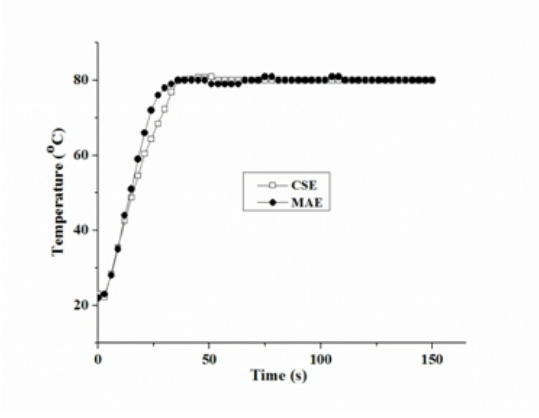
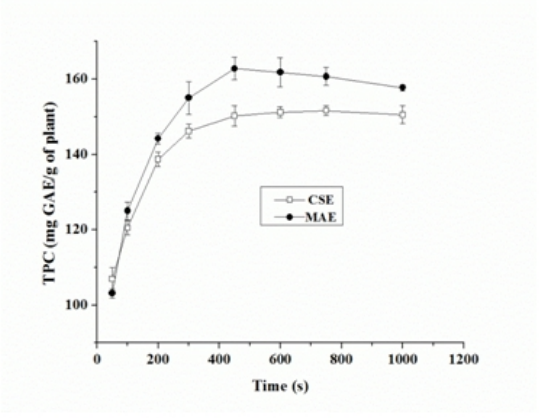
Sea buckthorn leaves

MAE/CSE extraction



Bulk temperature profile for MAE and CSE experiments at 80 °C bulk temperature profile

TPC determination



High efficiency of MAE vs CSE

New insights into the role of selective and volumetric heating during microwave extraction: Investigation of the extraction of polyphenolic compounds from sea buckthorn leaves using Microwave-Assisted Extraction and Conventional Solvent Extraction

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Abstract:

We report a direct comparison of microwave heating and conventional heating in solvent extraction by using exactly the same reaction conditions (including heating rate) in the extraction of polyphenols from dried sea buckthorn leaves. We have for the first time decoupled the effects of bulk heating rate and mixing regime from the fundamental microwave heating mechanism. We show that although microwave selective heating can increase the yield and quality of the polyphenols extracted, if the same bulk heating rate is applied there is no difference in treatment time and therefore theoretical energy requirements of the process. The first implication of these results for process intensification is that if microwave selective heating can be enhanced in scaled up processes through electromagnetic design, the extract yield and quality may be increased further. The second implication is that conventional extraction processes could be designed to provide the same heating rate and hence treatment time as microwave extraction, but any potential energy and space savings would have to be balanced against the increase in capital cost and complexity of the equipment. That said, the very small penetration depth of microwaves into ethanol/water solvent also poses design challenges in the scale up of microwave equipment.

Abbreviations:

MAE, Microwave-Assisted Extraction; CSE, Conventional Solvent Extraction; TPC, Total Phenolic Content; GAE, Gallic Acid Equivalent

Keywords:

microwave-assisted extraction (MAE), sea buckthorn leaves, polyphenolic compounds, antioxidant activity, selective heating; dielectric properties

1. Introduction

Microwave-assisted extraction (MAE) shows great potential in the intensification of natural product extraction processes: extraction times and solvent consumption can be significantly lower when compared with Conventional Solvent Extraction (CSE), higher yields and improved quality of the extracts can be achieved, and, due to smaller equipment and faster start-up and shut down times, energy requirements can be reduced [1]. However, despite the existence of over 2,000 research papers and numerous patents in this area [2], there are very few industrial processes that utilize MAE, and those that do are relatively small processes (e.g. up to 800 kg/day of solid biomass [3]). A survey of chemical, pharmaceutical and related industries [4] indicates that perceived difficulties in scale-up are the reason for the low uptake of microwave technologies, but that most companies are keeping a watching brief in microwave technology due to the potential for "real benefits to be gained by using microwave heating if the scale-up issues could be solved".

Microwaves heat volumetrically and selectively. Volumetric heating allows the system to be heated instantaneously throughout, and is responsible for the rapid heating times achieved in microwave processes. Selective heating enables some components in heterogeneous systems to heat more rapidly than others, resulting in thermal gradients, which can change the heat and mass transfer regimes during solvent extraction. The two most commonly cited reasons for the enhanced extraction results achieved by MAE are that (a) rapid selective heating of biomass samples during MAE can lead to structural damage of the plant matrix, thereby enhancing component extraction, and (b) the heat and mass transfer act in the same direction (as opposed to conventional processes, where heat transfer works from the outside in) [5]. A recent paper evaluated the effect of selective electromagnetic heating on mass transfer gradients during MAE;

it reported that a temperature difference in the order of 1˚C could result in a large enough chemical potential differential between the plant matrix and the bulk to cause solvent to move into the cell structure, increasing internal cell pressures to the point where disruption occurs. Extraction efficiency is sensitive to a range of variables including heating source, biomass characteristics, target extract, solvent, plant to solvent ratio, mixing regime, temperature, time and heating rate. This multivariate dependency has led to the common practice of laboratory scale solvent extraction being optimized using response surface optimization of multiple variables, which is only applicable to the specific extract and substrate pair in the specific experimental configuration investigated, and is therefore not applicable to scale-up. To date, a direct comparison of MAE and CSE changing only one variable (the heating source) has not been published for any feedstock, as the mixing regime (caused by using a different reactor geometry) and/or heating rate weren't controlled. In this paper, we report a direct comparison of microwave heating and conventional heating in solvent extraction by using exactly the same reaction conditions (including heating rate) in the extraction of polyphenols from dried sea buckthorn leaves. By doing this, we have for the first time decoupled the effects of bulk heating rate and mixing regime from the fundamental microwave heating mechanism, and therefore determine the potential for microwaves to increase yields and theoretical energy requirements in this plant-extract system, and this will inform process selection and scale-up.

Polyphenolic compounds, one of the most widely occurring groups of phytochemicals, are of considerable physiological and morphological importance in plants [6]. All plants produce polyphenolic compounds because they play a key role in plant defense [7], against ultraviolet light [8] or against plant pathogens and animal aggression [9]. Polyphenolic compounds from plant tissues are strongly associated with health benefitting properties, including reducing the

risk of certain types of cancer, cardiovascular and [10] neurodegenerative diseases [11, 12]. Because of their health benefits, in recent years there has been specific focus on the extraction of polyphenolic compounds from inexpensive or residual biomass that is not used as food [13]. Sea buckthorn leaves represent one of these non-food residual sources, because this bush is cultivated in general for its fruits [14].

Sea buckthorn (*Hippophae rhamnoides* L., family: Elaeagnaceae) is a bush that grows widely in various regions of Asia, Europe and North America [15-17]. This plant is cultivated for its fruits, which represent an important source of vitamins A, C, E, K, flavonoids, carotenoids, organic acids, and oils [18]. Sea buckthorns leaves, which constitute an inexpensive and currently unused waste biomass, are rich in polyphenolic compounds, but few studies are available on their pharmacological effects, as compared to fruits and seeds [19, 20]. Sea buckthorn leaf extract presents antioxidant, immunomodulatory, anti-stress [21, 22], anti-inflammatory [23] and a significant wound-healing activity [24]. Few studies have been published in the literature about the extraction of polyphenolic compounds from sea buckthorn leaves [18, 25].

A major challenge in recent decades has been the extraction of polyphenolic compounds from different sources of biomass with a high yield without modifying the natural structure of these compounds [6, 26]. To address this challenge, attention has been focused on developing efficient and environmentally friendly extraction techniques. These extraction techniques need to use less solvent, shorter time of extraction and less energy compared with conventional techniques [27].

MAE has been successfully used to extract polyphenolic compounds from a range of leaves, including sea buckthorn leaves [28], Dalmatian sage (*Salvia officinalis)* [29], *Eucalyptus*

robusta [30], blueberry [31], cherry laurel (*Prunuslaurocerasus*) [27, 32], mastic (*Pistacialentiscus)* [33, 34], olive [35, 36] and sweet potato *(Ipomoea batatas)* [37]. Where comparisons were made with other solvent extraction technologies such as CSE [31-36], Ultrasound Assisted Extraction (UAE) [31-34], and supercritical fluid extraction [36], MAE was found to give comparable or higher yields of Total Phenolic Compounds (TPC), and comparable [33, 34] or superior antioxidant activity [32].

In our previous study [28], we demonstrated the successful extraction of polyphenols from sea buckthorn leaves using MAE and CSE. The highest extracted TPC yields obtained for MAE and CSE were comparable, at 144 and 143 mg GAE/g plant material respectively (i.e. the results were within error bars). Therefore it is unclear from those results whether microwave heating offers benefits to extraction yields in this process. The work also showed that the maximum TPC could be achieved in 450 s using MAE, versus around 1800 s using CSE, suggesting that microwaves may lead to energy savings in a scaled up process. However, this may have been due to the slower heating profile applied in the CSE experiments, and therefore it is unclear from these results whether microwave volumetric and/or selective heating leads to lower theoretical energy requirements in this process. The work was carried out in a Biotage Initiator device, which is a multimode microwave applicator with no impedence matching, meaning that the power absorbed by the sample may be significantly lower than that applied. The work carried out in this paper addresses the uncertainties arising from our previous results by (a) using a single mode microwave cavity with a stronger applied electric field together with an impedance matching device, which maximizes the absorbed power into the sample, thereby increasing any benefit offered by microwave selective heating and allowing measurement of the energy absorbed by the sample/solvent system, (b) comparing conventional heating and

microwave heating with the same temperature profile and identical reaction vessel (for the first time) in order to directly compare the extraction yields and theoretical energy requirements of the two processes.

The aim of this work is therefore to carry out a direct comparison of MAE and CSE changing only one variable at a time, using as a model the extraction of polyphenols from dried sea buckthorn leaves. The objectives of this work are to (1) optimize MAE of polyphenolic compounds from dried sea buckthorn leaves as a function of ethanol concentration in the solvent, plant to solvent ratio, extraction time, extraction temperature and stirring rate, and (2) compare MAE and CSE using the same bulk heating rate to determine whether MAE offers theoretical benefits over CSE for this application in terms of extraction yields, extract quality and theoretical energy requirements, and (3) relate the findings to the dielectric properties of the system in order to contribute to fundamental understanding of microwave extraction mechanisms. How the new fundamental understanding can be applied to scale-up methodology is also discussed.

2. Materials and Methods

2.1. Plant material

Fresh sea buckthorn (*Hippophae rhamnoides* L.**)** leaves were collected from Furculesti - Alexandria (S.C. Hofigal Export Import SA) in Romania, in June 2014. The plant material was dried at 60 ˚C in an oven and, after drying, the material was milled using a coffee grinder and the ground material was passed through a fine sieve (<0.5mm) to give a product with a uniform particle size. The fine powder was stored immediately in airtight bottles to avoid any moisture reabsorption, and the bottles were stored in a dark place at 4 ˚C to prevent the composition changes of plant material [38].

2.2. Extraction procedure

2.2.1. Microwave-assisted extraction (MAE)

Extraction of total polyphenolic compounds from sea buckthorn leaves by MAE was performed using a microwave system consisting of a Miniflow 200SS batch reactor manufactured by Sairem capable of generating 200 W of microwave energy at 2.45 GHz. The sample was then heated in a sealed pyrex reactor with a volume of 50 mL (vapour to liquid ratio ~4:1) within a TE_{10n} single mode cavity in WE340 waveguide terminating in a short circuit. A single mode microwave cavity was chosen for its ability to supply a well-defined standing wave pattern, which enables the reactor to be placed in the position of maximum electric field strength. A single manual tuning stub was used for impedance matching purposes to maximize the power absorbed into the sample. Stirring of the sample was provided by an external magnetic stirrer placed at the bottom of the microwave cavity (IKA – magnetic stirrer RH digital GmbH & Co KG, Staufen, Germany) (**Fig.1**). The temperature inside the reactor was measured using an optical fiber. 0.5 grams of sea buckthorn leaf powder were mixed with an ethanol:water solvent (plant to solvent ratio 1:10 – 1:30 w/v). The extraction process was performed at different extraction temperatures, extraction time and stirring rates. In some of the experiments the boiling point of the mixture was exceeded by 1 – 2 ˚C (i.e. those in which the set temperature was 80˚C and the concentration of ethanol in the solvent was 90%). However, the vessel was sealed, thereby suppressing any distillation of ethanol from the solvent, and after extraction the reactor was cooled before opening. These measures ensured that the ratio of ethanol to water in the solvent mixture was maintained throughout the experiment. The mixture obtained was filtered through a Whatman No. 1 filter paper (Whatman International Ltd., England, UK) and the filtrate was then stored in airtight bottles in a dark place at 4 ˚C to prevent the degradation of the

polyphenolic compounds extracted during storage [39, 40]. All the extraction experiments were carried out in triplicate.

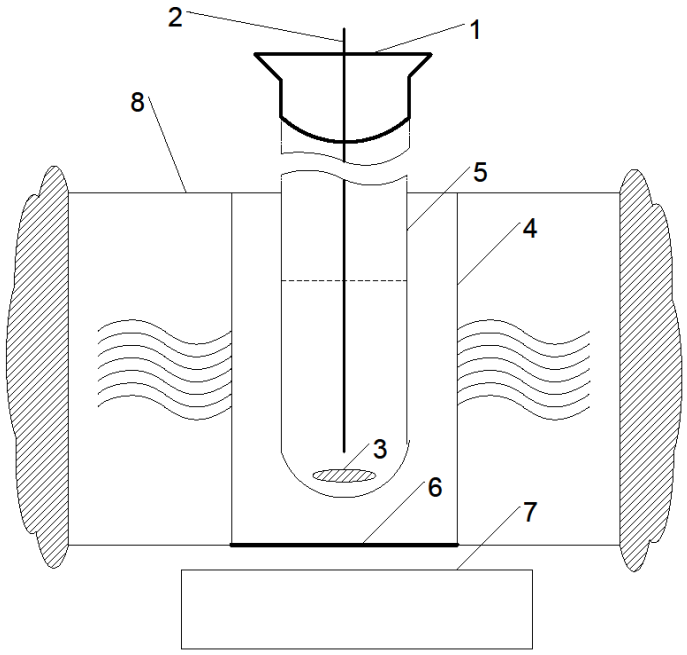


Fig. 1. Schematic diagram of MAE (1-rubber stopper, 2-optical fiber, 3- magnetic stirrer, 4 - TE_{10n} single mode cavity, 5- pyrex reactor, 6-aluminium lid and quick release clamp, 7-stirrer plate, 8- WE340 waveguide)

2.2.2. Conventional Solvent Extraction (CSE)

The CSE experiments were designed to replicate the MAE experimental conditions as closely as possible. 0.5 g of sea buckthorn leaf powder were introduced into the same reactor used for MAE, mixed with ethanol:water solvent and immediately placed in a heating bath. The reactor was immersed in a 150 ˚C ethylene glycol bath until the set temperature was reached, and then immediately transferred to a water bath at the set temperature. This method was found to achieve a very similar bulk heating profile to the MAE experiments, as shown in **Fig.2**. Although the heating rate during MAE was slightly higher than CSE between 15 and 30 seconds, the time

to reach the set point was the same. The mixture stirring was ensured by a stirring plate. The temperature inside the reactor was measured using an optical fiber. Immediately after the extraction, the mixture was filtered through a Whatman No.1 filter paper (Whatman International Ltd., England, UK) and the filtrate was then stored in airtight bottles in a dark place at 4 ˚C.

To our knowledge, this is the first time that a direct comparison of MAE and CSE using the same reactor geometry and bulk temperature profile has been published. This means that any differences in the extraction results can be directly attributed to heating method, rather than other factors such as thermal degradation, which can be caused by different bulk heating profiles.

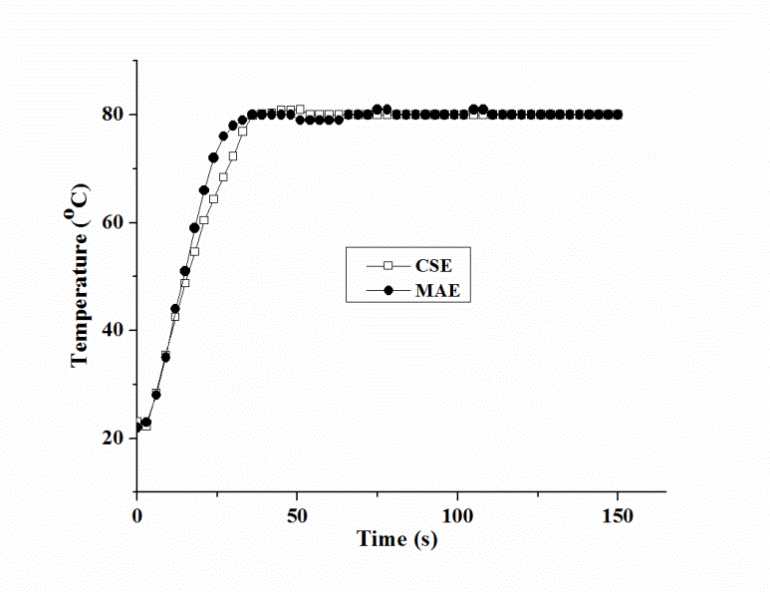


Fig.2. Bulk temperature profile for MAE and CSE experiments with an 80 ˚C set point

2.3. Determination of total phenolic content

The Total Phenolic Content (TPC) was determined by the modified Folin-Ciocalteu colorimetric method [41]. 7.5 mL of ultrapure water, 0.5 mL of Folin-Ciocalteu reagent 1N (Sigma Aldrich) and 0.5 mL of calibration standards (0, 1, 1.5, 2, 2.5, 3, 3.5, 4 and 4.5 mg L^{-1} of gallic acid (Sigma Aldrich) or diluted sample extracts were added to the test tubes and stirred (at

300rpm) for 3 min. After stirring, 1.5 mL of sodium carbonate solution (200 g L^{-1}) (Sigma Aldrich) was added and the mixture was kept in the dark for 1 hour. The absorbance was measured at 760 nm using a spectrophotometer (Shimadzu UVmini 1240) and the results were expressed in milligram of gallic acid equivalent (GAE) per gram of dry plant. All analyses were carried out in duplicate.

2.4. HPLC analysis

The extracted samples contained both polyphenolic compounds and also some nonpolar compounds, therefore a liquid-liquid extraction step using diethylether (Sigma Aldrich) was employed in order to remove these compounds, which cause serious matrix interferences [42]. Before HPLC analysis, 0.5 mL aliquot of the extract was mixed with 0.5 mL diethyl ether and shaken for 10 minutes at 600 rpm using a magnetic stirrer. After the extraction, the diethylether layer was separated and stored at 4 °C.

HPLC analyses were carried out on a system consisting of: UV-2075 Detector, PU-2080plus Pump, LG-2080 Gradient Unit, DG-2080_4 Degasser (from Jasco Analytical Instruments). Separation was realised on a Teknokroma Nucleosil 100 C18 column (250 x 0.4 mm, 10 μm). Analyses were performed at room temperature, with a flow rate of 0.5 mL/min using 2% v/v acetic acid (solvent A) (Sigma Aldrich) and methanol (solvent B) (Chromasolv - Sigma Aldrich) under the following gradient program: 0-8 min 70% A, 8-19 min 60%A and 19-30 min 50% A. Identification of the compounds was performed on the basis of the retention time of polyphenols used as standards. Standard stock solutions were prepared by dissolving gallic acid (1.3 mg) (Sigma Aldrich), catechin (1.6 mg) (Fluka), caffeic acid (1.5 mg) (Fluka), p-cumaric acid (1.5 mg) (Sigma Aldrich), ferulic acid (1.3 mg) (Sigma Aldrich), rutin (1.6 mg) (Fluka), quercetin (1.5 mg) (Fluka) in 16 mL ethanol 50% (Sigma Aldrich). The stock solutions

were diluted successively and analyzed in order to construct the calibration curve. On the basis of the retention time, four compounds were identified (from the compounds used for the calibration curve) and other two unidentified; these two were considered as derivates of rutin and their concentration was calculated according to it.

2.5. Antioxidant activity

The antioxidant activities of the samples analyzed by HPLC were determined theoretically using the antioxidant activities of the standard phenols present in the samples. The antioxidant activities of each phenolic compound present in the samples were correlated with the concentration of the compound in the samples [43, 44].

2.6 Dielectric Property Measurements

Dielectric property measurements of high dielectric loss materials were performed using an Agilent 85070E Dielectric Probe Kit. The experimental set-up consisted of a performance coaxial probe equipped with an electronic calibration (ECal) module. The coaxial probe was connected to an Agilent N5232A PNA-L (Purpose Network Analyser) operating at 300 kHz–20 GHz via a high quality coaxial cable.

Sea buckthorn leaf powder was rehydrated to its original moisture content (67 %w/w) with solvent. The moist powder was placed in small aluminium pans, wrapped in aluminium foil and heated in an oven to the desired temperature. Dielectric measurements were performed immediately after taking the samples out of the oven. The probe was pressed firmly onto the moist powder to ensure there was good contact. When measuring the solvent, the probe tip was immersed in the test liquid. Measurements were carried out over a temperature range of 20–80 ˚C and a solvent composition of 50% ethanol, and dielectric loss (ε'') was recorded using Agilent Technologies 85070 software. All measurements were done in triplicate and error bars

are plotted as ± one standard deviation. The moist powder measurements were limited to 70 ˚C due to experimental difficulties with evaporation at higher temperatures.

A cavity perturbation method was used to measure the dielectric properties of dry sea buckthorn leaf powder, following the method of Navarrete et al. [45]. Briefly, the apparatus consisted of a cylindrical copper cavity connected to an HP 8753B vector network analyser (VNA). A linear stage (driven by a step motor) and a furnace were also incorporated to the apparatus. The cavity resonates at spot frequencies (896–915 MHz and 2450 MHz). Quartz tubes were used to hold the samples. This technique is well suited for low loss materials (which mean low moisture in the case of plant material).

3. Results

3.1. Absorbed power and temperature profiles during MAE

The evolution of microwave power and temperature for a typical MAE experiment are shown in **Fig.3**. From the graph it can be seen that the set temperature (80 ˚C) was reached after 30 s of applied power, this being the heating phase. Based on the data in **Fig.3**, the energy absorbed by the system during the heating phase and holding phase were 3167 J and 2413 J respectively, totaling 5580 J.

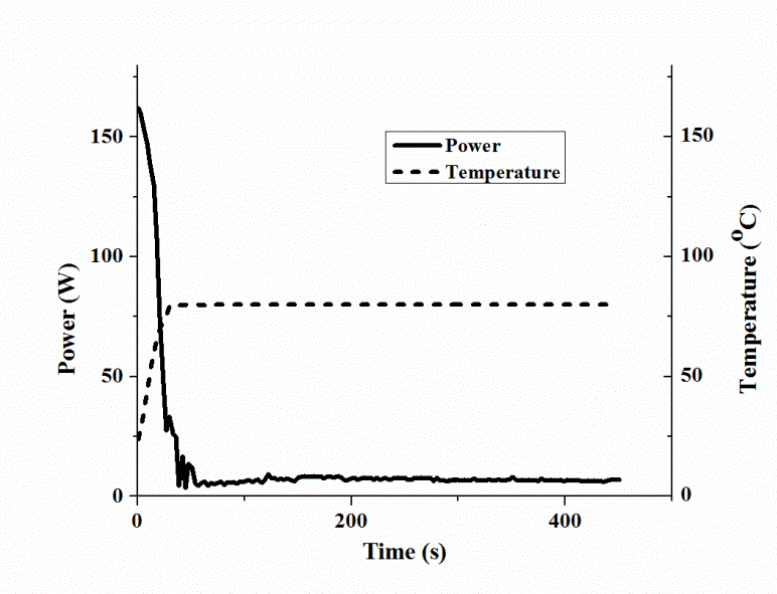


Fig. 3. Absorbed power and temperature profiles for a typical MAE experiment (extraction temperature 80 ˚C, extraction time 450 s, stirring rate 1200 rpm and ratio of plant to liquid 1:20 (w/v), 50% ethanol (v/v)).

It is important to note that the temperature measured during microwave heating experiments is the bulk temperature only; the local temperature of the plant material may be hotter or cooler than the bulk, and that this depends on the relative dielectric properties of the solvent and sample, which change during processing. The dielectric constant and dielectric loss describe the ability of a material to absorb microwaves and convert them to heat, respectively. The loss tangent is the ratio of the dielectric loss to the dielectric constant, and gives an indication of the efficiency with which electromagnetic radiation is converted to heat at a specific frequency and temperature [46]. Published values for the loss tangent of water and ethanol at 2.45 GHz and 25 ˚C are 0.123 and 0.941 respectively [46]. This means that pure water is considered a medium microwave absorber while pure ethanol is considered a strong microwave absorber, and the ability of the solvent to absorb microwaves and convert them to

heat is expected to increase with ethanol concentration. The dielectric properties of water and organic solvents decrease with increasing temperature [46], and therefore the maximum loss tangent of the solvent is expected to occur at the start of heating. The dried sea buckthorn leaves are poor microwave absorbers on their own: the loss tangent measured at 2.47 GHz using the cavity perturbation method as detailed in Section 2.6 ranged from 0.03 to 0.06 from room temperature up to 80 ˚C. However, the salts within the plant matrix would be expected to mobilize in the presence of the solvent, thereby contributing to the microwave-heating ability of the system; Singh et al. have observed that the presence of a plant matrix, as well as temperature and solvent concentration, affect dielectric properties of alcohol-water mixtures [47]. To summarize, the relative temperatures of the plant and solvent are expected to change during microwave processing, and this will be discussed later in the paper.

The penetration depth (D_p) is also an important factor in microwave processing; it is defined as the depth into the material at which the power flux has decreased by 37%, and calculated according to **Equation 1** below, where λ_0 is the free space microwave wavelength, ε' is the dielectric constant and ε" is the dielectric loss [48].

$$D_p = \frac{\lambda_0}{2\pi\sqrt{(2\varepsilon')}} \cdot \frac{1}{\left[\sqrt{\left\{1+\left(\frac{\varepsilon''}{\varepsilon'}\right)^2\right\}^{0.5}-1}\right]} \qquad [1]$$

At 25 ˚C and 2.45 GHz, the penetration depth of pure water is 13 mm and ethanol is 9 mm (based on published values [46]). The diameter of the reactor used in the microwave experiments was 30 mm. The means that the power flux in the centre of the reactor at the start of

heating is expected to be less than 37% of that at the outside of the sample, and that this effect becomes more marked as the ethanol concentration increases.

3.2. The effect of different ethanol concentration on the extraction of TPC

The solvent ethanol concentration was varied between 10 and 90% (v/v) in 10% increments to investigate the influence of the ethanol proportion on the extraction of TPC, and the other reaction conditions were as follows: extraction temperature 80 ˚C, extraction time 450 s, stirring rate 1200 rpm and ratio of plant to liquid 1:20. **Fig.4** shows similar trends for both MAE and CSE extraction. In both cases, TPC extraction was significantly influenced by the ethanol concentration in water. In case of a volume percentage of ethanol lower than 50% (v/v) the TPC content increased with the ethanol percentage. At an ethanol volume percentage in the solvent higher than 50% (v/v) the TPC content decreased with the ethanol concentration increase. Extraction of the polyphenolic compounds is closely tied to physicochemical interactions exposed during the extraction and the persistence and strength of these phenomena depend on the properties of the solvent (solubilization power, solubility in water, purity, polarity, etc.) [49]. Because polyphenolic compounds are polar molecules, it was necessary to use a polar solvent. Ethanol has a relative polarity of 0.654 compared with 1.0 for water [50], and they can be blended in any proportion to manipulate the polarity of the solvent. The solubility of gallic acid in ethanol at 25 ˚C is 12 times higher than its solubility in water at the same temperature. At 60 ˚C the solubility of gallic acid in ethanol, is still 3 times higher than that of water [51]. The Hildebrand solubility parameter (δ) can be estimated by using the Fedor group contribution method [52]. The solubility parameter of gallic acid, catechin, cafeic acid and rutin (which are the polyphenolic compounds identified in the extracted products as reported in Section 3.5) were

calculated using **Equation 2,** where $\sum(\Delta e)_i$ is the summation of cohesive energies (cal/mol) and $\sum(\Delta v)_i$ is the summation of molar volumes (cm^3/mol).

$$\delta(cal/cm^3)^{1/2} = \sqrt{\frac{\sum_i (\Delta e)_i}{\sum_i (\Delta v)_i}} \qquad [2]$$

The obtained data are presented in the **Table 1.**

Table 1. Hildebrand Solubility Parameters at 25 ˚C ($MPa^{1/2}$)

Solvent	
Ethanol	26.1
Water	48
50% Ethanol	37.1
Phenolic compound	
Gallic acid	39.08
Catechin	32.72
Cafeic acid	31.74
Rutin	44.02

Table 1 shows that the values of Hildebrand parameter for polyphenolic compounds identified in our samples are very close to the value of the solvent selected (ethanol 50%). According to Raynie, 2010 for an efficient extraction it is important to use solvent that have δ values similar to those of the solutes interest [53].

Comparing the MAE and CSE results, it is observed that CSE outperforms MAE up to 30% ethanol, yielding up to 13 mgGAE/g plant material (12%) more than MAE. However, there is a crossover around 35% ethanol above which MAE yields more TPC than CSE (up to 24 mgGAE/g plant material, or 25%). The maximum extraction for both cases is at 50% ethanol, where MAE yields 13 mgGAE/g plant material, or 8.5%, more than CSE. Ethanol is a stronger microwave absorber than water (as discussed in **Section 3.1**), which means that the differential temperature between the sea buckthorn and the solvent is likely to vary as the ethanol concentration in the solvent varies. If mass transfer in this system is in part driven by chemical

potential gradients caused by selective heating, as was proposed for MAE of okra [54], the concentration of ethanol in the solvent is likely to affect this mechanism. It could even be that the solvent is hotter than the sea buckthorn at high ethanol concentrations and cooler than the sea buckthorn at low ethanol concentrations, resulting in the chemical potential gradient between the plant matrix and the solvent reversing directions. According to the thermodynamic theory presented by Lee et al. [54], slight temperature differences can lead to significant mass transfer between the solvent and plant matrix.

According to the results presented, the highest TPC content was obtained at a concentration of ethanol in water of 50% in both conventional and microwave-assisted extraction, therefore this percentage was used for the remaining experiments. This is consistent with the MAE extraction of polyphenols from *Pistacialentiscus* [33], but significantly different from that of sweet *Ipomoea batatas* leaves, where 70% ethanol was found to be optimal [37]. However, both of those studies used reaction vessels equipped with reflux rather than controlling the temperature, and so differences in temperature may have influenced those results (as the dielectric properties of the solvent change with composition, and also the applied power and treatment time were different in the two studies). Another explanation is that the solubility parameter of the *Ipomoea batatas* leaf extracts differs from *Pistacia lentiscus* and sea buckthorn leaf extracts.

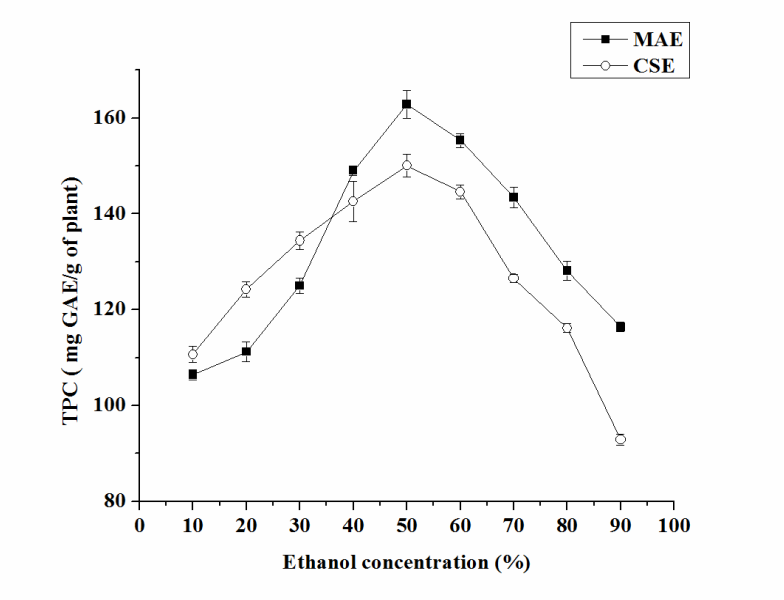


Fig. 4. Effect of ethanol concentration in water on the extraction of TPC (extraction temperature 80 ˚C, extraction time 450 s, stirring rate 1200 rpm and ratio of plant to liquid 1:20 (w/v), mean± 1 S.D. (n = 6, triplicate extraction and duplicate analysis))

3.3. The effect of plant to solvent ratio on the extraction of TPC during MAE

Extraction is expected to improve with decreasing plant to solvent ratio as osmotic pressure increases with increasing concentration gradient. However, in order to develop a sustainable process, the solvent use should be minimized. Therefore, the optimal plant to solvent ratio will be a compromise between these two factors. In order to investigate the influence of different plant to solvent ratios on TPC, several plant to solvent ratios (1:10, 1:15, 1:20, 1:30) were investigated (**Fig.5**), keeping the other four factors constant (extraction time 450 s, extraction temperature 60 ˚C, 900 rpm stirring rate and 50% (v/v) ethanol concentration). The TPC content increased with decreasing plant to solvent ratio. This occurred rapidly below a plant to solvent ratio of 1:15. Between 1:15 and 1:30 plant to solvent ratio, the increase in TPC with

plant to solvent ratio started to level off. The highest TPC value (143.7± 4.2 mg GAE/ g of plant) was obtained for the samples extracted using a plant to solvent ratio of 1:30; compared with the results obtained for the samples extracted using 1:20 plant to solvent ratio, the difference is of only 6%. Therefore, in the remaining experiments, a 1:20 plant to solvent ratio was selected, and this is comparable with plant to solvent ratios found in other optimization studies for the MAE of polyphenols from leafy materials [27, 33, 37].

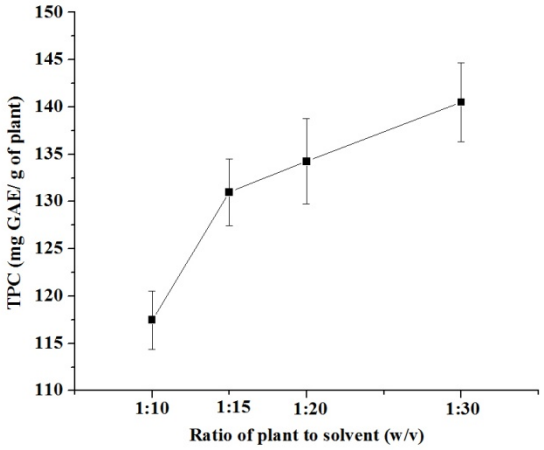
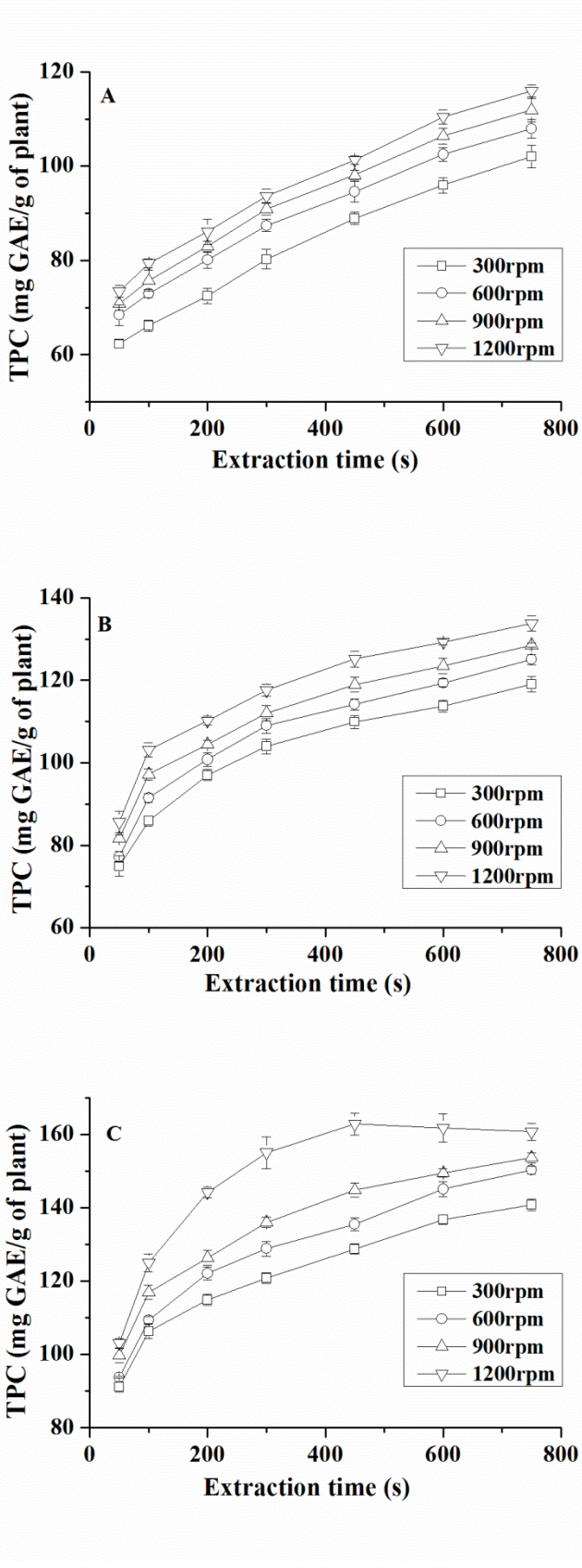


Fig. 5. Effect of ratio solid to liquid on the extraction of TPC during MAE (extraction temperature 60 ˚C, extraction time 450 s, stirring rate 900 rpm, ethanol 50% (v/v), mean±S.D. (*n* = 6, triplicate extraction and duplicate analysis))

3.4. The effect of extraction temperature, time and the stirring rate on the extraction of TPC during MAE

The yield of TPC was dependent on temperature, extraction time and stirring rate. These variables were found to be interdependent, and so are addressed together in this section. Extraction times of 50,100, 200, 300, 450, 600 and 700 s, stirring rates of 300, 600, 900 and 1200 rpm and extraction temperatures of 40 ˚C, 60 ˚C and 80 ˚C were investigated, and the results are shown in **Fig.6**A-C. Based on the results presented in **Sections 3.2** and **3.3**, the ethanol concentration and plant to solvent ratio were fixed at 50% (v/v) and 1:20 (w/v)

respectively. The results indicate that the extraction of TPC increases with increasing extraction time and stirring rate for extraction temperatures of 40 ˚C and 60 ˚C, and **Fig.6**A and **Fig.6**B suggest that further increases in extraction time and stirring speed would be required to maximize TPC extraction. However, in the case of an extraction temperature of 80 ˚C (**Fig.6**C), the TPC yield reached its maximum (163 mg GAE/g of plant) in 450 s at 1200 rpm stirring rate, and further increases in extraction time led to a slight reduction in TPC yield. This decrease of the TPC at a long time of extraction at high temperature and high stirring rate is likely to be caused by hydrolyzation and oxidation of some polyphenols [55]. In all cases, TPC yield increases with temperature, and this can be attributed to the fact that the temperature increase leads to a decrease in the surface tension of the solvent and its viscosity, allowing deeper penetration of the solvent into the sample matrix, thus solubilizing more of the polyphenolic compounds [56]. There may also selective heating effects influencing the extraction, and this will be discussed in the following sections.

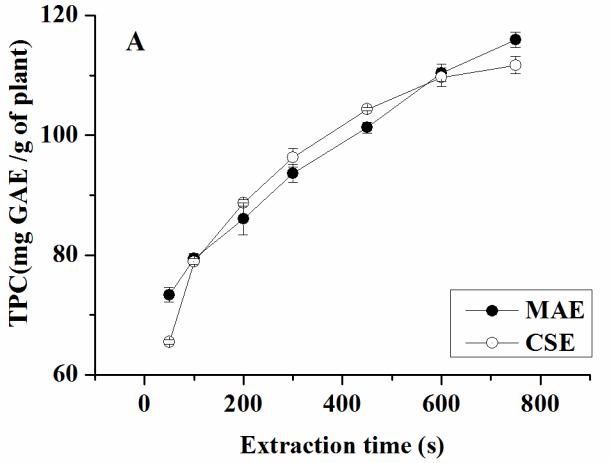

A
TPC (mg GAE/g of plant)
Extraction time (s)
300rpm
600rpm
900rpm
1200rpm
B
TPC (mg GAE/g of plant)
Extraction time (s)
300rpm
600rpm
900rpm
1200rpm
C
TPC (mg GAE/g of plant)
Extraction time (s)
300rpm
600rpm
900rpm
1200rpm

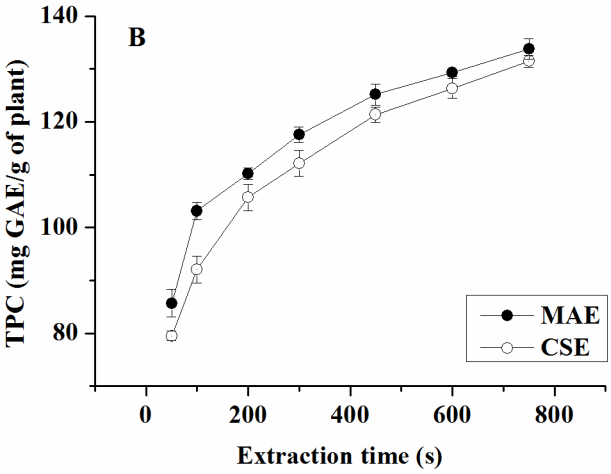
1

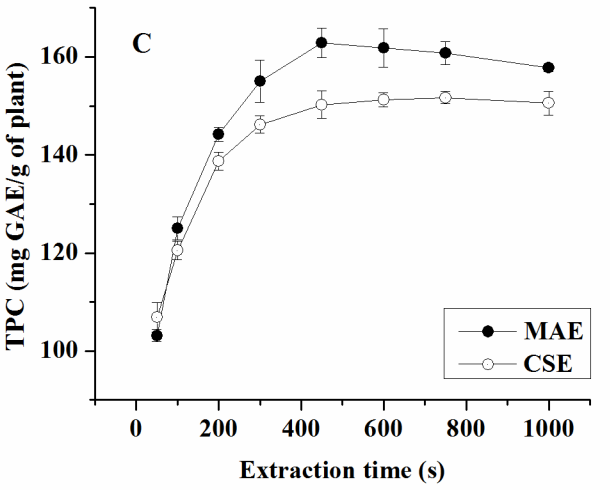
Fig.6. Effect of different extraction time and different stirring rate on the extraction of TPC during MAE (extraction temperature 40 °C (**A**), extraction temperature 60 °C (**B**), extraction temperature 80 ˚C (**C**), ethanol concentration 50%, ratio plant: solvent 1:20 (w/v), mean±S.D. (*n* = 6, triplicate extraction and duplicate analysis))

3.4. Comparison microwave-assisted extraction (MAE) vs. conventional solvent extraction (CSE)

In order to assess the performance of MAE and also to aid in understanding of the extraction mechanisms in this system, CSE was carried out using the same bulk temperature profile as in the MAE experiments (see **Section 2.2.2** for method details) for a stirring speed of 1200 rpm, plant to solvent ratio of 1:20 (w/v) and solvent ethanol concentration of 50%. **Fig.7**A-C presents a comparison of the results obtained for TPC extraction from MAE and CSE.


A
TPC(mg GAE /g of plant)
120
100
80
60
0
200
400
600
800
Extraction time (s)
MAE
CSE


B
TPC (mg GAE/g of plant)
140
120
100
80
0
200
400
600
800
Extraction time (s)
MAE
CSE


C
TPC (mg GAE/g of plant)
160
140
120
100
0
200
400
600
800
1000
Extraction time (s)
MAE
CSE

1

Fig.7. TPC for MAE and CSE (extraction temperature 40 ℃ (**A**), extraction temperature 60 ℃ (**B**), extraction temperature 80 ℃ (**C**), stirring rate 1200 rpm, ethanol concentration 50%, ratio plant to solvent ratio 1:20 (w/v), mean±S.D. (n = 6, triplicate extraction and duplicate analysis)

Fig.7A-C illustrates two very important points. This is the first time that MAE and CSE experiments with the same heating rates have been compared, and it is clear from **Fig.7**C that the extraction time is the same whether the system is heated *via* microwaves or heating bath. The second point is that the maximum TPC yield, which in both cases is achieved at 80˚C, is 163 mg GAE/g in MAE compared with 150 mg GAE/g in CSE, which is an increase of 8%. This supports results in the literature [35], and by using the same heating profile in both sets of experiments confirms, for the first time, that there is a selective heating effect when microwaves are used (as all other experimental parameters were the same), and this leads to an increase in TPC yield. However, **Fig.7**A-C also shows that the TPC yield for MAE and CSE is the same at 40 ˚C; at 60 ˚C MAE yields are slightly than CSE, while at 80 ˚C the advantage of MAE is significant (as stated above). **Fig.8** shows the dielectric loss of the 50% ethanol solvent from 20 – 80 ˚C and the wet powder (dried sea buckthorn leaves wet to their original moisture content with 50% ethanol solvent) from 20 – 70 ˚C. Of course these measurements do not exactly represent the dielectric properties of both phases throughout the extraction process, as they will change during processing, but they give a good indication of the relative dielectric properties of the solid and solvent phase and therefore the propensity for selective heating at different solvent concentrations.

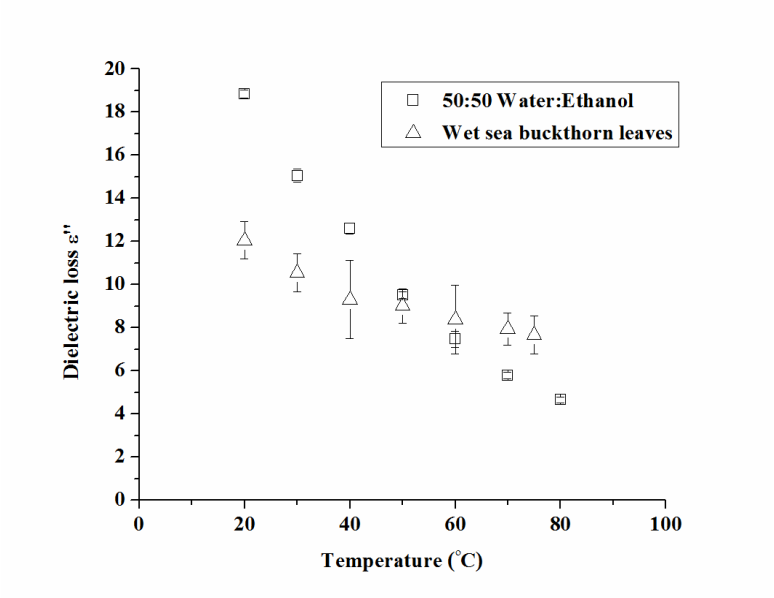


Fig.8. Dielectric loss, ε", of solvent of 50% ethanol concentration from 20 -80 ˚C and wet sea buckthorn leaves (67% solvent) from 20 – 75 ˚C; mean±S.D. (n = 3)

The dielectric loss, which indicates the ability of a material to convert microwave energy into heat, is higher in the solvent up to 40 ˚C, the same at 50 ˚C, and higher in the plant matrix at and above 60˚C; in other words, the plant matrix will only be selectively heated above 50˚C. These results correspond directly with the results in **Fig.7**A-C, which show that microwave heating only offers an increased yield at and above 60˚C, and that this advantage increases with temperature. This result supports the findings of Lee at al. [54] and suggests that increasing the electric field in the microwave cavity design will lead to further increases in yield as the selective heating effect will be more pronounced.

3.5. Compositional analysis of the extracted products and the effect of processing conditions

HPLC was used to identify and quantify polyphenolic compounds. The total mass of polyphenolic compounds identified *via* HPLC showed the same trends as the TPC identified

using the modified Folin-Ciocalteu colorimetric method. However, there was a large disparity between the magnitudes of total phenolics detected. For instance, at 40 ˚C, 1200 rpm and 600 s, the TPC *via* the Folin-Ciocalteu method was 110 mg GAE/g plant, while the total mass of polyphenols detected using HPLC was 18.9 mg/g plant. This discrepancy is in line with data presented in other papers [28, 42], and is likely to be the result of extraction of phenolic acids in their free and bound forms as their esters and glycosides. The ratio of free and bound forms may be of 1 to 6. The bound phenolic acid reacts with Folin-Ciocalteu reagent for TPC, but can not be determined by HPLC until a more complex hydrolysis procedure in which some compounds may be lost [57]. Also,some non-phenolic compounds like amino acids, proteins, nucleotide bases, unsaturated fatty acids, carbohydrates etc., can be extracted and are reactive towards the Folin-Ciocalteu reagent [58]. Therefore, the TPC results give an excellent indication of the trends of polyphenolic extraction, but they do not directly quantify the polyphenolic compounds extracted. The discrepancy was slightly higher at 80 ˚C (where HPLC accounted for around 15% of the GA equivalent) than at 40 ˚C (where HPLC accounted for around 17% of the GA equivalent), but the overall trends for higher polyphenolic compounds extraction with increasing temperature, time and stirring rate were still observed.

According to our previous study [28], the TPC values determined by the Folin-Ciocalteu method are close to the TPC values determined by the Differential Pulse Voltammetry (DPV) method (~70%), and are significantly higher than those obtained using the HPLC method (~16%). This strengthens our supposition that not all the extracted polyphenols can be identified by the HPLC method, because most of them are in their bound form, but the Folin-Ciocalteu method is representative for TPC analysis, and the HPLC method is helpful to determine the types of polyphenolic compounds extracted.

Fig.9A and B shows the results of the HPLC analysis for the samples extracted using MAE at 40 ˚C and 80 ˚C. From this data, it can be observed that the same polyphenolic compounds were identified in all samples, and their extraction trends largely followed those of the TPC. At 40 ˚C, the polyphenolic profile of the extract did not change with time or stirring rate; a general trend for an increase in the detected polyphenolics with time and stirrer speed was observed. At 80 ˚C, extraction of all identified compounds increased, but this increase was more marked in cafeic acid, rutin and its derivatives (unknown 1 and 2) than the more prevalent compounds gallic acid and catechin. A further change in extract composition was observed at 80 ˚C and the higher stirring speed of 1200 rpm. Referring to **Fig. 6**C, it can be seen that 80 ˚C and 1200 rpm were the only conditions under which a maximum in TPC yield was observed. At 300 s, maximum TPC yield had almost been reached, while by 600 s overall yield had decreased slightly. This corresponds with a slight decrease in gallic acid and catechins, and an increase in rutin from around 0.5 mg/g plant to 3 mg/g plant between 300 and 600 s processing time. Rutin is strongly linked to the sample matrix owing to its complex glycosidic structure [59], and therefore its extraction is likely to increase with temperature and time.

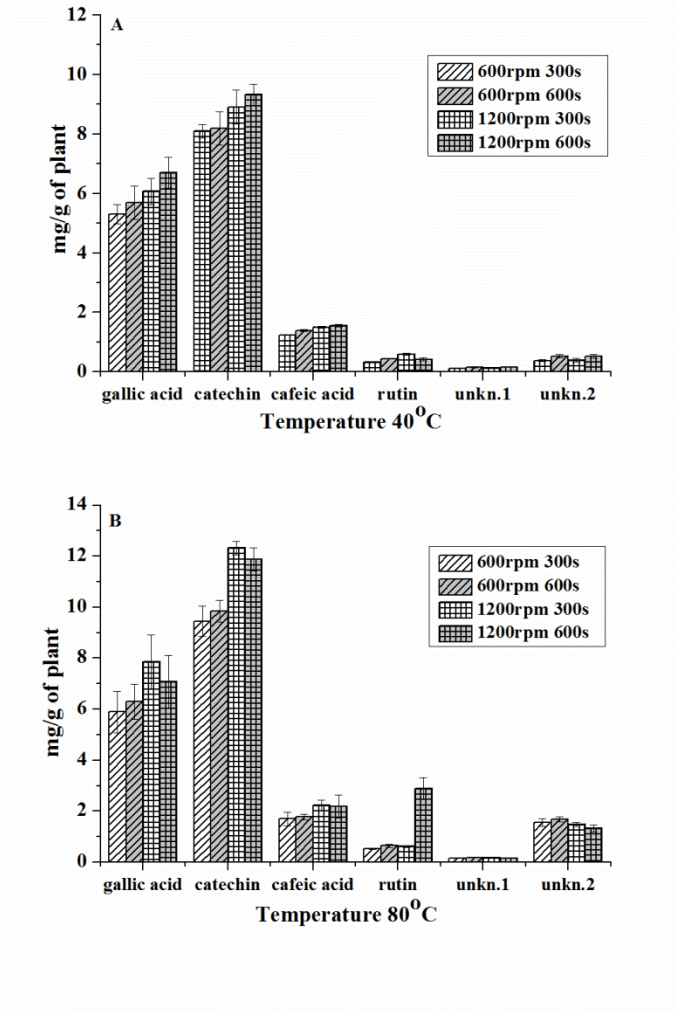


Fig.9. Selected polyphenols determined by HPLC analysis - in extracts of sea buckthorn leaves (extracted by MAE at 40 ˚C (**A**) and 80 ˚C (**B**) mean±S.D,(n=3)).

The results of the HPLC analysis comparing extracts of MAE and CSE under the optimized conditions of 80 ˚C, 1200 rpm and 450 s are shown in **Fig.10**. The calculated antioxidant activity of these extracts for MAE and CSE were 93.6 ± 0.5 and 71.5 ± 1.0 µmolTrolox/g respectively. The results agree with what would be expected from **Fig.10**, that the overall extraction of polyphenolic compounds is higher using MAE than CSE. The composition of polyphenolic compounds in the MAE and CSE extracts is also slightly different, with the

extraction of rutin proportionally higher in MAE (representing 6.6% of the MAE extract versus only 3.5% of the CSE extract). The composition of the other polyphenolic compounds in the MAE and CSE extracts were similar. The enhanced extraction of rutin by MAE is likely related to the strong links that rutin has to the sample matrix of the sample [59]. Rutin is difficult to extract *via* conventional methods and results presented in **Fig.9**A and B show that increasing MAE temperature improves rutin extraction. **Figs.7** and **8** suggest that there is a selective heating effect causing the difference in extraction results between MAE and CSE experiments (as the bulk temperature profile and all other experimental variables were the same). This could have enhanced the extraction of rutin in two ways. First, the sample may have been hotter than the bulk temperature measured in the sample. This would enhance extraction through the conventional mechanisms of decreasing surface tension and viscosity. Second, the chemical potential gradients [60] generated by selective heating (either of the sample or the solvent) could have led to mass transfer into or out of the plant matrix: if the sample was hotter than the solvent, the chemical potential would be higher in the solvent and therefore solvent would move into the sample, causing swelling and potentially rupturing the plant matrix, releasing rutin; if the solvent was hotter than the sample, the conventional solvent extraction mechanism of liquid moving out of the sample into the solvent would be enhanced. Either mechanism could lead to increased extraction of more recalcitrant compounds such as rutin. With the increase in severity of the extraction conditions, the ratio between the free radical and bonded polyphenols can be modified. This can be observed in the case of rutin which is bonded very strong with the plant matrix. The increase in free radical polyphenols can also increase the antioxidant activity [61].

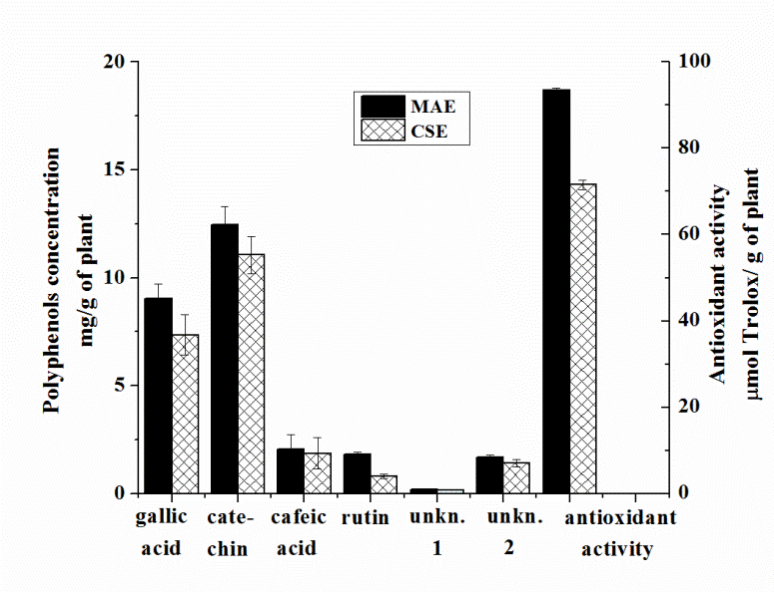


Fig. 10. MAE vs CSE - Selected polyphenols determined by HPLC analysis in extracts of sea buckthorn leaves and antioxidant activity (Extraction temperature 80 ˚C, extraction time 450 s and 1200 rpm stirring rate, mean±S.D, (n=3)).

4. Discussion

4.1 Microwave extraction mechanism

Many literature sources discuss the possible mechanisms of MAE when compared with CSE. In general, solvent extraction works by diffusion of the solvent into and through the cell walls and into the cells, where the target compounds are dissolved and then transported out of the plant matrix, through the solvent film surrounding the particle and into the bulk solvent [5]. In CSE, increasing temperature increases extraction rate in a variety of ways, such as enhancing desorption of solutes from the matrix, and reducing viscosity and interfacial surface tension, which favours the diffusion of solvent through the solid matrix and increases solubility. MAE is thought to enhance solvent extraction through volumetric heating, which means that heat transfer and mass transfer are both working from the "inside out" rather than heat transfer having to come more slowly from the outside [5], which could enhance extraction in the same ways as mentioned for CSE above. However, in this work we have shown that a CSE experiment can be designed to provide the same bulk heating rate as MAE, and that in this case the extraction rates are the same. The second common hypothesis is that microwave heating can lead to physical

effects such as cell disruption, whether this is by superheating of the cell structures containing the target compounds [62] or by changes in the chemical potential differences between phases caused by selective heating [54]. This work supports this hypothesis, clearly confirming that for MAE to offer a fundamental advantage in solvent extraction, the plant matrix must be selectively heated. Further work needs to be done on the clarification and quantification of this mechanism. For instance, it is not known whether cell rupture is actually required, or whether the change in thermodynamics (described by chemical potential gradients) caused by temperature differences in the plant matrix inherently leads to increased yields without the need for cell disruption.

The results reported here (**Fig. 2**) show that the set power is only applied for around 30 s, after which time the set point is reached and the power is reduced to maintain the set temperature. This could possibly support the theory that some type of cell disruption early in the treatment is responsible for the difference in results between MAE and CSE, and that the extraction time is dictated by diffusion of the solvent and solute through the plant matrix, which is not changed by selective heating. However, it is also plausible that the small power that is applied throughout the holding phase maintains a temperature difference between the plant matrix and the solvent, and this changes the thermodynamics and therefore the equilibrium yield. Further work is needed to clarify this.

4.2 Implications for engineering design and scale-up

The existing examples of scale-up MAE processes have tended to use non-polar solvents [62]; this has the advantages of maximizing the selective heating of the plant matrix and the penetration depth (as non-polar solvents are microwave “transparent”), leading to low energy requirements and scope for larger reactor dimensions. However, the use of “greener” solvents such as water and ethanol, or even solvent free processing [63], is increasingly preferred by industry. In this paper we have shown for the first time that even in polar solvents under certain conditions the plant matrix can selectively heat. Scale-up therefore must concentrate on

identifying the processing conditions under which selective heating can be maximized and identifying which processes offer an economic advantage. This will mean: (1) operating within the temperature range of selective heating; (2) quantifying the mechanism by which selective heating increases yield and using this knowledge to optimize power delivery to the materials through electromagnetic design; (3) understanding the limitations of penetration depth in the design, and (4) understanding the theoretical energy requirements of the system. This last point is elaborated on below based on new findings in this paper.

In this plant-extract system, microwave heating does not directly result in a faster extraction time, and this means that the theoretical energy requirements of CSE and MAE for this process should be expected to be similar (although the yield produced for the same energy will be higher in a microwave process). The theoretical energy requirements can be approximated as the energy required to heat the solvent from 24 ˚C to 80 ˚C (225 J/g for 50% ethanol based on composition-enthalpy diagrams [64]) plus the heat losses during treatment, any exothermic or endothermic processes resulting from extraction (e.g. heat of mixing as components diffuse through the plant matrix, and the heat of the adsorption/desorption processes), and any energy absorbed by the system (e.g. microwave power absorption by system components). The heat losses for the Miniflow system were calculated from the thermal profile for the microwave experiment in **Fig.2** using **Equations 3 – 6** [65] and using the following assumptions.

- Stationary state: Instantaneous thermal equilibrium without tangential heat flows across the surface boundaries.

- Losses independently considered for each axis, i.e. radius and height for the cylindrical container.
- Thermal expansions/contractions are negligible, thus heat exchange surface area is constant.
- The lack of Pyrex on the top of the fluid does not affect the heat exchange between the fluid and the surrounding air.
- Thermal gradients across the thickness of the Pyrex are negligible.
- The fluid is heated homogeneously across its volume.

$$q_L = U\,A(T_F - T_A) = \frac{K_P}{e_P} A(T_F - T_P) = (h_N + h_R)\,A(T_P - T_A) \quad [3]$$

$$\frac{1}{U} = \frac{e_P}{K_P} + \frac{1}{h_N + h_R} \quad [4]$$

$$h_N = 1.18\left(\frac{T_P - T_A}{d/2}\right)^{0.25} \quad [5]$$

$$h_R = \sigma F \frac{{T_P}^4 - {T_A}^4}{T_P - T_A} \quad [6]$$

A (m^2) is the heat exchange area, T_F, T_P and T_A (K) are the temperatures of the fluid, the Pyrex and the at the ambient air respectively, K_P (J/g K) is the thermal conductivity of Pyrex [66], e_P (m) is the thickness of the Pyrex (2 mm), σ is the Boltzmann constant, F is the grey vision factor (0.95) [65], d is the diameter of the Pyrex container (37 mm), h_N and h_R (J/m^2 s) are the air natural convection and radiation heat transfer coefficients and U (J/m^2 s) is the global heat transfer coefficient. Using this method, the total heat losses for the MAE heat profile shown in **Fig.2** were estimated to be 1571 J, which accounts for around 30% of the total energy absorbed by the system during extraction. In a scaled up system, the proportional heat losses would vary depending on the design of the system, and the other energy requirements would be

fixed by the solvent composition, plant to solvent ratio, and target biomass and extract. If an industrial CSE process is to be designed that exploits the fast heating rate demonstrated in these experiments, a large contact area would be required, therefore increasing complexity and capital cost compared with a conventional Continuously Stirred Tank Reactor. However, the microwave process would also be limited by the penetration depth of the microwaves, and so a long tubular reactor would likely be indicated, potentially leading to significant heat losses. Therefore, the overall capital and operational costs of the processes would depend on the design configuration chosen as well as the selection of heating technology.

5. Conclusions

The aim of this work was to carry out a direct comparison of MAE and CSE changing only one variable at a time, using as a model the extraction of polyphenols from dried sea buckthorn leaves. An extract with a TPC of up to 162 mg GAE/g of plant material and an Antioxidant Activity of 93.6 ± 0.5 μmolTrolox/g was extracted under the optimal conditions investigated here, which were MAE using 50% aqueous ethanol, 1:20 plant to solvent ratio, treatment temperature 80˚C, time 450s and stirrer rate 1200 rpm.

For the first time, MAE results were compared with CSE using exactly the same experimental parameters; the bulk heating profile, reactor geometry, stirrer speed, solvent composition and plant to solvent ratio were the same. It was found that maximum yield could be achieved within the same timeframe as the MAE experiments, but that the CSE extract has a lower TPC (150 mg GAE/g) and Antioxidant Activity (71.5 ± 1.0 μmolTrolox/g). Many authors cite short treatment times leading to reduced energy requirements as potential advantages of MAE compared with CSE. However, in the small system investigated here, we have shown that

the theoretical energy requirements to heat a given amount of sample at the same plant to solvent ratio are the same (because the bulk temperature in both experiments was the same for the same amount of time). Of course, in a larger CSE system, heat transfer limitations would mean that the heating profile achieved here would not be easily achieved. Therefore, in both cases (CSE and MAE), the efficiency achieved here will be compromised if the systems are scaled up without careful consideration of heat transfer and, in the case of MAE, dielectric properties and penetration depth.

Even though there is no advantage in terms of theoretical energy requirement per mass of biomass treated, MAE does offer higher yield of polyphenolic compounds and superior Antioxidant Activity compared with CSE. **Fig.7**, which shows extraction results where the only experimental variable was the heating source (microwave versus hot bath), and all other experimental variables were the same, suggests that this is a result of selective heating affecting the extraction mechanism, and the dielectric properties presented in **Fig.8** support the conclusion that the plant matrix was hotter than the bulk temperature measured in the sample. The difference in the yields achieved in this work using a single mode cavity together with impedance matching, compared with the results from the multimode cavity without impedance matching used in our previous research [28], illustrate the importance of microwave cavity design and suggest that further increases in yield and possibly extract quality could be achieved in a higher electric field cavity, which would enhance the selective heating effect.

More work needs to be done on understanding the effect of electric field strength on extraction and the potential commercial value of the extracts before a techno-economic assessment of whether the selective heating advantage of MAE can be translated into a driver to

commercialize this technology for the valorization of biomass byproducts such as sea buckthorn leaves can be made.

Acknowledgements

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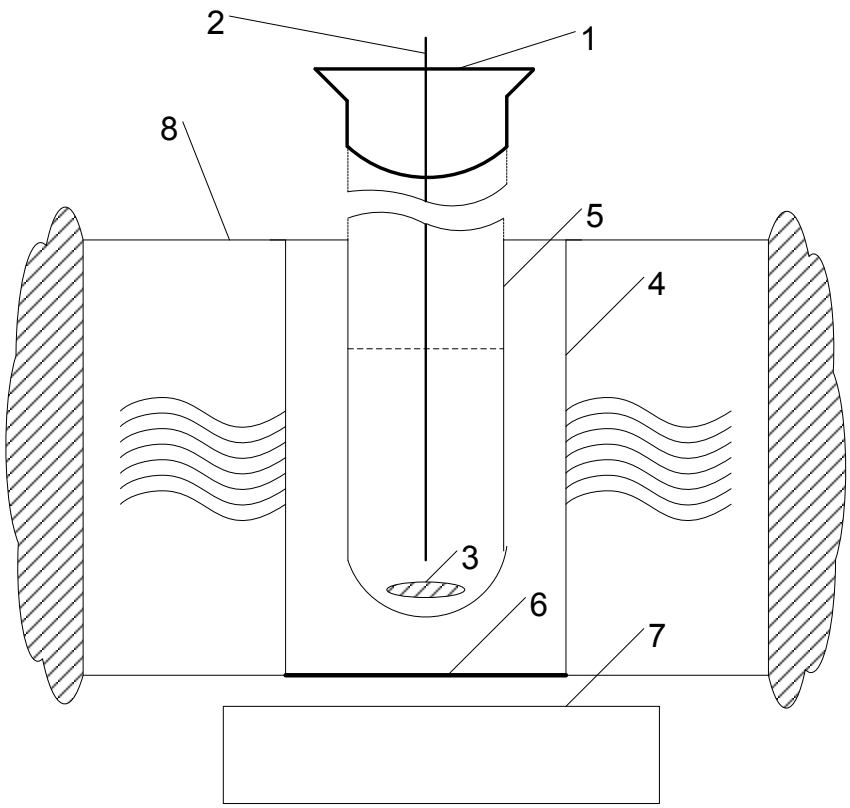


Fig. 1. Schematic diagram of MAE (1-rubber stopper, 2-optical fiber, 3- magnetic stirrer, 4 - TE_{10n} single mode cavity, 5- pyrex reactor, 6-aluminium lid and quick release clamp, 7-stirrer plate, 8- WE340 waveguide)

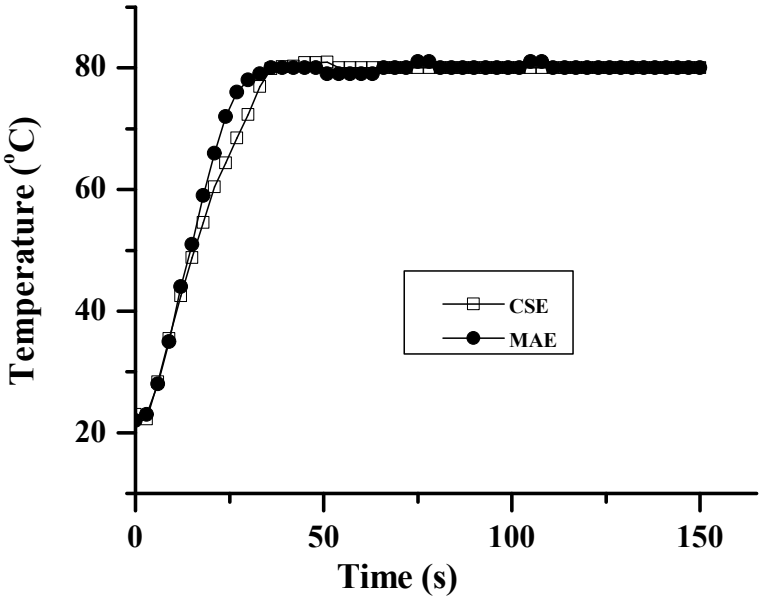


Fig.2. Bulk temperature profile for MAE and CSE experiments with an 80 ℃ set point

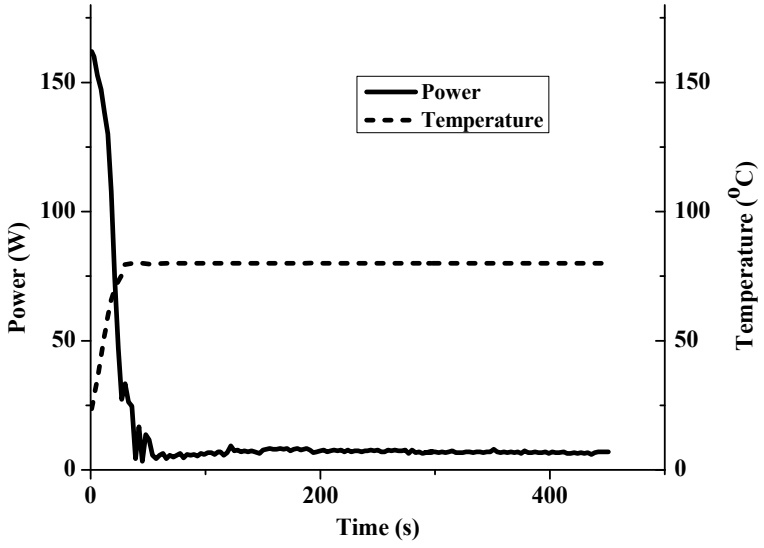


Fig. 3. Absorbed power and temperature profiles for a typical MAE experiment (extraction temperature 80 ˚C, extraction time 450 s, stirring rate 1200 rpm and ratio of plant to liquid 1:20 (w/v), 50% ethanol (v/v)).

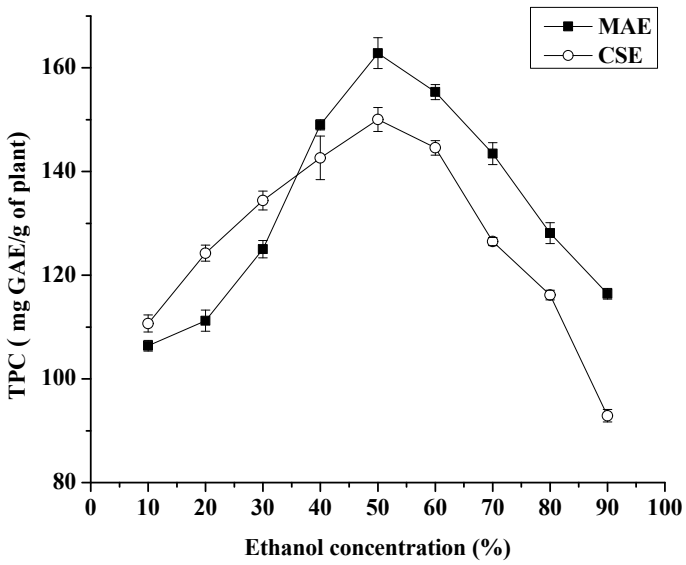


Fig. 4. Effect of ethanol concentration in water on the extraction of TPC (extraction temperature 80 ˚C, extraction time 450 s, stirring rate 1200 rpm and ratio of plant to liquid 1:20 (w/v), mean± 1 S.D. (n = 6, triplicate extraction and duplicate analysis))

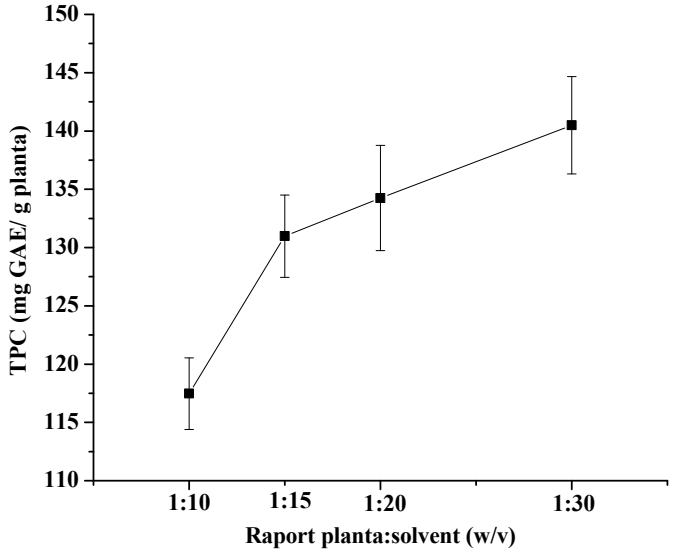


Fig. 5. Effect of ratio solid to liquid on the extraction of TPC during MAE (extraction temperature 60 ˚C, extraction time 450 s, stirring rate 900 rpm, ethanol 50% (v/v), mean±S.D. (*n* = 6, triplicate extraction and duplicate analysis))

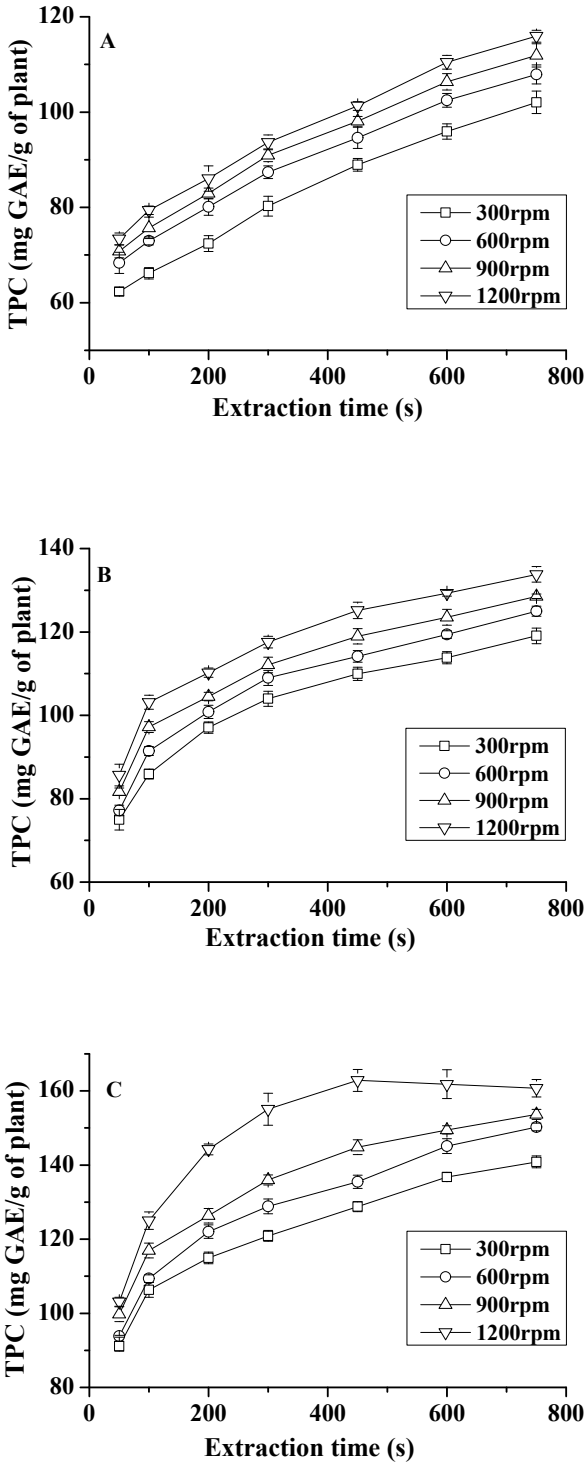
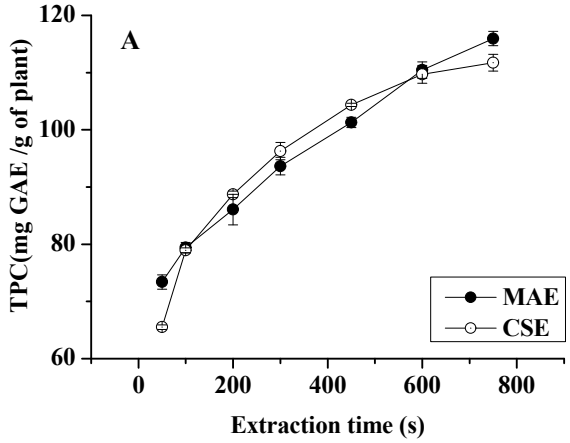
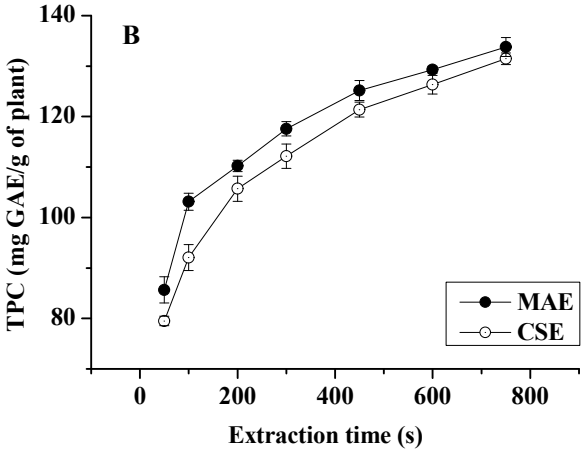


Fig.6. Effect of different extraction time and different stirring rate on the extraction of TPC during MAE (extraction temperature 40 °C (**A**), extraction temperature 60 °C (**B**), extraction

temperature 80 ˚C (**C**), ethanol concentration 50%, ratio plant: solvent 1:20 (w/v), mean±S.D. (*n* = 6, triplicate extraction and duplicate analysis))


A
TPC(mg GAE /g of plant)
120
100
80
60
0
200
400
600
800
Extraction time (s)
MAE
CSE


B
TPC (mg GAE/g of plant)
140
120
100
80
0
200
400
600
800
Extraction time (s)
MAE
CSE

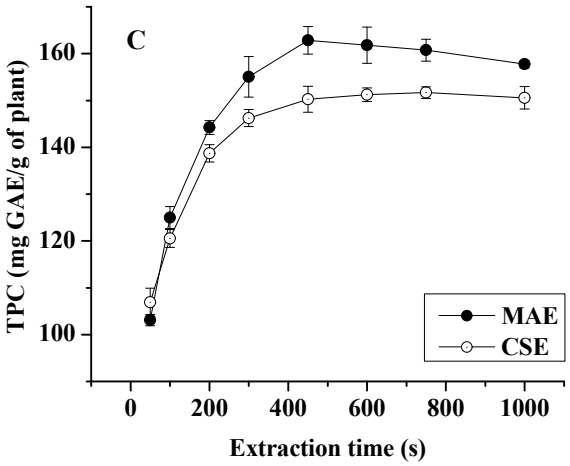

C
TPC (mg GAE/g of plant)
160
140
120
100
0
200
400
600
800
1000
Extraction time (s)
MAE
CSE

Fig.7. TPC for MAE and CSE (extraction temperature 40 ℃ (**A**), extraction temperature 60 ℃ (**B**), extraction temperature 80 ℃ (**C**), stirring rate 1200 rpm, ethanol concentration 50%, ratio plant to solvent ratio 1:20 (w/v), mean±S.D. (*n* = 6, triplicate extraction and duplicate analysis))

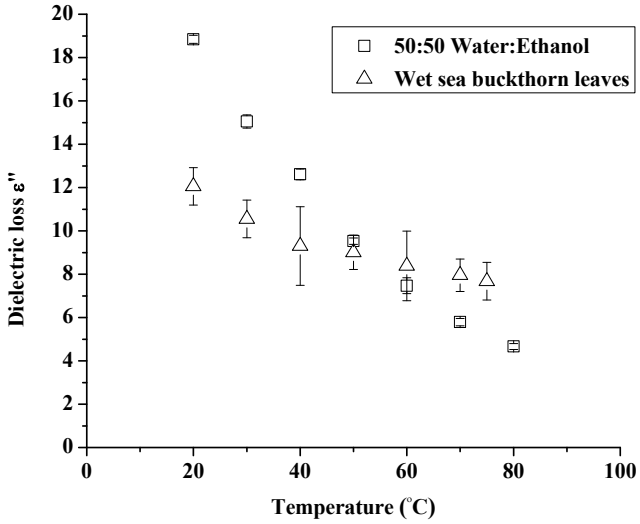


Fig.8. Dielectric properties of solvent of 50% ethanol concentration from 20 -80 ˚C and wet sea buckthorn leaves (67% solvent) from 20 – 75 ˚C (b) dielectric loss, ε", (; mean±S.D. (*n* = 3, measurements were performed in triplicate

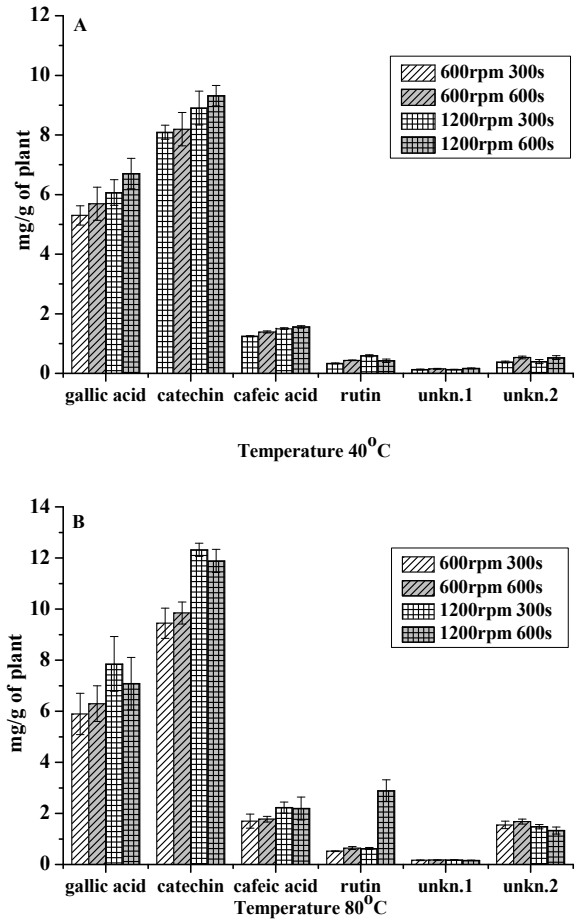


Fig.9. Selected polyphenols determined by HPLC analysis - in extracts of sea buckthorn leaves (extracted by MAE at 40 ºC (**A**) and 80 ºC (**B**) mean±S.D,(n=3)).

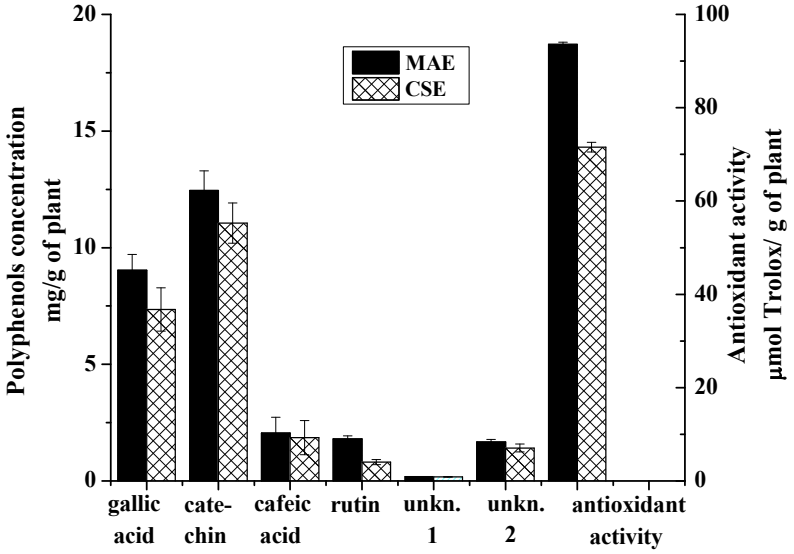


Fig. 10. MAE vs CSE - Selected polyphenols determined by HPLC analysis in extracts of sea buckthorn leaves and antioxidant activity (Extraction temperature 80 ˚C, extraction time 450 s and 1200 rpm stirring rate, mean±S.D, (n=3)).

Highlights

- A direct comparison of MAE and CSE using the exactly same experimental parameters.
- A selective heating effect when MAE is used, which leads to an increase in TPC.
- The same polyphenolic compounds were identified in all samples.
- An increase in the detected polyphenolics with time and stirrer speed.