carried out with the addition of LPS to the medium. Fig. 1 showed that these LPS treated cells (positive control), after an incubation period of 24 h, presented an important increase in the release of TNF-α, IL-1β and IL-6, compared to non-activated controls (negative control). When THP-1/M were activated with LPS in presence of 5 and 10 µg/mL of original extract and SAF fractions, a decrease in TNF-α secreted level was observed (Fig. 1), compared with positive control. Moreover, 5 µg/mL of original extract inhibited TNF-α secretion in a 40%. Regarding P10 and P15 fractions, P15 presented a decrease in TNF-α superior to P10 and similar to that obtained with original extract. However, it should be noted that the greatest decrease in TNF-α secretion was achieved in presence of S10 and S15 fractions. Thus, 5 µg/mL of S15 fraction reduced TNF-α release in a 70%.

In the same way, the original extract and SAF fractions inhibited the IL-1β secretion by activated cells, at both concentrations employed, although separator fractions showed the greatest inhibition (Fig. 1). Thus, meanwhile 10 µg/mL of original extract decrease IL-1β release in a 40%, the same concentration of S15 showed an inhibition near to 70%. In addition, 5 µg/mL of S15 reduced IL-1β secretion by a 60%. The obtained results for the IL-6 release in presence of samples (Fig. 1) were similar to those obtained for TNF-α and IL-1β, since S10 and S15 fractions were the most active and produced an important decrease in the IL-6 release (near to basal levels).

These results indicated that all SAF fractions presented anti-inflammatory activity, although separator fractions were much more active than precipitation vessel ones. The anti-inflammatory activity of precipitation vessel fractions could be related to its content in phenolic compounds, more specifically with dicaffeoylquinic acids, luteolin, apigenin and its glycosides, since the anti-inflammatory effects of these compounds have been previously described (Liang & Kitts, 2016; Wang et al., 2014; Wang et al., 2017). Moreover, Francisco et al. (2014) indicated that luteolin-7-*O*-glucoside presented a certain anti-inflammatory activity, but lower than that observed with the luteolin aglycone in LPS-stimulated macrophages. Similarly, Choi et al. (2014) reported that apigenin presented higher anti-inflammatory activity than other naturally occurring *C*-glycosylated derivatives of apigenin. These results could explain the higher inhibition of TNF-α, IL-1β and IL-6 secretion found when using P15 compared with P10, since P15 contained a higher amount of luteolin and apigenin aglycones, whereas P10 presented a higher quantity of luteolin-7-*O*-glucoside and apigenin-7-*O*-glucoside.

However, the higher anti-inflammatory activity found in separator fractions, could not be related to its phenolic compounds content, due to the low quantity of these compounds presented in these fractions. Thereby, it's relevant to notice that conditions used to obtain the separator fractions allowed the enhancement of these fractions in essential oil components. Since essential oils from *A. millefolium* have been reported to present anti-inflammatory activity (Chou, Peng, Hsu, Lin, & Shih, 2013; Kazemi, 2015), S10 and S15 were analyzed by GC–MS in order to establish a relationship between the composition and the anti-inflammatory activity of these fractions.

3.4. GC–MS characterization of separator fractions

In order to identify the compounds involved in the anti-inflammatory activity found in separator fractions (S10 and S15), a characterization by GC–MS of these fractions, along with the original extract was performed. The identification of the main volatile compounds presented in the sample (Table 3) was performed based on the comparison of their mass spectra and retention index (RI). As can be

Table 3
GC–MS identification, peak area contribution (%), and retention index (RI) of compounds found in original extract and separator fractions. S10 (separator fraction at 10 MPa) and S15 (separator fraction at 15 MPa).

RI	Compound	Original	S10	S15
997	Yomogi alcohol	2.1	2.0	2.2
1028	Eucalyptol	4.3	3.5	3.4
1037	γ-Vinyl-γ-valerolactone	1.8	1.6	1.5
1058	Artemisia ketone	11.0	9.9	9.4
1070	1,2-Epoxylinalool	0.9	0.8	0.8
1079	Artemisia alcohol	1.0	0.8	0.8
1084	*cis*-Linalool oxide	0.8	0.6	0.7
1099	*β*-Linalool	0.9	0.9	0.9
1136	Camphor	13.8	12.5	12.3
1141	*cis*-Verbenol	0.9	0.7	0.7
1160	Borneol	8.7	8.9	8.6
1171	2-Methyl-2-octen-4-ol	1.0	0.9	1.0
1174	Terpinene-4-ol	0.5	0.4	0.5
1182	*p*-Cymen-8-ol	0.7	0.8	0.9
1188	3,7-dimethyl-1,5-Octadiene-3,7-diol	5.9	6.1	6.2
1200	Verbenone	0.5	0.6	0.6
1212	Fragranol	0.4	0.4	0.4
1218	2-Hydroxycineole	0.6	0.6	0.7
1236	*trans*-Chrysanthenyl acetate	2.4	2.5	2.5
1250	Piperitone	0.9	0.9	1.0
1261	(5*E*)-5,9-Dimethyl-5,8-decadien-2-one	1.5	1.4	1.3
1276	2,6-Dimethyl-1,7-octadiene-3,6-diol	8.6	9.3	9.3
1280	Bornyl acetate	0.8	1.0	1.0
1284	n.i.	7.2	8.7	8.5
1393	Jasmone	1.6	1.6	1.5
1412	*β*-Caryophyllene	1.4	1.6	1.5
1569	Spathulenol	0.9	1.2	1.1
1578	Caryophyllene oxide	4.9	6.0	5.8
1640	*β*-Eudesmol	1.2	1.2	1.5
1810	Saussurea lactone	3.4	3.2	3.2
1845	Hexahydrofarnesyl acetone	3.0	3.2	3.4
2069	n.i.	6.5	6.2	6.7
Σ AUC		24.41 10^6	42.80 10^6	53.40 10^6

n.i.: no identified; AUC (area under curve).

observed, 30 identified and 2 non-identified compounds were found in S10 and S15. In general, a very similar GC–MS profile was obtained for both fractions, being camphor, artemisia ketone, borneol and 2,6-dimethyl-1,7-octadiene-3,6-diol the most abundant compounds in both fractions. Original yarrow extract also presented a similar profile to S10 and S15, although total chromatographic area (expressed as Σ AUC) was much higher for the fractions. As expected, separator fractions have been enriched in essential oil components (1.75 times for S10 and 2.18 for S15), in comparison to the original extract. Regarding main components, camphor and borneol were shown to reduce TNF-α, IL-1β and IL-6 secretion in THP-1 macrophages stimulated with LPS or ox-LDL (oxidized low-density lipoproteins) (Arranz et al., 2014; Arranz et al., 2014). Similarly, Rungqu et al. (2016) reported that an essential oil containing a 37.5% of artemisia ketone presented an important anti-inflammatory activity, evaluated in rats, using egg albumin-induced paw edema. Therefore, the anti-inflammatory activity exhibited by S10 and S15 fractions could be mainly related to the presence of these three compounds (camphor, borneol and artemisia ketone) that represented approximately 30% of the fractions. However, the contribution to other anti-inflammatory compounds presented in smaller quantities, such as eucalyptol and β-linalool, to this activity cannot be ruled out. Thus, the enrichment of S10 and S15 fractions in compounds that exhibit anti-inflammatory activity, regarding to the original extract, would explain the higher anti-inflammatory activity of these fractions. Accordingly, S15 fraction that presented a higher enrichment in essential oil compounds than S10, also presented a higher anti-inflammatory activity.

4. Conclusion

Supercritical anti-solvent fractionation of an ethanolic yarrow extract resulted an adequate method to improve its antioxidant and anti-inflammatory activities. Thus, a selective precipitation of phenolic compounds increased its antioxidant activity twice, compared to original extract, especially when fractionation was carried out at 10 MPa. Regarding anti-inflammatory activity, separator fractions presented higher anti-inflammatory activity than precipitation vessel ones. This fact was related to separator fractions enrichment in essential oil compounds with anti-inflammatory activity. Being more active, in this case, the separator fraction obtained when pressure was 15 MPa.

Therefore, this study pointed out the feasibility of SAF process as a green technology in order to achieve a fractionation of compounds with different biological activities. This fact could be a useful tool for food or nutraceutical products design.

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References

Antonisamy, P., Subash-Babu, P., Albert-Baskar, A., Alshatwi, A. A., Aravinthan, A., Ignacimuthu, S., ... Kim, J.-H. (2016). Experimental study on gastroprotective efficacy and mechanisms of luteolin-7-O-glucoside isolated from *Ophiorrhiza mungos* Linn. In different experimental models. *Journal of Functional Foods, 25*, 302–313.
Arranz, E., Jaime, L., García-Risco, M. R., Fornari, T., Reglero, G., & Santoyo, S. (2014). Anti-inflammatory activity of Rosemary extracts obtained by supercritical carbon dioxide enriched in carnosic acid and carnosol. *International Journal of Food Science and Technology, 50*, 674–681.
Arranz, E., Jaime, L., Lopez De Las Hazas, M. C., Vicente, G., Reglero, G., & Santoyo, S. (2014). Supercritical sage extracts as anti-inflammatory food ingredients. *Industrial Corps and Products, 54*, 159–166.
Benedek, B., Gjoncaj, N., Saukei, J., & Kopp, B. (2007). Distribution of phenolic compounds in Middleeuropean taxa of the *Achillea millefollium* L., aggregate. *Chemistry & Biodiversity, 4*, 849–857.
Chafer, A., Fornari, T., Berna, A., & Stateva, R. P. (2004). Solubility of quercetin in supercritical CO2 + ethanol as a modifier: Measurements and thermodynamic modelling. *Journal of Supercritical Fluids, 32*, 89–96.
Chebil, L., Humeau, C., Anthoni, J., Dehez, F., Engasser, J. M., & Ghoul, M. (2007). Solubility of flavonoinds in organic solvents. *Journal of Chemical & Engineering Data, 52*, 1552–1556.
Chinnarasu, C., Monte, A., Fernández-Ponce, M. T., Casas, L., Mantell, C., Pereyra, C., & Martínez De La Ossa, E. J. (2015). Precipitation of antioxidant fine particles from *Olea europaea* leaves using supercritical antisolvent process. *Journal of Supercritical Fluids, 97*, 125–132.
Choi, J. S., Islam, M. N., Ali, M. Y., Kim, E. J., Kim, Y. M., & Jung, H. A. (2014). Effects of C-glycosylation on anti-diabetic, anti-alzheimer's disease and anti-inflammatory potential of apigenin. *Food and Chemical Toxicology, 64*, 27–33.
Chou, S. T., Peng, H. Y., Hsu, J. C., Lin, C. C., & Shih, Y. (2013). *Achillea millefolium* L. essential oil inhibits LPS-induced oxidative stress and nitric oxide production in RAW 264.7 macrophages. *International Journal of Molecular Sciences, 14*, 12978–12993.
Cissé, M., Vaillant, F., Pallet, D., & Dornier, M. (2011). Selecting ultrafiltration and nanofiltration membranes to concentrate anthocyanins from roselle extract (Hibiscus sabdariffa L.). *Food Research International, 44*, 2607–2614.
Dias, M. I., Barros, L., Dueñas, M., Pereira, E., Carvalho, A. M., Alves, R. C., ... Ferreira, I. C. F. R. (2013). Chemical composition of wild and comercial *Achillea millefolium* L. and bioactivity of the methanolic extract, infusion and decoction. *Food Chemistry, 141*, 4152–4160.
Fernández-Ponce, M. T., Casas, L., Mantell, C., & de la Ossa, E. M. (2015). Use of high pressure techniques to produce Mangifera indica L. leaf extracts enriched in potent antioxidant phenolic compounds. *Innovative Food Science & Emerging Technologies, 29*, 94–106.
Francisco, V., Figueirinha, A., Costa, G., Liberal, J., Lopes, M. C., García-Rodriguez, C., ... Batista, M. T. (2014). Chemical characterization and anti-inflammatory activity of luteolin glycosides isolated from lemongrass. *Journal of Functional Foods, 10*, 436–443.
Giorgi, A., Mingozzi, M., Madeo, M., Speranza, G., & Cocucci, M. (2009). Effect of nitrogen starvation on the phenolic metabolism and antioxidant properties of yarrow (*Achillea collina,* Becker ex Rchb.). *Food Chemistry, 114*, 204–221.
Kammerer, J., Boschet, J., Kammerer, D. R., & Carle, R. (2011). Enrichment and fractionation of major apple flavonoids, phenolic acids and dihidrochalcones using anion exchange resins. *LWT- Food Science and Technology, 44*, 1079–1087.
Kazemi, M. (2015). Chemical composition and antimicrobial, antioxidant activities and anti-inflammatory potential of *Achillea millefolium* L., *Anethum graveolens* L., and

Carum copticum L. essential oils. *Journal of Herbal Medicine, 5*, 217–222.
Kim, J. Y., Cho, J.-Y., Ma, Y.-K., Park, K. Y., Lee, S.-H., Ham, K.-S., ... Monn, J.-H. (2011). Dicaffeoylquinic acid derivatives and flavonoid glucosides from glasswort (*Salicornia herbacea* L.) and their antioxidant activity. *Food Chemistry, 125*, 55–62.
Liang, N., & Kitts, D. D. (2016). Role of cholrogenic acids in controlling oxidative and inflammatory stress conditions. *Nutrients, 8*, 16–35.
Marqués, J. L., Porta, G. D., Reverchon, E., Renuncio, J. A. R., & Mainar, A. M. (2013). Supercritical antisolvent extraction of antioxidants from grape seeds after vinification. *Journal of Supercritical Fluids, 82*, 238–243.
Meneses, M. A., Caputo, G., Scognamiglio, M., Revenchon, E., & Adami, R. (2015). Antioxidant phenolic compounds recovery from *Mangifera indica* L. by-products by supercritical antisolvent extarction. *Journal of Food Engineering, 163*, 45–53.
Mohammadhosseini, M., Sarker, S. D., & Akbarzadeh, A. (2017). Chemical composition of essential oils and extracts of *Achillea* species and their biological activities: A review. *Journal of Etnopharmacology, 199*, 257–315.
Mosmann, T. (1983). Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays. *Journal of Immunological Methods, 65*, 55–63.
Murga, R., Sanz, M. T., Beltran, S., & Cabeza, J. L. (2003). Solubility of three hydroxycinnamic acids in supercritical carbon dioxide. *Journal of Supercritical Fluids, 27*, 239–245.
Natolino, A., Da Porto, C., Rodríguez-Rojo, S., Moreno, T., & Cocero, M. J. (2016). Supercritical antisolvent precipitation of polyphenols from grape extract. *Journal of Supercritical Fluids, 118*, 54–63.
Pedan, V., Fischer, N., & Rohn, S. (2016). Extraction of cocoa proanthocyanidins and their fractionation by sequential centrifugal partition chromatography and gel permeation chromatography. *Analytical and Bioanalytical Chemistry, 408*, 5905–5914.
Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., & Rice-Evans, C. (1999). Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radical Biology & Medicine, 26*, 1231–1237.
Reverchon, E., & de Marco, I. (2006). Supercritical fluid extraction and fractionation of natural matter. *Journal of Supercritical Fluids, 38*, 146–166.
Rungqu, P., Oyedeji, O., Nkeh-Chungag, B., Songca, S., Oluwafemi, O., & Oyedeji, A. (2016). Anti-inflammatory activity of the essential oils of *Cymbopogon validus* (Stapf) Stapf ex Burtt Davy from eastern cape, South Africa. *Asian Pacific Journal of Tropical Medicine, 9*, 426–431.
Sánchez-Camargo, A. P., Mendiola, J. A., Valdés, A., Castro-Puyana, M., García-Cañas, V., Cifuentes, A., ... Ibáñez, E. (2016). Supercritical antisolvent fractionation of rosemary extracts obtained by pressurized liquid extraction to enhance their antiproliferative activity. *Journal of Supercritical Fluids, 107*, 581–589.
Shaheen, N., Lu, Y., Geng, P., Shao, Q., & Wei, Y. (2017). Isolation of four phenolic compounds from *Mangifera indica* L. flowers by using normal phase combined with elution extrusion two-step high speed countercurrent chromatography. *Journal of Chromatography B, 1046*, 211–217.
Singleton, V. L., Orthofer, R., & Lamuela-Reventos, R. M. (1999). Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. *Methods in Enzymology, 299*, 152–178.
Song, Y. S., & Park, C. M. (2014). Luteolin and luteolin-7-O-glucoside strengthen antioxidative potential through the modulation of Nrf2/MAPK mediated HO-1 signaling cascade in RAW 264.7 cells. *Food and Chemical Toxicology, 65*, 70–75.
Tadíc, V., Arsic, I., Zvezdanovic, J., Zugic, A., Cvetkovic, D., & Pavkov, S. (2017). The estimation of the traditionally used yarrow (*Achillea millefolium* L. Asteraceae) oil extarcts with anti-inflammatory potential in topical application. *Journal of Ethnopharmacology, 199*, 138–148.
Trumbeckaite, S., Benetis, R., Bumblauskie, L., Burdulis, D., Janulis, V., Toleikis, A., ... Jakstas, V. (2011). *Achillea millefolium* L. s.L. herb extract: Antioxidant activity and effect on the rat Herat mitochondrial functions. *Food Chemistry, 127*, 1540–1548.
Villalva, M., Jaime, L., Aguado, E., Nieto, J. A., Reglero, G., & Santoyo, S. (2018). Anti-inflammatory and antioxidant activities from the basolateral fraction of Caco-2 cells exposed to a rosmarinic acid enriched extract. *Journal of Agricultural and Food Chemistry, 66*, 1167–1174.
Villanueva, D., Ibánez, E., Reglero, G., Turner, C., Fornari, T., & Rodriguez-Meizoso, I. (2015). High catechins/low caffeine powder from green tea leaves by pressurized liquid extraction and supercritical antisolvent precipitation. *Separation and Purification Technology, 148*, 49–56.
Villanueva-Bermejo, D., Zahran, F., Troconis, D., Villalva, M., Reglero, G., & Fornari, T. (2017). Selective precipitation of phenolic compounds from *Achillea millefolium* L. extracts by supercritical anti-solvent technique. *Journal of Supercritical Fluids, 120*, 52–58.
Visentín, A., Cismondi, M., & Maestri, D. (2011). Supercritical CO2 fractionation of rosemary ethanolic oleoresins as a method to improve carnosic acid recovery. *Innovative Food Science & Emerging Technologies, 12*, 142–145.
Vitalini, S., Beretta, G., Iriti, M., Orsenigo, S., Basilico, N., Dall'Acqua, S., ... Fico, G. (2011). Phenolic compounds from *Achillea millefollium* L., and their bioactivity. *Acta Biochimica Polonica, 58*, 203–212.
Vitas, J. S., Cvetanović, A. D., Mašković, P. Z., Švarc-Gajić, J. V., & Malbaša, R. V. (2018). Chemical composition and biological activity of novel types of kombucha beverages with yarrow. *Journal of Functional Foods, 44*, 95–102.
Wang, J., Liu, Y. T., Xiao, L., Zhu, L., Wang, Q., & Yan, T. (2014). Anti-inflammatory effects of apigenin in lipopolysaccharide-induced inflammatory in acute lung injury by suppressing COX-2 and NF-kB pathway. *Inflammation, 37*, 2085–2090.
Wang, S., Cao, M., Xu, S., Zhang, J., Wang, Z., Mao, X., ... Liu, C. (2017). Effect of luteolin on inflammatory responses in RAW264.7 macrophages activated with LPS and INF-ϒ. *Journal of Functional Foods, 32*, 123–130.
Wijngaard, H., Hossain, M. B., Rai, D. K., & Brunton, N. (2012). Techniques to extract bioactive compounds from food by-products of plant origin. *Food Research International, 46*, 505–513.