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Title: Effect of ultrasound transducer design on the acoustically-assisted supercritical fluid extraction of antioxidants from oregano.

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[1]

[1] CO_2 – carbon dioxide, FRAP - ferric reducing/antioxidant power; GA – gallic acid; HPLC – high pressure liquid chromatography; NUS – no ultrasound; PU – power ultrasound; SFE – supercritical fluid extraction; T1 – transducer 1; T2 – transducer 2; T3 – transducer 3; T4 – transducer 4; TE – Trolox equivalents; TPC – total phenol content; US – ultrasound; USFE – ultrasound assisted supercritical fluid extraction

Abstract

Power ultrasound is applied in food technology to intensify extraction processes, due to the phenomena ultrasonic energy induces in the medium, enhancing mass transfer. The purpose of this work was the acoustic characterization of four transducers of different geometries and the evaluation of their performance in the ultrasonically assisted supercritical fluid extraction of antioxidants from oregano. The transducers differed in the amount of energy transmitted into the medium. Designs varied from the base model (T1), a larger cylindrical headmass (T2), a stepped circular section sonotrode (T3) and a multiplate configuration (T4). The highest nominal power density provided according to the calorimetric method was for T4 (151.6±7.1 W/L). The T2 produced a more uniform acoustic field and a higher acoustic pressure (150.6±20.5 kPa). Both parameters had an impact on total phenolics and antioxidants extraction with CO_2 under supercritical conditions (35 MPa, 35 °C, 2.3% ethanol as co-solvent). T4 and T2 were equally efficient (4.0±0.2 and 4.2±0.2 mg GA/g) for phenolic extraction, and with respect to antioxidant capacity, the best performance was that of T4 (26.4±1.1 µmol TE/g). Of the antioxidant compounds extracted, flavones and flavanones were identified. Therefore, transducer geometry influenced the amount and distribution of energy transmitted into the medium, thus determining the efficiency of the extraction process.

Keywords: acoustically assisted extraction, antioxidants, phenolics, supercritical fluids, transducer, ultrasound.

1. Introduction

Power ultrasound (PU) generates structural, physical or chemical changes in the medium through which it propagates. Due to these properties, PU has been proposed as an emerging technology for food processing, such as microbial inactivation, drying, sonocrystallization, and extraction [1]. PU induces phenomena in the medium, such as acoustic streaming and cavitation, that lead to intense agitation and mass transfer enhancement [2]. Such intensification effects have been observed during the application of ultrasound in food drying [3], pork meat salting in sodium chloride [4] and innovative methods for sample preparation by solid phase microextraction [5–9].

One of the applications of ultrasound is the intensification of bioactive compound extraction from vegetal substrates under atmospheric conditions, requiring immersion of the sample in a liquid solvent. To generate ultrasound, piezoelectric transducers are commonly used, since they are compact and relatively inexpensive. These transducers are composed of piezoelectric ceramics that vibrate at a particular frequency, creating mechanical energy when are supplied with electrical energy [10]. Although ultrasonically assisted extraction has been the subject of many studies, problems arise when trying to compare different transducers. In most cases, the only information regarding ultrasound equipment performance is the amount of electrical energy supplied to the apparatus. This is not an accurate approach since ultrasound performance will depend on various factors such as the medium's physicochemical properties, temperature, pressure, generator efficiency and the size and geometry of the transducer, and consequently the acoustic fields are not easy to characterize [11,12].

To determine the actual amount of power delivered by a transducer, various methods have been developed by measuring the effects of ultrasound on the fluid, such as thermal, mechanical, optical and chemical effects [13]. Among these procedures, the use of calorimetry stands out as a relatively simple and feasible way to determine the electrical/acoustic energy conversion rate by measuring the rise in the

medium's temperature during the period of ultrasound application [14]. However, through this approach there is no distinction between waste heat and acoustically generated heat [13]. On the other hand, hydrophones are useful for acoustic field characterization, since ideally the device itself has minimal effect on the acoustic field [12]. However, hydrophones pose the disadvantage that the cavitational effects may generate interference during the measurement [14]. In consequence, an adequate analysis of the acoustic energy transferred to the medium requires more than one technique.

One of the recent applications of ultrasound is as part of the intensification of the supercritical fluid extraction (SFE) of high added value compounds, such as antioxidants [15], essential oils, seed oils, oleoresins, carotenoids, phenolics and tocopherols, among others [16]. Considering that the extraction yields of SFE can be low and the kinetics slow, PU has been proposed as a means of improving the feasibility of industrial application through an increase in the yield or a decrease in the extraction time by means of phenomena such as compression/decompression, acoustic streaming and cavitation [17]. Ultrasound can be incorporated into the extraction vessel in different ways: the probe can be introduced into the SFE cell but the ceramics are placed outside (Fig 1A) or the whole transducer is placed into the extraction cell (Fig**Error! Reference source not found.** 1B). A third possibility is the non-direct application of ultrasound in the extraction cell, either with a horn-type device (Fig 1C) or with an ultrasound bath (Fig 1D) using a fluid as the coupling medium. At present, ultrasonically-assisted supercritical fluid extraction (USFE) has been studied for the purpose of recovering natural products, such as seed oils [18,19], antioxidants from plant sources [20,21], and capsaicinoids [21,22]. The use of probes placed inside the extraction cell is common, ideally inserting the whole transducer in the cell (Fig 1B), such as in the extraction of ginsenoides from ginseng [23] or cocoa butter from cocoa beans [24], and can be implemented at industrial scale [19,25].

The use of ultrasound during SFE helps to promote cell disruption [26] and enhance solute diffusivity [25], accordingly intensifying the process. However, the effect of the geometry of the ultrasonic transducer/probe on the ultrasound-assisted supercritical fluid extraction (USFE) process has not been analyzed. Therefore, the aim of this work was to evaluate the effect of transducer geometry on the ultrasound intensity, acoustic pressure and USFE intensification of total phenolics and antioxidant compounds from oregano.

2. Materials and methods

2.1. Transducer design

Four customized transducers of different geometries (Fig 2 T1-T4) were designed and constructed by the ASPA Group of the Universitat Politècnica de València, Spain. In every case, sandwich-type piezoelectric ultrasonic transducers were considered. The transducers consisted of a metal tail mass ([1], Fig 2, T1), a pair of piezoelectric ceramics ([2], Fig 2, T1; an external diameter of 36.8 mm, an internal diameter of 12.5 mm, a thickness of 5 mm, a resonance frequency of 30 kHz) and an aluminum (ASTM 7075) head mass ([3], Fig 2, T1). Although aluminum has a non-ideal acoustic response, it was chosen to facilitate mechanization of the head mass. In every case, the tail mass (carbon steel) and the piezoelectric ceramics were the same and the design differed in the head mass only, which is the element that directly irradiates the ultrasound vibration to the solvent and product subjected to extraction. Transducers 1 (Fig 2, T1) and 2 (Fig 2,T2) were rod-shaped head mass transducers, differing in the length of the head mass (18.5 mm and 35.8 mm, for T1 and T2, respectively). Transducer 3 (Fig 2, T3) consisted of a stepped circular section head mass with an input (surface attached to the piezoelectric ceramics) diameter and length of 36.8 mm and 15.8 mm, respectively, and an output diameter and length of 18.0 and 30.0 mm, respectively. Transducer 4 (Fig 2, T4) was a multiplate circular head mass mechanized as a whole piece. As can be observed in Fig 2, this arrangement allowed T4 to have five irradiation faces with a diameter of

36.8 mm. In all cases tail and head mass were attached to the ceramics in a sandwich-type configuration using a carbon steel screw (M12x1.25). The length of the transducers were limited by the dimensions of the extraction vessel (50 mm diameter, 120 mm length) and the need of filling the extraction cell with the sample to be processed. Although the efficiency of the transducers was limited by the fact of using a length different of a multiple of half of the wavelength emitted from ceramics (λ/2), in all cases the energy supplied by the transducers allowed to observe cavitation in water.

2.2. Characterization of the acoustic field

The energy delivered to the medium by each transducer was estimated by two methods: calorimetry and acoustic pressure, as reported in previous works [14,27]. Due to the highly complex nature of conducting the measurements in a supercritical fluid, water in atmospheric conditions was used as a medium. This procedure allows the comparison between the customized transducers designed in this work and provides a reference of energy for comparison with other studies.

For the calorimetric assessment, the transducer was submerged in a thermally isolated vessel containing 160 mL of distilled water at room temperature. Ultrasound was applied for 90 s through an ultrasound generator (60 W and 30 kHz, FSP300-60BTV, FSP Group Inc., Taoyuan City, Taiwan) coupled to a power meter (WT300-760401, Yokogawa Iberia S.A., Madrid, Spain), as reported by Cárcel [14]. The increase in water temperature during this time was recorded using a type-K thermocouple (0.15 s response time) placed at the bottom of the water vessel and connected to a data logger (HP Data Logger 34970 A, Hewlett-Packard Española, S. A., Madrid, Spain). Ultrasound power was calculated following

$$P = mC_p \frac{dT}{dt}$$

Equation 1:

$$P = mC_p \frac{dT}{dt}$$

Equation 1

where *P* represents the ultrasonic power (W), C_p is the water heat capacity and *dT/dt* is the increase in temperature with respect to time. Nominal power density (W/L) was determined by dividing *P* by the volume of water used in the experiment. Each measurement was carried out four times.

The acoustic pressure (kPa) was determined using a hydrophone (TC4013, Reson A/S, Slengerup, Denmark) connected to a digital oscilloscope (Tektronix TDS 420 A, Tektronix Inc. Oregon, USA). The hydrophone was placed in a rectangular, non-thermally isolated container with distilled water at a 15 mm distance from the bottom (Fig 3A). Measurements were taken at room temperature at equidistant points along the length and the width of the vessel, creating a mesh of 9 nodes, which covered an area of 120x100 mm under the transducers (where most of the acoustic energy was focused. The sonotrodes were located in the central node, as shown in Fig 3B. The points were previously marked on the surface of the container to facilitate the hydrophone location. Three measurements were taken in each node and the experiment was repeated three times for each transducer. The sound pressure for each transducer was calculated by averaging the measurement in each node.

2.3. Ultrasonically-assisted supercritical fluid extraction (USFE)

Extraction experiments were carried out in a customized supercritical fluid pilot-scale plant designed and built by the ASPA group of the Universitat Politècnica de València, Spain (Fig 4) to work at pressures and temperatures up to 70 MPa and 70 ºC, respectively. The plant design included a CO_2 tank at room temperature (1, Fig 4), an ethanol reservoir at room temperature (2, Fig 4), an ethanol pump (3, Fig 4, ISCO 100DM, Teledyne ISCO, VERTEX Technics, S.L., Barcelona, Spain), a chiller reservoir for liquid CO_2 at -7 ºC (4, Fig 4), a pump for liquid CO_2 (5, **Error! Reference source not found.**Fig 4, LDB1, Lewa, Leonberg, Germany) connected to a variable speed drive in order to control CO_2 pressure (1.5 kW, 2 hp, 240V,

ATV12HU15M2, Schneider Electric Spain, Barcelona, Spain), a T-section where ethanol and CO_2 were mixed, (6, **Error! Reference source not found.**Fig 4), a temperature-controlled water bath (7, Fig 4**Error! Reference source not found.**), an extraction vessel (8, Fig 4), an extraction cell (9, Fig 4), a separation vessel (10, Fig 4), a manual valve for extract collection (12, Fig 4), an ultrasound transducer (13, Fig 4) and an ultrasound generator (14, Fig 4, (60 W and 30 kHz, FSP300-60BTV, FSP Group Inc., Taoyuan City, Taiwan) coupled to a power meter (WT300-760401, Yokogawa Iberia S.A., Madrid, Spain). An immersed electrical resistance, a type-K thermocouple and a digital controller (E5CK, OMRON, Madrid, Spain) regulated the water temperature. Pressure (P, Fig 4) and temperature (T, Fig 4) gauges were installed in the extractor to monitor supercritical conditions during the process.

Every experiment was carried out at under the same conditions of pressure (35 MPa), temperature (35 ºC) and co-solvent ratio (2.3%) during 60 minutes, placing 5.5 ± 0.5 g of dry oregano inside the extraction cell. Extract samples were collected at 15, 30 and 60 minutes. Dry oregano was purchased at a local market in Valencia (Terra Verda, Valencia, Spain) in February 2017. For each transducer, analyses were carried out with (US) and without ultrasound (NUS). The chiller reservoir was supplied with carbon dioxide gas (99.9%, Abelló Linde S.A., Barcelona, Spain), where it was liquefied at -7 ºC. Ethanol (96% pharma grade, AppliChem GmbH, Darmstadt, Germany) was compressed to the operation pressure and programmed to flow at 0.5 mL/min. Compressed liquid CO_2, at a flow rate of 1 ± 0.1 kg/h, was mixed in a T-section with the co-solvent and the mixture was injected into the extraction unit in supercritical state. The extract was isolated from the gaseous CO_2 at the separator, where the supercritical fluid was decompressed to 6.0 ± 0.5 MPa and the gas was recirculated to the chiller reservoir. Total polyphenol content and antioxidant capacity were evaluated and the extracts were evaporated to dryness under reduced pressure.

2.4. Total polyphenol content (TPC) and antioxidant activity evaluation

Total polyphenol content (TPC) was evaluated by the Folin-Ciocalteau method based on Gao et al. (2000) [28]. The extracts were appropriately diluted in pharma-grade 96% ethanol for analysis. A spectrophotometer (Helios Gamma+, Thermo Scientific, Madrid, Spain) was used to record absorbance at 765 nm and the concentration was calculated using a gallic acid (Sigma-Aldrich, St. Louis, MO, USA) standard curve. Ethanol mixed with all the reagents was used as blank. The results were expressed as milligrams of gallic acid/gram of oregano (mg GA/g).

The antioxidant capacity of the extracts was measured by the ferric reducing/antioxidant power assay (FRAP) [29]. The absorbance was recorded at 595 nm and the antioxidant capacity was evaluated using a Trolox (6-hydroxy- 2,5,7,8-tetramethylchroman-2-carboxylic acid, Sigma-Aldrich) standard curve. Ethanol mixed with all the reagents was used as blank. The results were expressed as micromoles of Trolox equivalents per gram of oregano (μmol TE/g).

2.5 Compound identification

Compounds in the extract were identified by high pressure liquid chromatography with diode array and mass detector as previously reported [30]. The dry samples were re-suspended in HPLC grade (BDH, Poole, UK) methanol:water (1:1 v/v) with the aid of an ultrasound bath before analysis.

2.6. Statistical analysis

Every extraction was performed in triplicate. The results were reported as the average ± the standard deviation and were subjected to analysis of variance, followed by Tukey's post-hoc test to determine statistical differences with a 95% level of significance. The correlations were carried out by means of Spearman's analysis. The statistical tests were carried out using STATGRAPHICS 5.1 (Statistical Graphics Corp., The Plains, USA).

3. Results

3.1. Characterization of the acoustic field

Ultrasound transducer characterization obtained through calorimetry (Table 1) showed that T4 and T3 imprinted the greatest nominal power density into the medium, 151.6±6.1 W/L and 136.57±6.16 W/L, respectively. The transducer supplying the lowest amount of power was T1, with only 83.5±17.7 W/L, but it also presented the greatest variability. In turn, T2 transmitted an intermediate level of power (116.4±7.7 W/L), being significantly ($p<0.05$) different to the rest of the transducers.

The four transducers evaluated in this work showed significant differences ($p<0.05$) in the average acoustic field pressure (Table 1). T1 (Fig 5A) and T3 (Fig 5C) showed the lowest average acoustic pressure (84.5 ± 3.2 kPa and 83.2 ± 5.4 kPa; Table 1). T1 distributed the pressure evenly across the horizontal plane. Contrastingly, T3 focused most of the acoustic energy in the center of the field, at levels between 80 and 110 kPa, while a lower pressure was found at the edges (50-80 kPa, Fig 5C). T2 (Fig 5B) showed the highest average acoustic pressure (150.6 ± 20.5 kPa, Table 1) mostly concentrated around the edges (140-200 kPa). However, it presented a variable distribution, as we can see zones of lower pressure (110-140 kPa in the center. T4 provided an average acoustic field of 99.3 ± 12.8 kPa (Table 1), with most of the acoustic pressure concentrated around the center (Fig 5D, 110-140 kPa), an extensive area of intermediate pressure (80-110 kPa) and a small area of low pressure at the right-hand edge of the field (50-80 kPa). The acoustic pressure measured in this work only indicates the average energy on a layer of water located 72 mm from the transducer's surface. The differences found in Table 1 are due to the different length and shape of the head mass of the sonotrode, which influences both ultrasound power and distribution. However, nominal power density shows the average energy supplied per volume of treated medium, which probably is a better indicator of the ultrasound intensification effect during supercritical fluid extraction.

3.2. Effect of the type of transducer on the USFE of phenolic compounds and the antioxidant capacity

The transducer geometry, extraction time and application of ultrasound (US) were all significant ($p<0.05$) factors that affected the extraction of total phenolics. Additionally, the first-order interactions between transducer geometry and US application, as well as between transducer geometry and time, were significant ($p<0.05$). The best performances were reached with T4 (4.2±0.2 mg GA/g) and T2 (4.0±0.2 mg GA/g), according to Tukey's test, followed by T1 (3.0±0.2 mg GA/g) and T3 was the least efficient (2.1±0.2 mg GA/g). In general, the use of ultrasound allowed us to obtain significantly ($p<0.05$) larger amounts TP (US, 3.6±0.1 mg GA/g) than when no ultrasound (NUS, 3.0±0.1 mg GA/g) was applied. For T1 (Fig 6A), ultrasound application only produced a small increase in TPC, reaching maximum values after 60 min (4.6±0.3 mg GA/g NUS and 5.0±0.1 mg GA/g with US). Using T2 (Fig 6B), the extraction of phenolics after 60 min changed from 5.0±0.6 (NUS) to 6.8±0.5 mg GA/g (US). As regards T2 behavior, it is also noteworthy to point out that the increase by ultrasound application occurs from the first 15 min of the process and is intensified throughout extraction. T3 (Fig 6C) presented unexpected behavior: the amount of total phenols obtained during the whole extraction process (60 min) was lower when using ultrasound (US, 2.9±0.09 mg GA/g and NUS, 3.6±0.4 mg GA/g). Overall, T4 (Fig 6D) produced the best results, allowing up to 6.9±0.5 mg GA/g (US) to be extracted after 60 min. Interestingly, the geometry of the transducer, and consequently the energy supplied and its distribution, produced an effect even when not applying US, obtaining up to 6.3±1.0 mg GA/g (NUS). Therefore, the geometry of T4 probably affected the solvent turbulence in the extraction cell, improving the contact between the solvent and sample.

As for the antioxidant capacity, the geometry of the transducer, ultrasound application and extraction time were significant ($p<0.05$) factors, along with first-order interactions between transducer and

ultrasound ($p<0.05$) and transducer and time ($p<0.05$). Tukey´s test indicated that T4 was able to extract with the highest amount of antioxidants (26.4±1.1 µmol TE/g), followed by T2 (22.3±1.1 µmol TE/g), while T3 (18.2±1.1 µmol TE/g) and T1 (15.4±1.1 µmol TE/g) were less effective and similar to each other (Table 1). In the same way, the antioxidant capacity obtained by ultrasound application (US, 24.0±0.6 µmol TE/g) was higher than when no ultrasound (NUS, 17.1±0.6 µmol TE/g) was used. In the case of T1 (Fig 7A), no ultrasound effect was observed throughout the extraction, obtaining maximum values of 24.9±6.6 µmol TE/g when using US and 22.6±0.5 µmol TE/g with NUS. Not only a greater antioxidant capacity was observed for T2 extractions (Fig 7B), but also a better efficiency of US, obtaining 41.8±1.9 µmol TE/g after 60 min of US, compared to 22.4±2.5 µmol TE/g with NUS. In this case, the intensification effect was observed since the first 15 min of the process, increasing gradually during extraction. The performance of T3 (Fig 7C) was similar to T1, where US only produced a small increase in the antioxidant capacity (30.9±8.0 with US compared to 25.3±1.1 µmol TE/g NUS, after 60 min of extraction). Finally, T4 allowed extraction of 43.7±2.5 µmol TE/g after 60 min with US, compared to 34.7±7.0 µmol TE/g with NUS (Fig 7D).

3.3. Identification of compounds in the USFE extracts

The most abundant compounds identified in the extracts (Fig 8) belong to the flavonoid family of phenolic compounds: a dihydroxy-trimethoxyflavone derivative and sakuranetin, a methylated form of the flavonoid naringenin. Dihydroxy-trimethoxyflavones and sakuranetin have both been previously reported in European oregano species, [31]. Other compounds in the extracts included the flavonoids tetrahydroxy-dimethoxyflavone derivative, velutin (luteolin-7,3'-dimethyl ether), luteolin-methyl ether, xanthomicrol, genkwanin (apigenin-7-*O*-methyl ether), diosmetin 7-*O*-rutinoside and rosmarinic acid, all of which have been reported in oregano extracts [32,33] and in other herbs from the Lamiaceae family [34]. The phenolic profile of the extracts was not altered by the application of ultrasound (Fig 8).

4. Discussion

When comparing the overall performance of the four transducers, T4 and T2 provided the most effective extraction of total phenolic compounds and antioxidant capacity, while T1 was the one with the poorest results. T4 transducer was designed with a multiplate configuration, which resulted in a higher surface area of head mass in contact with the medium. It supplied the highest nominal power density and therefore the best extraction scores corresponded to this transducer. Delivered energy was not the only factor that makes T4 the best ultrasonic transducer since, even without US, its geometry provided a better SC-CO_2 distribution throughout the oregano samples and improved the extraction of phenolic and antioxidant compounds due to increased turbulence and contact area. With respect to acoustic pressure, the assay only indicated the energy on a layer of water located 72 mm from the transducer surface. Consequently, T4 showed a lower acoustic pressure than T2, which showed the best energy distribution on that plane. The hydrophone test is only able to measure the acoustic pressure emitted by the surface facing the hydrophone; therefore, this technique could not account for all the energy generated by each ultrasound transducer. When comparing T3 with T2, the better extraction results of T2 could be explained by its higher acoustic pressure (the highest of all the transducers), regardless of its lower volumetric power. This indicates that the large amount of energy delivered to the medium by T3 is mainly focused on the tip of the head mass (Fig 5C), while the T2 transducer, uniformly distributed the energy over the whole oregano surface (Fig 5B).

The impact of transducer geometry on its efficiency has been reported for sonochemical reactions, where it was shown how the ultrasound effect is more intense at the tip of the transducer [35]. It has also been reported that the acoustic field distribution is essentially not homogenous, with the highest acoustic pressure found at the tip of the probe [36]. Bearing these facts in mind, T2 and T4 have the most convenient geometry for an adequate propagation of the acoustic energy in the supercritical fluid

(Fig 5). Moreover, the design of T4 not only permits energy distribution throughout the whole mass of the product to be extracted, but also a better SC-CO_2 circulation around the sample, since it has four surfaces emitting acoustic energy to the medium. Hence, for this application the use of focused sonotrodes does not favor the extraction. In ultrasound assisted extraction at atmospheric conditions it has also been determined that extraction performance is greatly influenced by differences in acoustic field distribution [37]. Accordingly, power and distribution are important factors to characterize if the best system performance is to be exploited.

Regarding the intensification effect for each transducer, increases of 35% and 86% were observed in total phenolic content and antioxidant capacity when using T2, when compared with extraction without US. With T4, increases of 9% and 26% in these two variables were obtained. The use of T1 led to increases of 8% and 10% in TPC an AOXC respectively, while T3 produced a 19% reduction in total phenolic content, but a 6% increase in antioxidant capacity. To date, there are no reports of USFE from oregano; however, in other matrices it has been shown that US has an intensification effect on the extraction of various types of compounds. In a study dealing with the extraction of phenolic compounds from peppers (*Capsicum baccatum* L. var. *pendulum*), a SFE system at 15 MPa and 40 °C without a co-solvent brought about an increase of 48% in total phenolics when ultrasound was applied. The transducer ceramics and probe were located inside in the extraction cell. Contrastingly, the antioxidant capacity, when measured by means of the FRAP and DPPH methods, decreased 2-fold and 6-fold, respectively, compared to extracts without ultrasound. The authors attributed the loss in the antioxidant capacity to the potential degradation of compounds caused by US application [21]. It was interesting to observe that, compared to the results found by Dias et al., (2016), T2 and T4 were more effective at extracting compounds with antioxidant potential, although the increase in total phenolics was less pronounced. Dias et al. (2016) obtained amounts ranging from 0.07-0.13 mg GA/g for total phenols, which are considerably lower than the results in our study (2-4 mg GA/g). In a different study, the

extraction of phenolics from blackberry bagasse using SFE at 40°C and 20 MPa showed an 8% increase in total phenolic content when ultrasound was applied, but a reduction in the antioxidant capacity evaluated using the DPPH and ABTS methods was also seen [20]. However, the authors reported that adding water as a co-solvent (5-10%), allowed a better performance, increasing the TPC by up to 10 times and the antioxidant capacity by up to 5 times [20]. These results indicate that the presence of a co-solvent is required for the extraction of these compounds, and it possibly affects the performance of the USFE as well. In both aforementioned cases, the type of transducer was a sonotrode type probe. In this study, this was the most inefficient transducer type in terms of its distribution of acoustic energy in the medium, which possibly explaining the limited results observed by Dias et al. (2016) and Pasquel Reategui et al. (2014) [20,21].

Regarding oregano, phenolic-rich extracts with antioxidant capacity were obtained by supercritical fluid extraction under conditions of 15 MPa, 40 and 60 °C and 7% ethanol as co-solvent [38]. The authors identified dihydroflavonols, flavanones, flavones and flavonols in these extracts. In the present study, the two most abundant compounds corresponded to the flavone and flavanone families, and were most likely responsible for the antioxidant capacity of the extracts. The use of a co-solvent, such as ethanol, is critical for the purpose of obtaining a phenolic-rich extracts, since use of pure CO_2 could decrease the antioxidant capacity of the extracts, as it was observed in the case of shiitake mushrooms [39].

5. Conclusions

The results from this study have shown that ultrasound assisted supercritical fluid extraction is a feasible way to obtain extracts rich in phenolic compounds with antioxidant capacity from dry oregano. This work allowed us to ascertain the relationship between the geometry of the transducers with its performance in USFE. The intensification effect observed in USFE with the different transducers was mediated by the intensity of the delivered acoustic energy and its distribution in the medium, and various levels of

nominal power density (83-150 W/L), acoustic pressure (83-150 kPa) and acoustic field distribution were observed. Ultimately, these two parameters generated a 9-38% increase in the total phenolic content and 10-86% increment in antioxidant capacity of the extracts. Flavonoid profile of the extracts was not altered, since the same major compounds were identified in every extract. Although T3 and T4 provided a similar nominal power density, the best performance was obtained through the multiplate circular sonotrode (T4), which increased the irradiation surface and improved the distribution of the ultrasonic energy throughout the entire treated volume of oregano, while T3 tends to concentrate the energy in a smaller area, decreasing the overall extraction efficiency. This sonotrode arrangement also improved the performance of the supercritical fluid extraction without ultrasound since it improved the turbulence and the contact area between solvent and solids. Custom made sonotrodes can be used to optimize both power density and its distribution, overcoming the limitations of commercial transducers. Further studies using different matrices would allow the performance of the transducers with materials of different composition and structure to be determined, and further research should aim to improve transducer design for the purposes of process optimization.

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Conflicts of Interest

The authors declare that they have no competing financial interests.

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Figure 1

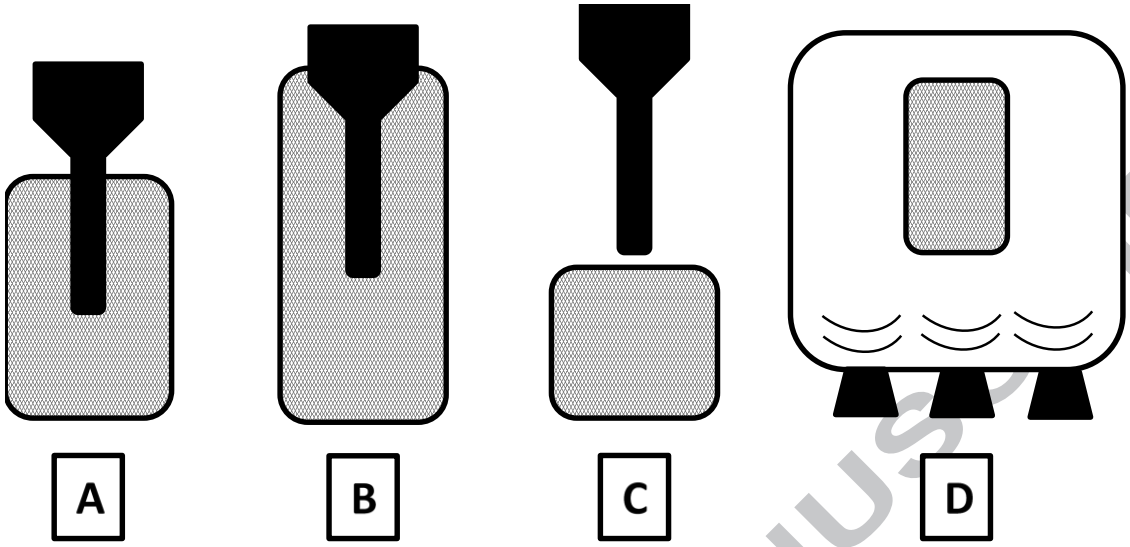
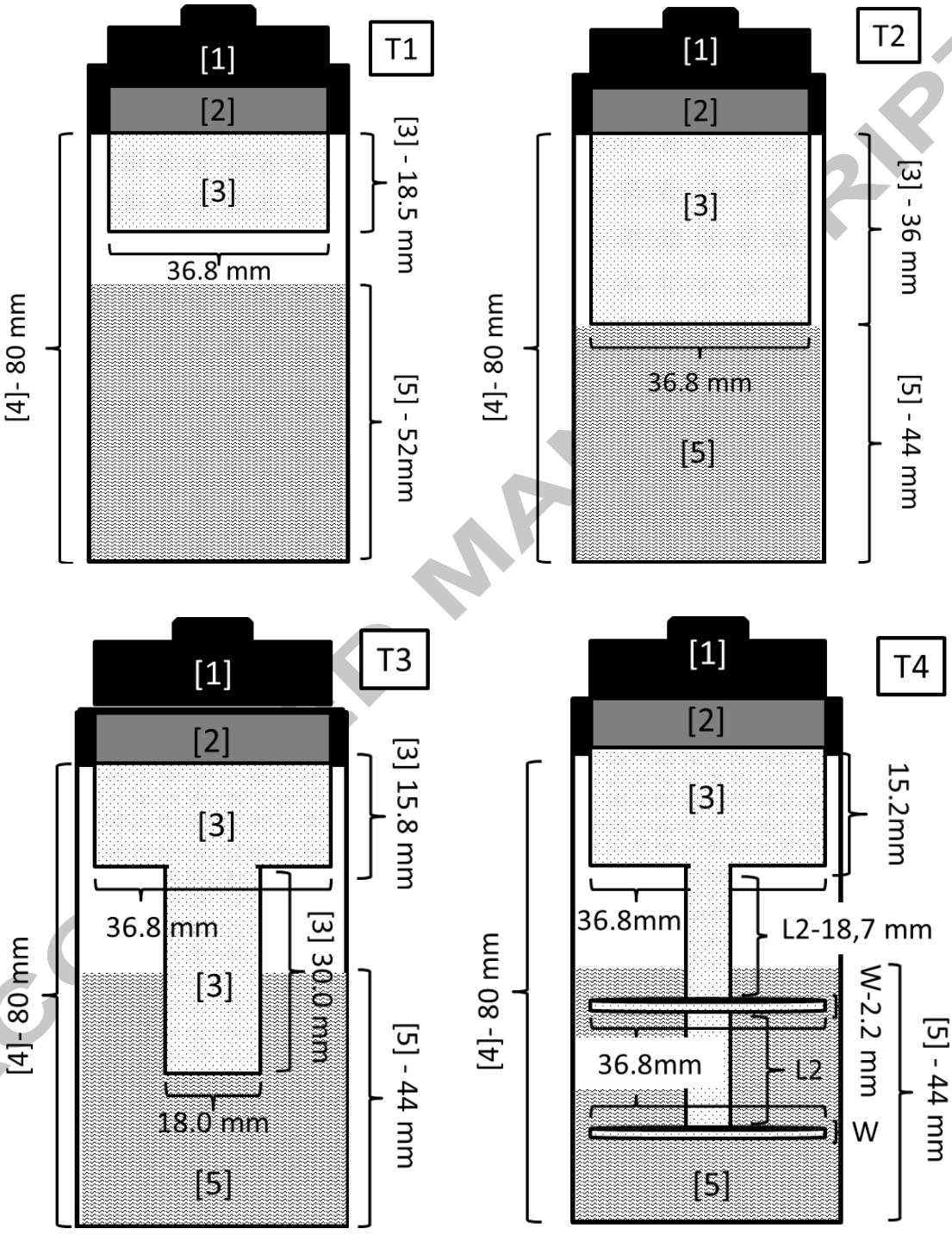


Figure 2



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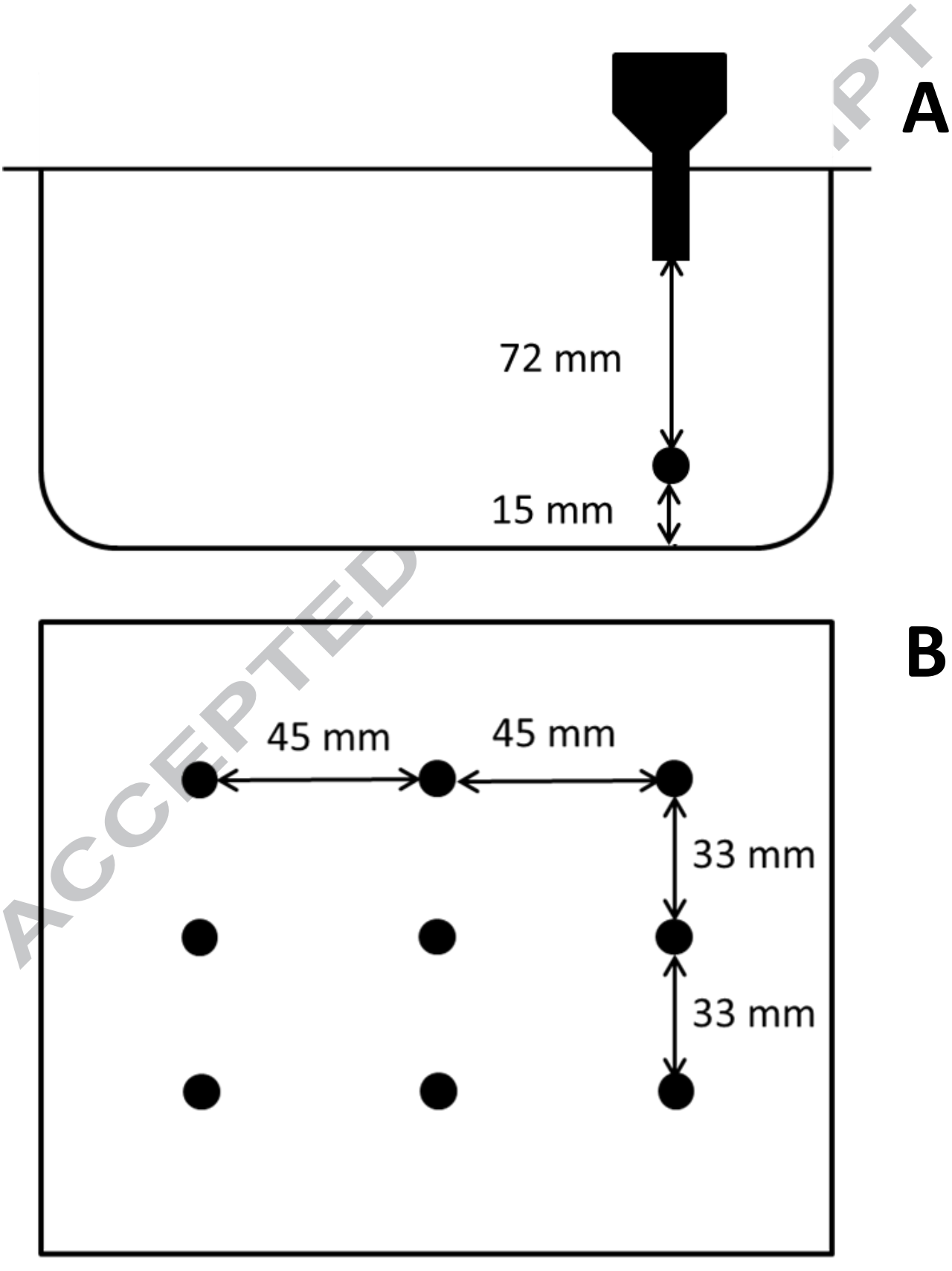

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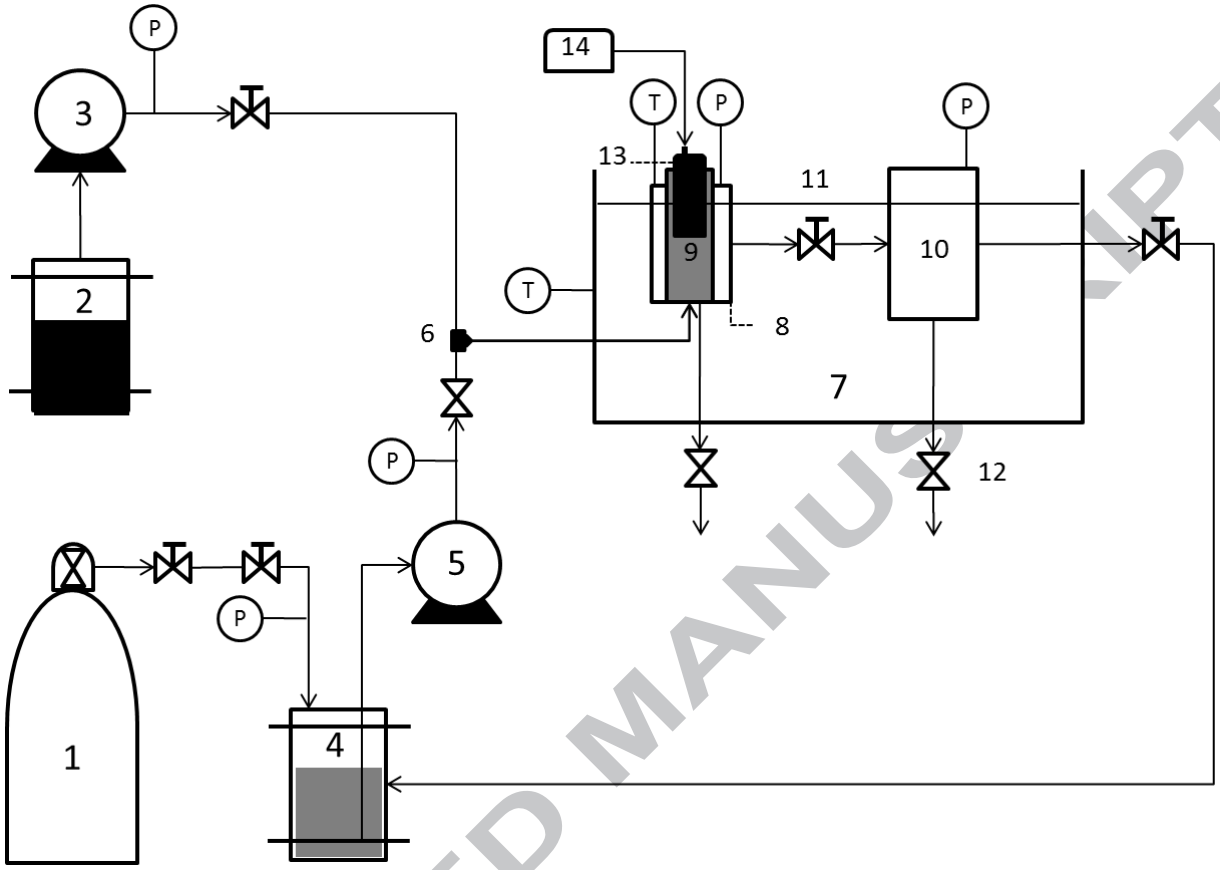

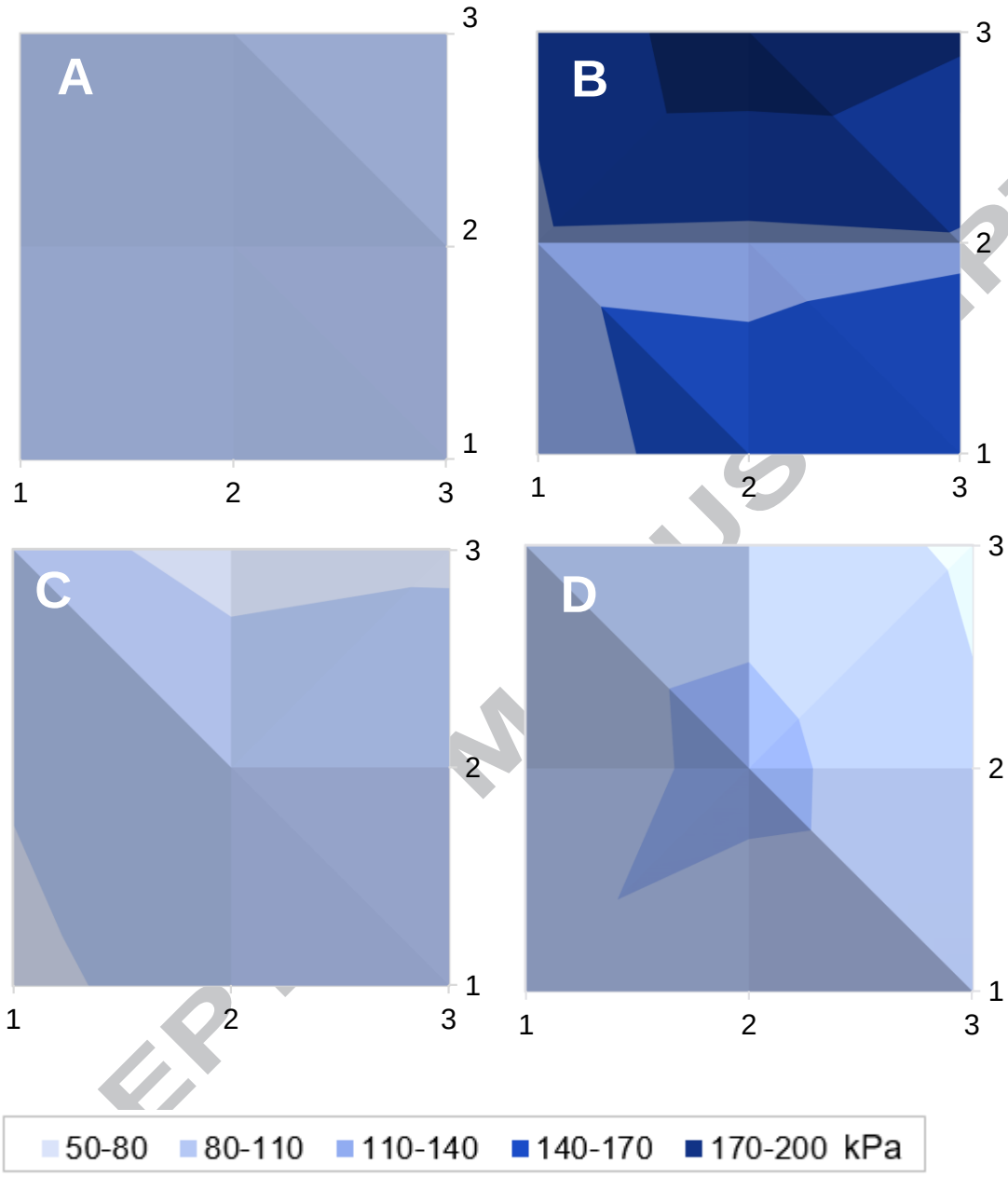

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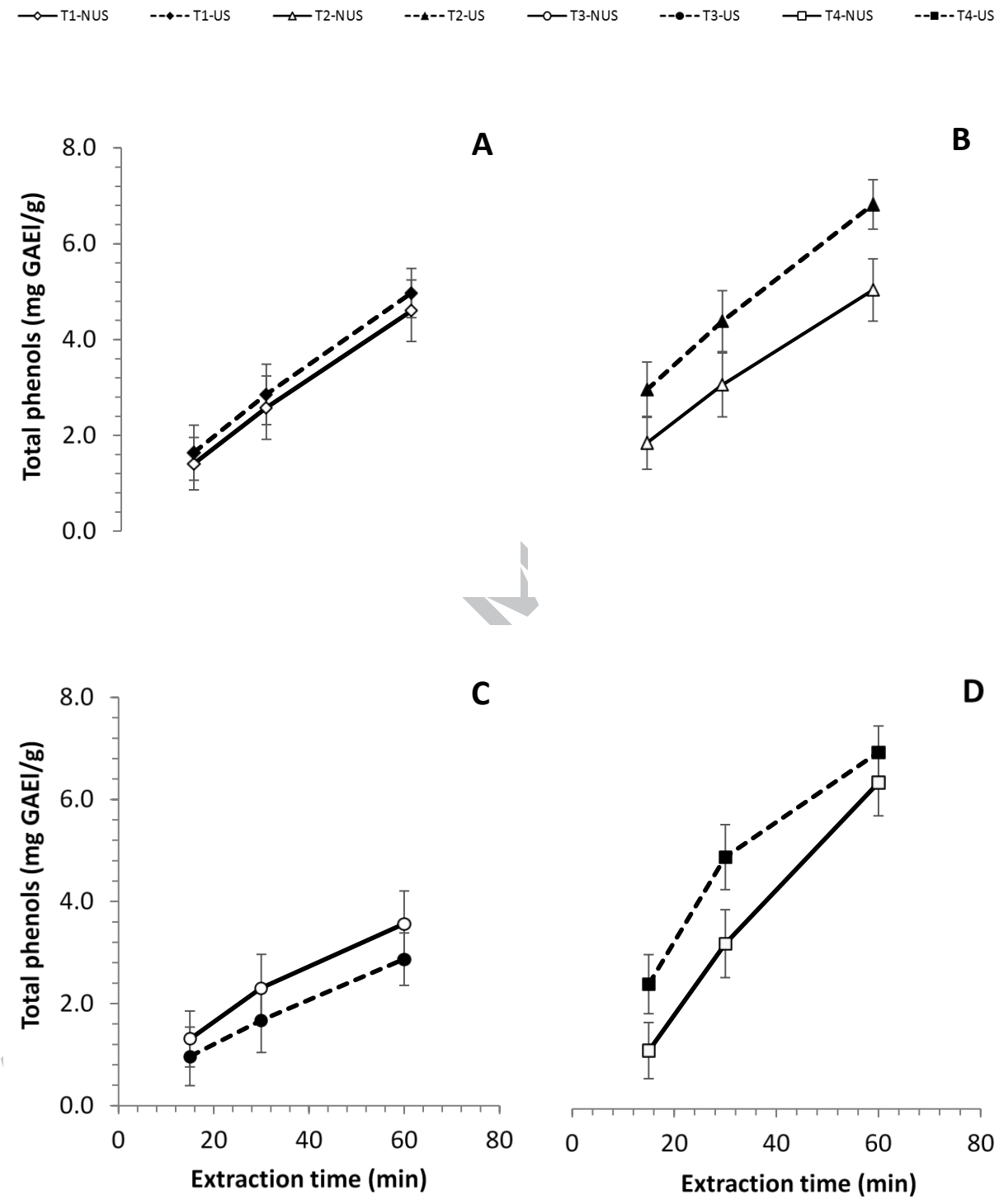


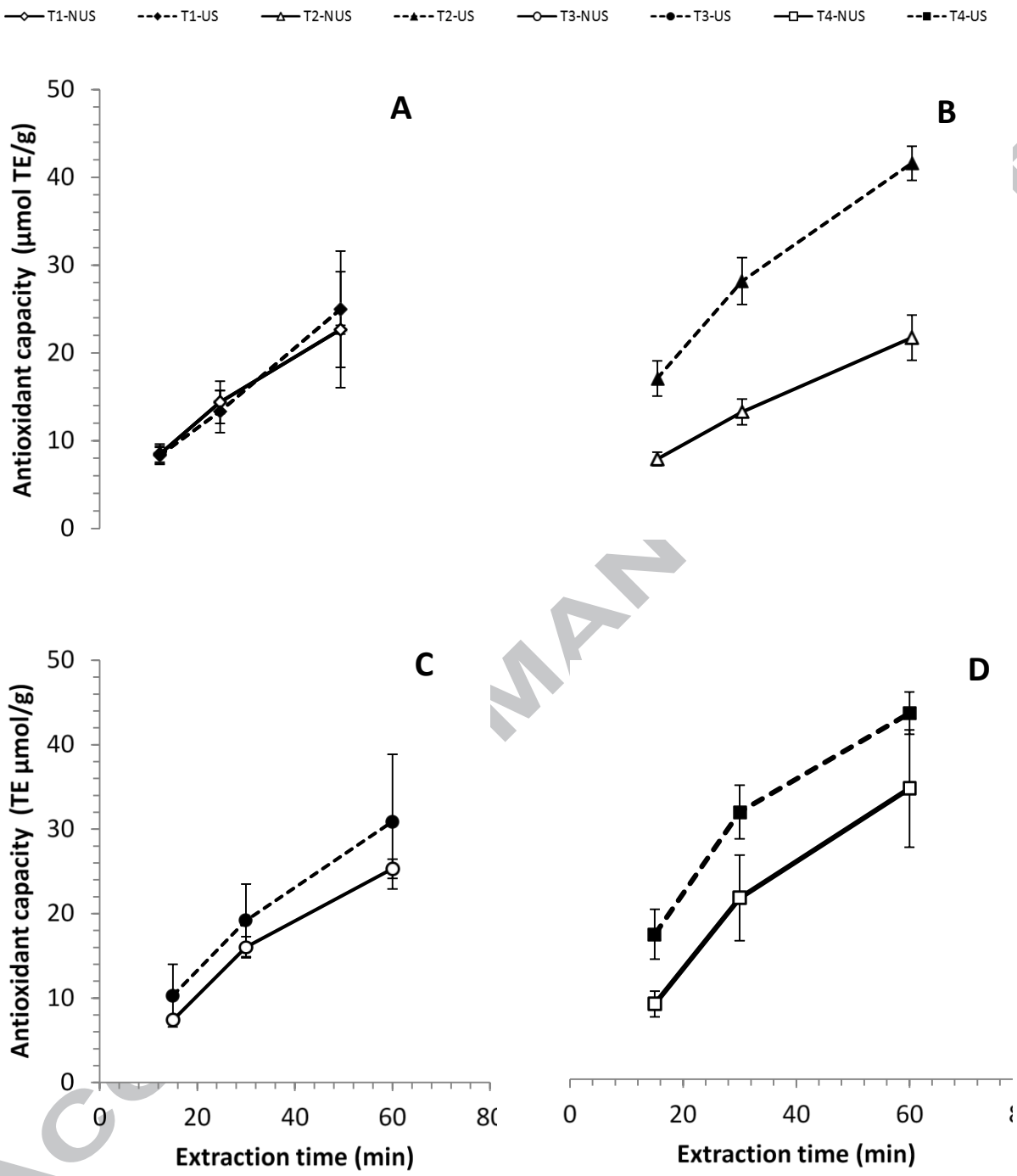
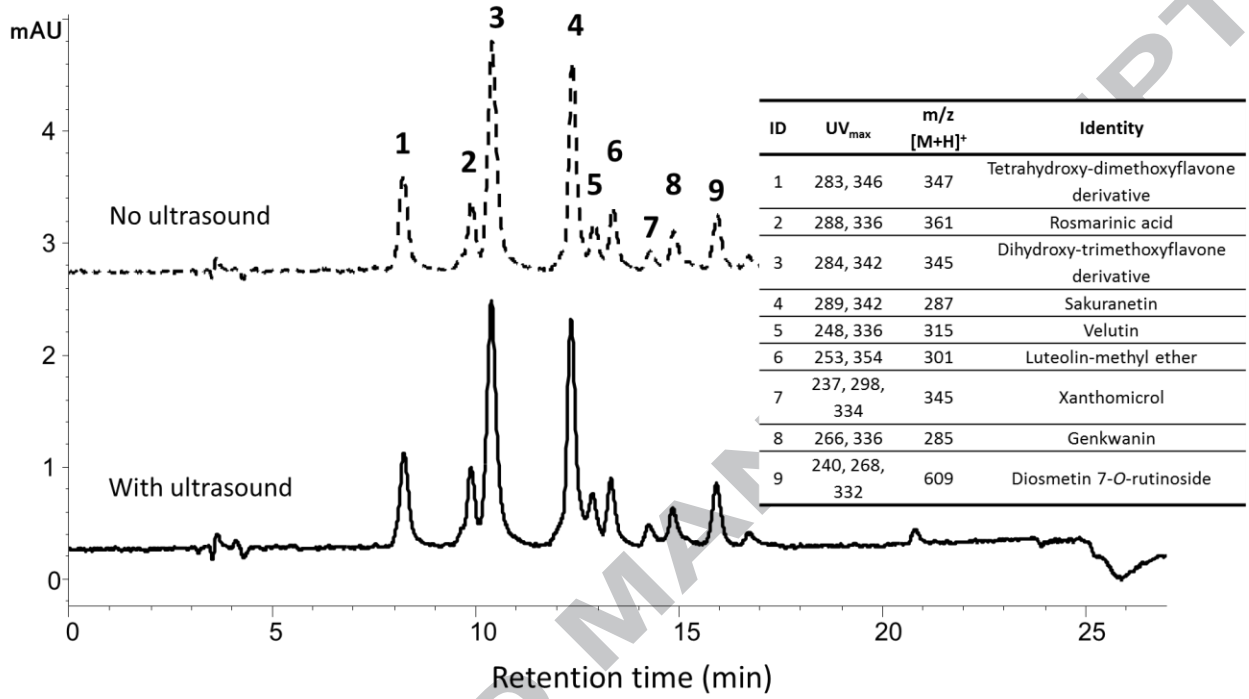


Figure 8



ID	UV_{max}	m/z $[M+H]^+$	Identity
1	283, 346	347	Tetrahydroxy-dimethoxyflavone derivative
2	288, 336	361	Rosmarinic acid
3	284, 342	345	Dihydroxy-trimethoxyflavone derivative
4	289, 342	287	Sakuranetin
5	248, 336	315	Velutin
6	253, 354	301	Luteolin-methyl ether
7	237, 298, 334	345	Xanthomicrol
8	266, 336	285	Genkwanin
9	240, 268, 332	609	Diosmetin 7-*O*-rutinoside

Figure captions

Fig 1: Typical transducer arrangements used for ultrasonically-assisted extraction: (A) the probe is placed inside the extraction vessel and the piezoelectric ceramics are outside; (B) both the probe and ceramics are inside the extraction vessel; (C) the transducer and probe are outside the extraction cell; (D) ultrasound is applied through a water bath. In both C and D, water is used as the coupling method.

Fig 2: Ultrasound transducer geometries designed in this study: (T1) Transducer 1: short rod-shaped head mass; (T2) Transducer 2: long rod-shaped head mass; (T3) Transducer 3: stepped circular head mass; (T4) Transducer 4: multiplate circular head mass. [1] tail mass, [2] ceramics, [3] head mass, [4] height of the extraction cell, [5] height of extraction cell filled by the sample.

Fig 3: Experimental setup for acoustic pressure measurement using a hydrophone. (A) distance at which the transducer was submerged for each node; (B) top view of the grid mesh where measurements were taken.

Fig 4: Supercritical fluid extraction pilot plant coupled to ultrasound delivering system. (1) CO2 tank; (2) ethanol reservoir; (3) ethanol pump; (4) liquid CO2 reservoir; (5) CO2 pump; (6) mixing T for supercritical ethanol and CO2; (7) temperature-controlled water bath, (8) extraction unit; (9) extraction vessel; (10) separation unit; (11) microvalve; (12) separation unit valve; (13) ultrasound transducer; (14) ultrasound generator, (P) pressure gauge, (T) thermocouple.

Fig 5: Acoustic pressure distribution measured by a hydrophone for (A) transducer 1; (B) transducer 2; (C) transducer 3; (D) transducer 4. The numbers on axes x and y (1, 2, 3) indicate the node mesh where the sound pressure was measured according to Fig. 3A and the color scale shows the ranges of sound pressure (bar) level.

Fig 6: Amount of total phenols extracted from oregano with (US) and without ultrasound (NUS) using: (A) transducer 1; (B) transducer 2; (C) transducer 3; (D) transducer 4.

Fig 7: Antioxidant capacity in oregano extracts obtained with (US) and without ultrasound (NUS) using: (A) transducer 1; (B) transducer 2; (C) transducer 3; (D) transducer 4.

Fig 8: Compounds identified in oregano extracts obtained by supercritical fluid extraction with and without ultrasound. (1) tetrahydroxy-dimethoxyflavone derivative, (2) rosmarinic acid, (3) dihydroxy-trimethoxyflavone derivative, (4) sakuranetin, (5) velutin, (6) luteolin-methyl ether, (7) xanthomicrol, (8) genkwanin, (9) diosmetin 7-*O*-rutinoside.

Tables

Table 1. Ultrasonic power delivered by the four transducers measured by means of the calorimetric and hydrophone methods, total phenols and antioxidant capacity after 60 min USFE.

Transducer	Nominal power density (W/L)	Acoustic pressure (kPa)	Total phenols (mg GA/g)	Antioxidant capacity (µmol TE/g)
T1	83.5±17.7^{c}	84.5 ± 3.2^{c}	3.0±0.2^{b}	15.4±1.1^{c}
T2	116.4±7.7^{b}	150.6 ± 20.5^{a}	4.0±0.2^{a}	22.3±1.1^{b}
T3	136.6±6.2^{a}	83.2 ± 5.4^{c}	2.1±0.2^{c}	18.2±1.1^{c}
T4	151.6±7.1^{a}	99.3 ± 12.8^{b}	4.2±0.2^{a}	26.4±1.1^{a}

Average ± standard errors are shown. Different letters in the same column indicate significant differences between transducers ($p < 0.05$).

Highlights

- Stepped circular section sonotrode focused energy on the tip, losing extraction efficiency.
- High performance in multiplate sonotrode was due to better energy distribution.
- Multiplate transducer made SFE more efficient even without ultrasound.
- Supercritical fluid extraction was intensified by ultrasound application.