PRESSURIZED HOT WATER EXTRACTION OF BIOACTIVES

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ABSTRACT

In the last years, the fact that water is a "green" solvents capable of tunable their properties by changing temperature has tended to increase publications using pressurized hot water extraction (PHWE) as a "green" extraction technique. PHWE has been established as a special extraction technique employed in obtaining bioactive compounds such as polyphenols, diterpenes and polysaccharides, among others from natural sources. Therefore, the main goal of this review is to provide the reader with a brief description of PHWE fundamentals, an up-to-date overview on the use of PHWE to recover bioactive compounds (2015-present), following our previous work by Plaza and Turner (*Trends in Analytical Chemistry* 71 (2015) 39-57). The last and future trends in PHWE are presented such as its combination with ionic liquids or deep eutectic solvents as well as its coupling with drying steps in order to get higher extraction yields and dried particle formation from extracts, respectively.

Keywords: Bioactive compounds, diterpenes, extraction, polyphenols, polysaccharides, pressurized liquid extraction, subcritical, superheated, water.

Abbreviations: BMIMAc, 1-Butyl-3-methylimidazolium acetate; DES, deep eutectic solvents; HPLC, high performance liquid chromatography; ILs, ionic liquids; MS, mass spectrometry; PHWE, pressurized hot water extraction; PLE, pressurized liquid extraction; RSM, response surface methodology; SWE, subcritical water extraction, UHPLC, ultra-high performance liquid chromatography; WEPO, water extraction and particle formation on-line.

1. Introduction

Nowadays there is an increasing concern by the consumers for naturalness and plant-based products. A high interest is focused in fruits and vegetables and their by-products as source of bioactive compounds. For instance, these bioactives such as polyphenols, polysaccharides, terpenes, carotenoids, sterols, among others, have been extracted from plants [1]. The growing scientific researches show that these compounds are closely associated to human health and disease prevention because they possess a good range of bioactivities, including antioxidant, antiviral, anti-inflammatory, and anticancer properties [2,3]. Therefore, this is one of the reasons that has made that the extraction of bioactives from natural sources be one of the most studied topics currently.

The extraction methods that have usually been employed to recover bioactive compounds are conventional including Soxhlet extraction, maceration, or steam distillation extraction [4]. The main disadvantages of these extraction methods are that they use large volumes of potentially hazardous solvents, are time-consuming, have low selectivity, and have a risk of decomposition of thermolabile compounds [4]. Today, society claims for the employ of sustainable processes in order to protect the environment. Closely related to this point, it is important to develop "green" extraction processes enabling to reduce solvents, energy, wastes and environmental pollution while obtaining the enhancement of yields [5]. Then, one of the main points to be considered is the solvent selection because the degree of environmental impact depends on the way that natural resources are harvested; energy usage; and emissions to air and water from the production and use of solvents, transportation, and disposal or recycling [6]. That is why, water can be treated as a potentially "green" solvent since is non-toxic to health and the environment, and is the safest and least expensive solvent. In addition, its transportation is achieved by an already built infrastructure and it is not needed to carry out the pretreatment of drying the raw material before the extraction which implies that water has a minimum

environmental impact in terms of extraction processes. The fact that water is one of the few "green" solvents capable of tunable their properties by changing the temperature has contributed to grow in the number of publications using pressurized hot water extraction (PHWE) as a "green" extraction technique in the last years. PHWE (also called subcritical water extraction (SWE), superheated water extraction and pressurized liquid extraction (PLE) or accelerated solvent extraction using water as a solvent) consists on the employ of water submitted to high temperatures (usually above its boiling point) and enough pressures to keep the water in the liquid state. Accordingly, PHWE uses water in liquid state as a solvent at temperatures over its boiling point (100 °C, 0.1 MPa) and under its critical point (374 °C, 22.1 MPa) [7].

Thus, the goal of the present review is to provide an up-to-date overview on the applications of PHWE to extract bioactive compounds (2015-present), following our previous work [7]. The new PHWE applications are introduced such as its use together with ionic liquids or deep eutectic solvents to obtain higher extraction yield as well as its coupling with drying steps in order to form dried particles from extracts. In addition, a brief description of PHWE fundamentals and an outline of its future trends are presented.

2. Fundamentals of pressurized hot water extraction

In PHWE, the use of high temperatures and pressure is wanted in order to maintain water in liquid state during all the extraction process. The physicochemical properties of water change dramatically under these conditions.

There are interrelated factors such as solubility, mass transfer and matrix effect that influence the extraction efficiency. Temperature, pressure, extraction time and flow rate are the main parameters affecting these extraction factors in PHWE. Among all these parameters, temperature is the most crucial one because it is involved in extraction efficiency and

selectivity. For example, the dielectric constant of water (also named as relative static permeability, ε) decreases with increasing temperature (see **Figure 1**). Then the solvent polarizability can be adjusted by modifying the temperature in PHWE. Therefore, liquid water at high temperature is a solvent with lower polarizability/polarity and density than water at ambient conditions. On the other hand, when the temperature increases, the surface tension and viscosity decrease, and the diffusivity increases. All these water properties that change when the temperature of the water increases allow faster mass transfer and improve wetting of the matrix. Furthermore, the high temperature reduces intermolecular interactions that links the analyte to the matrix improving its desorption to the water. This, in turn, may lead to a more complete extraction and faster extraction process.

Figure 1.

However, the use of high temperature may display some disadvantages. For instance, an increase of solubility of other compounds beyond the desired ones might happen, besides thermolabile compounds can be degraded and other reactions such as Maillard and caramelization in the matrix may take place which could produce unwanted and toxic compounds [9]. Thus, in PHWE, it is of great importance to know the optimum extraction temperature of each analyte in order to increase solubility and mass transfer and decrease degradation, undesirable chemical reactions and selectivity loss.

Extraction time, which begins with the water in contact with the matrix at the desired extraction temperature and pressure, is other critical parameter to consider in PHWE. Generally, the static mode is the most employed type of extraction. In this mode, the solvent is not replaced during the extraction unless more extraction cycles are used, and in this case the solvent is replaced partially or completely after some time [10]. Therefore, the compounds still linked to the matrix and the water can achieve an equilibrium during the extraction time. At this point the extraction

115 efficiency will not increase but the degradation of desired compounds and the formation of
116 unwanted chemical reactions might happen more easily [7]. One way to minimize chemical
117 reactions, to avoid the equilibrium and to have a more complete extraction (theoretically)
118 during PHWE is to work in a continuous flow mode in which the water is replaced
119 continuously. In this extraction mode, the flow rate must be taken into account because it will
120 determine the extraction time. Mostly the flow rate must be the adequate to enable the
121 solubilisation of the compounds in water through brief contact time between the water and the
122 sample. Even if the use of a higher flow rate is the way to minimize degradation and unwanted
123 chemical reactions, the employ of high flow rates means high volumes of water and it could be
124 a disadvantage for analyte determination [10]. In addition, when dried sample matrices are
125 used, too high flow rates are not recommended because there is not enough time to hydrate and
126 swell the sample matrix avoiding the solubilisation of the bioactive compounds.

127 On the other hand, pressure itself has a limited effect on the physicochemical properties as well
128 as on the extraction efficiency of water as long as it is kept in the liquid state during PHWE
129 [10]. So, it would be good to state the minimum pressure to keep water in liquid state. For
130 instance, it may be deduced that for maintaining water as a liquid at 150 ºC, a pressure of about
131 0.5 MPa (considering the vapour pressure curve for water) needs to be applied [6]. However,
132 a pressure of 5-10 MPa is usually employed except when the saturation pressure of water is
133 used.

134 There are other parameters, in addition to the ones described above, that may affect the
135 extraction efficiency in PHWE. For instance, the physical state of the sample can have
136 influence on the mass transfer. This is why the matrix is sometimes treated before PHWE
137 because usually a larger surface arises greater accessibility of water to the compounds.
138 However, the particle size must be the appropriate in order to increase the contact surface
139 evading channelling effect such as particle agglomeration. Moisture content and solvent-to-

sample ratio in static extraction mode should be taken into account to get higher extraction yields [10]. Moreover, the use of some organic and inorganic modifiers, surfactants and additives can promote the solubility of the compounds in the extraction solvent and also affect the physical properties of the matrix and the desorption of analytes from the matrix.

For deeper knowledge of PHWE fundamentals, the readers are referred to our previous work [7].

3. The use of PHWE in chemical analysis of bioactive compounds in complex samples

Nowadays, PHWE has been considered as an appropriate extraction technique to obtain bioactive compounds. **Table 1** summarizes the PHWE applications disclosed from 2015 to the present. Many of them are related with the extraction of phenolic compounds, di- and triterpenes, and polysaccharides among others with bioactive properties.

Table 1.

3.1. PHWE of phenolic compounds

Phenolic compounds are a broad heterogeneous group, which contains from simple molecules to polymeric ones. The main groups of polyphenols are: flavonoids (flavonols, flavones, isoflavones, flavanones, anthocyanidins, and flavanols), phenolic acids, phenolic alcohols, stilbenes, and lignans [70]. Polyphenols are extensively spread through the plant kingdom and there are many studies that suggest their crucial role in the prevention of several diseases associated with oxidative stress, such as cancer, cardiovascular diseases, and neurodegenerative diseases [71,72]. Since all these bioactivities of polyphenols are associated with their chemical structure, researchers have focused their efforts on the appropriate extraction from natural sources [73].

In the last years, there are many works in the literature dealing with the extraction of phenolic compounds from different matrices using PHWE, which are summarized in **Table 1**. This table is not exhaustive, but it demonstrates the high interest shown by researchers to optimize the PHWE extraction process for obtaining phenolic compounds from different matrices. The parameters that are more often optimized are temperature, time, pressure and flow rate. Moreover, other parameters that can have influence on the extraction and were also considered in some works are solvent to solid ratio, agitation rate in static mode and the addition of modifiers. Temperature and time can be considered the most important ones. Usually, extraction temperatures of 80-150 °C and extraction times of 5-60 min were applied in general to extract phenolic compounds from plants by PHWE (see **Table 1**) [22-26]. The use of higher temperatures (>150 °C) should be carefully studied because of the degradation of phenolic compounds that can occur due to the fact that they are thermolabile compounds, as well as the occurrence of undesired chemical reactions in the sample matrix and the formation of new unwanted compounds with antioxidant capacity (see section 2). In many works in which the extraction conditions were optimized by spectrophotometric methods such as total phenolic and antioxidant assays, higher antioxidant capacities and total phenolic compounds have been detected in the extracts obtained at temperatures over 150 °C (150-201 °C) and at longer extraction times (up to 113 min) [12-19, 27,30]. Instead of the use of these analytical methods, more advanced analytical techniques are necessary to quantify phenolics and to carry out the optimization of the PHWE conditions.

As can be seen in **Table 1**, the static mode is the most employed type of extraction. The optimal extraction conditions of phenolics greatly vary when the extraction is carried out in dynamic mode because it reduces the residence time of the solvent in contact with the sample and higher temperature can be used thus avoiding the degradation of thermolabile compounds. For instance, the dynamic mode has been used to recover phenolics from black tea, pistachio hulls,

187 defatted orange peel and turmeric with optimal extraction temperatures of 150-170 °C at a flow
188 rates from 1 to 12 mL/min [37-40]. Lower temperatures (91 °C) were used to extract flavonols
189 such as kaempferol and quercetin, and vitamin C from *Moringa oleifera* leaves but it could be
190 because a lower flow rate was employed (0.3 mL/min) [43]. On the contrary, when flavonoids
191 were extracted from *Momordica foetida* the optimal extraction temperatures were higher (250
192 °C) because higher flow rates were running (5 mL/s) [44]. However, the extraction of
193 anthocyanins, which are extremely labile polyphenols, were operated a very low temperature
194 of 60 °C at 2 mL/min in *Morus nigra* L., while the optimum extraction temperature used was
195 higher (170 °C) at higher flow rate (3 mL/min) in *Lycium ruthenicum* Murr. [41,42].

196 On the other hand, the optimal extraction conditions greatly change, depending on the type of
197 phenolic compounds to be extracted. For instance, stilbenes such as resveratrol were extracted
198 from grape by-products being the optimal conditions 150-160 °C during 5-25 min [33,34]. The
199 optimal PHWE conditions to reach higher isoflavones yields from *Puerariae lobata* were
200 temperatures around 120 ºC [32]. However, the temperature had effect on the extraction of
201 isoflavones from soybean flour and soybean protein isolate depending on their derivative
202 structure [31]. For example, the malonyl forms were more temperature sensitive obtaining
203 higher concentrations at 60 ºC. While if the temperature exceeds 60 ºC, the conversion of
204 malonyl forms into glucoside forms took place and the conversion of glucosides into aglycones
205 when exceeding 160 ºC (**Table 1**) [31]. In general, the best PHWE conditions to extract
206 flavonoids are medium temperatures (85-126 ºC) and short extraction times in static mode (10-
207 30 min) [25-29].

208 *3.2. PHWE of terpenes*

209 PHWE has been used for the extraction mainly of di- and tri-terpenes. The diterpenes steviol-
210 glycosides (steviosides) which are a well-known low calorie sweetener alternative to sucrose,

have been extracted by PHWE from *Stevia rebaudiana* Bertoni leaves [38]. The temperature was the main PHWE parameter affecting the extraction of steviosides. Hence, the highest contents of stevioside and rebaudioside A by PHWE were achieved at 160 °C for 10 min using 3 extraction cycles [38] (see **Table 1**). This study indicated that PHWE is useful for recovering polar and nonpolar antioxidants and steviol glycosides.

In addition, different triterpenes have been extracted by PHWE in the static mode. For instance, ginsenosides have been reported to be the main active substances of ginseng, and there has been considerable research into the beneficial effects of ginsenosides, including anticancer and anti-inflammatory, among others [74,75]. Extraction temperature and time were optimized in order to obtain ginsenosides from red ginseng (*Panax ginseng* C.A. Meyer). The maximum yields of ginsenoside Rg3 and Rh2, which have 2 hydroxyl groups (200 °C), were obtained at a higher temperature compared to ginsenosides Rg2 and Rh1, which have three hydroxyl groups (150 °C). The antioxidant properties were maximized at 200 °C for 20 min because at this temperature Maillard reaction products were produced (**Table 1**). Extracts of red ginseng from PHWE had higher ginsenoside concentrations and antioxidant properties compared to extracts prepared using traditional extraction methods, including ethanol, hot water, and methanol [46]. PHWE was conducted at different temperatures ranging from 120 to 200 °C to extract ginsenosides from ginseng roots [47]. UHPLC-MS/MS analysis revealed that PHWE induced extensive hydrolysis of the ginsenosides, except for Rg2. In comparison, the extraction yields of R1, Rb1, Rb2, Rb3, Rc, Rd, Re, Rg1, and Ro decreased significantly with the increase of PHWE temperature. The optimum extraction temperature was between 140 and 160 °C for 20 min with two extraction cycles [47]. These findings suggested that PHWE is a promising environmentally friendly and efficient technology for extracting Rg2 from ginseng roots, but it has potency to induce extensive hydrolysis of other ginsenosides, such as Rb1 and Re.

The extraction of betulinic acid from birch bark by PHWE was optimized using response surface methodology (RSM). Betulinic acid is an interesting triterpenoid because it possesses medical activities [76]. Optimal extraction conditions were temperature at 184.5 °C, times of 27.37 min and a solvent-to-solid ratio of 59.60 mL/g finding less impurities in PHWE extracts in comparison with the ones obtained by conventional extraction techniques [48] (**Table 1**). Ursolic acid and its derivatives have been reported to have a number of bioactivities, including anti-inflammatory, hepatoprotective, anti-tumor, anti-viral, anti-HIV, anti-microbial, antimalarial, anti-diabetic, gastroprotective, and anti-hyperlipidemic effects [49]. Similar extraction conditions were used in order to extract ursolic acid from *Hedyotis diffusa* being the optimal extraction conditions at temperatures of 157 °C for 20 min and solvent-to-solid ratio of 30 mL/g [49]. However, higher extraction temperatures were allowed to use for extracting ursolic acid (200 °C) and corosolic acid (180 °C) from dry loquat leaves (*Eriobotrya japonica*) because PHWE in the dynamic mode was employed with high flow rates (41.66 and 33.33 mL/min, respectively) [50].

Saponins were extracted using PHWE from quinoa stalks [51] and ginseng [52]. Saponins are triterpenes glycosides which have potential antibiotic and antifungal properties, among other reported biological activities [51]. The greatest content of saponins was achieved at the extraction temperature of 195 °C and 207 °C, respectively. However, the extraction times were shorter for the extraction of saponins from quinoa stalks (1 min) than from ginseng (15 min) [51,52]. These compounds were less sensitive to the temperature, therefore, elevated extraction temperatures (200 °C) but with short extraction times (1-15 min) can be employed on saponins PHWE (**Table 1**).

3.3. PHWE of bioactive polysaccharides

In the last years, the interest of the extraction of different types of bioactive polysaccharides from different natural products, foods and food by-products by PHWE has increased. For instance, the extraction of bioactive polysaccharides from Pacific oyster *Crassostrea gigas* by PHWE in the static mode was optimized by RSM [53]. It was observed that a low temperature for longer extraction time and a high temperature for shorter extraction time increased the extraction yield being the optimal extraction conditions at temperature of 125 °C, solid-liquid ratio of 44.69:1 (ml/g), and extraction time of 14.93 min (see **Figure 2** and **Table 1**). These polysachharides have shown different biological activities including antioxidant, antihypertensive and hypoglucemic [53]. On the other hand, fucoidan, which is the characteristic polysaccharide from the brown macroalgae was extracted by PHWE from *Saccharina japonica* [55]. In order to determine the optimal extraction conditions for fucoidan, a desirability function method was applied and the best extraction conditions were 127 °C, 8.0 MPa, 12 min, solid-liquid ratio of 0.04 g/mL and 300 rpm of agitation speed (**Table 1**) [55]. 0.1 % NaOH was employed in all extractions because in a previous work, higher extraction yields of fucoidan were observed under these conditions [77]. The fucoidan got from the optimized conditions presented good antioxidant, modest antimitotic and moderate anti-proliferative activities in cell lines [55].

Figure 2

Pectic oligosaccharides were found to exhibit prebiotic functions [78]. Therefore, the extraction of these compounds from passion fruit peel by PHWE in the static mode has been studied [56,57]. In a first work, the effect of the extraction temperature and time on PHWE was studied. High yields of total oligosaccharides could be obtained by heating at 150 °C within 4.5 min or at 175 °C within 5.5 min [56]. The main advantage of this extraction method was that took short time and required no acid addition because under these extraction conditions pectin was predominantly hydrolysed and extracted. In other study, the effect of ethanol

283 addition (0-30%, v/v), time and temperature on PHWE of these pectic polysaccharides from
284 the same matrix was investigated [57]. Treatments at high temperature contribute to higher
285 yields while the addition of ethanol gave adverse effect, except for adding small amount of
286 ethanol at high temperature because adding ethanol at a low concentration resulted in milder
287 extraction condition. The highest extraction yields were obtained at 140 °C, 6.9 min using 10%
288 ethanol [57] (**Table 1**). Both works showed that PHWE could be used to extract pectic
289 polysaccharides from passion fruit peel with a yield comparable to that obtained using the hot
290 acid method but within a shorter time [56,57].

291 Polysaccharides are an abundant resource in coffee beans and have proved to show numerous
292 bioactivities. Despite their abundance, their activities are not always satisfactory mostly due to
293 their structure and large molecular size [58]. Molecular modifications of native polysaccharides
294 can overcome this problem. That is why PHWE was employed to modify native coffee
295 polysaccharides from green coffee beans at 180 °C, 3.0 MPa for 30 min and 220 °C, 6.0 MPa
296 for 15 min [58]. Also, PHWE was used to obtain bioactive polysaccharides from spent coffee
297 grounds (**Table 1**). This optimized method was similar to the one used for coffee beans using
298 similar extraction temperature (179 °C) and pressure (2.0 MPa) but shorter extraction times (5
299 min). In this case the sample was pretreated with ultrasound in order to obtain better results
300 [59]. The extracted polysaccharides showed very good antioxidant capacity and hypoglycemic
301 activity.

302 PHWE was applied to the isolation of prebiotic carbohydrates such as fructooligosaccharides
303 from Brazilian ginseng roots [54]. It was observed that at temperatures above 120 °C for 15
304 min the prebiotic carbohydrates content decreased. As it is described in section 2,
305 fructooligosaccharides were degraded at higher temperatures due to caramelization and
306 Maillard reactions [54]. To overcome this problem, low methoxyl pectin was extracted from
307 pomelo peel using PHWE in a dynamic mode [60]. The effect of pressure and temperature was

investigated by a face-centered central composite design and the optimal operating conditions were 120 °C and 3.0 MPa for 20 min with a flow rate of 1 mL/min (**Table 1**). The effect of the pressure on the PHWE of low methoxyl pectin was insignificant while the temperature played a significant role. The degradation of pectin was suggested at high temperature and/or prolonged extraction times [60].

In general, the optimal extraction conditions to achieve the highest content of polysaccharides were medium temperatures ranging from 120 to 150 °C for short times from 4 to 15-20 min. However, when the goal was to modify and / or hydrolyze the polysaccharides, the temperature and time increased until 220 °C and 30 min, respectively (see **Table 1**).

3.4. PHWE of other bioactive compounds

Protein extraction by PHWE has not been studied in deep and there is just a few works published about this topic. For example, PHWE was optimized for an efficient extraction of proteins from elderberry (*Sambucus nigra* L.) branches [61]. This extraction technique demonstrated to be suitable and reproducible at low extraction temperatures of 50 °C, pressures of 15 MPa and 5 min of extraction time (**Table 1**).

In addition, this extraction technique has been used to carry out the extraction of lipidic compounds from *Nannochloropsis salina* and *Camellia oleifera* Abel. seeds [62,63]. PHWE in dynamic mode was used to extract the crude oil from *N. salina*. The effect of the extraction parameters such as temperature, flow rate and sample loading on the extraction efficiency was considered being the best conditions 175 °C, 4 mL/min and 1 g, respectively (**Table 1**) [62]. Under these conditions, the fatty acid profile was compared to the one obtained by the conventional solvent extraction Folch method. These results showed that PHWE can be an option for the extraction of oil from biological resources. Furthermore, PHWE in static mode was optimised by RSM to collect *Camellia oleifera* Abel. seeds oil and tea saponins. The

highest yield of oil was obtained at 136 °C and for 32.03 min, and the highest saponins yield was found at very similar conditions as for the extraction of oil (121 °C for 32.07 min). Analytical results showed that extracted oil has a similar fatty acid profile and quality as that of the cold Soxhlet extraction oils. Additionally, oils extracted by PHWE were even more resistant to lipid oxidation and with more abundant valuable bioactive compounds than those of the cold-pressed oils [63]. These results support that PHWE could be an alternative and greener method for the extraction of oil than the conventional extractions methods [62,63].

4. PHWE of bioactive compounds by adding ionic liquids or deep eutectic solvents

Lately the combination of the use of ionic liquids (ILs) with PHWE has started to be used for the recovery of bioactive compounds due to an important demand to replace toxic organic solvents for green solvents [64-67]. The physicochemical properties of ILs (i.e. negligible vapour pressure, high thermal and electrochemical stability, wide solvating range, and strong miscibility with hydrophilic solvents) are adequate to achieve the extraction of bioactive compounds [79]. However, one of the main disadvantages of ILs is their high viscosity because ILs with long alkyl chain and large non-polar size part could contribute to increase the Van der Waal's interactions which it is not a good extraction medium [79]. In order to reduce this drawback, ILs could be mixed with a low-viscosity solvent such as water. Therefore, the combination of PHWE with ILs (PHWE + ILs) may be a desirable approach for ILs applications because ILs present high thermal stability up to 400 ºC [80]. Recently, the extraction of phenolic compounds from the brown seaweed *Saccharina japonica* by PHWE + ILs has been explored [64]. The optimized extraction parameters were temperature (100-250 ºC) and concentration of IL (0.25-1.00 M). The employed IL was 1-butyl-3-methylimidazolium tetrafluoroborate [C_4C_1im][BF_4] because it was identified as the most adequate catalyst for phenolic extraction. The highest total content of phenolics was determined at 175 ºC. Low IL concentration in PHWE gave high extraction capacity. The reason for the decrease in phenolic

content could be due to the increase of the viscosity at high concentrations of IL in water, thus reducing the penetration ability of extracting solvent in the matrix. PHWE +ILs was compared with conventional solid-liquid extraction (SLE) with water and with water + ILs, and PHWE for the extraction of phenolic compounds from this macroalga. As can be seen in **Figure 3**, SLE showed poor peak quality in both water and ILs extractions. In the case of PHWE, it seemed to give higher extracting capability than conventional SLE with water. However, in PHWE +ILs process, it was observed an increment on the extraction of phenolic compounds compared to SLE and PHWE (**Figure 3**). Phenolic compounds were believed to be extracted faster in PHWE +IL than PHWE [64].

Figure 3

In other work, PHWE + IL was exploited for recovering carrageenan from the red seaweed *Kappaphycus alvarezii*. Several extraction conditions with different ILs as catalyst were tested [65]. 1-Butyl-3-methylimidazolium acetate (BMIMAc) was selected because, it exhibited the highest percentage yield of κ-carrageenan compared to the rest of ILs tested. BMIMAc (1 %) at 150 °C/5 MPa exhibited the highest extraction yield of carrageenan. Extracted material by PHWE + IL was compared with extracts get by aqueous PHWE and conventional method being higher in PHWE + IL [65].

Furthermore, other polysaccharides such as fucoidan and alginate from the brown seaweed *S. japonica* were extracted combining deep eutectic solvents (DES) with PHWE (PHWE + DES) [66]. DES are preparations made from a eutectic mixture of Brønsted–Lewis bases and acids, which has several types of cationic or anionic groups [66]. DES are classified as a class of ILs. They have different properties such as low volatility, low melting point, and high thermal stability [81]. One of the main advantages of DES is that they do not have toxic quaternary ammonium salts and are biodegradable and inexpensive. Polyols, choline chloride, urea,

sugars, and organic acids are broadly employed to make DES [82]. That is the reason why a DES was added with enough water amount and it was used as an extraction solvent to extract polysaccharides from the *S. japonica* [66]. First, the DES choline chloride:glicerol (1:2) was selected because it showed higher yields of polysaccharides. The reason is that the steric hindrance of three hydroxyl groups of glycerol can greatly weaken the interactions between the polysaccharide and the chloride anion. The yield obtained by adding a DES in water as a catalyst was at least twice than that obtained from water/HCl as a solvent. After, the influence of the temperature (100-150 °C), pressure (1-5 MPa), water content (50%-70%), and liquid-to-solid ratio (30-50mL/g) was investigated. The optimal conditions to recover high yield of alginate and fucoidan were 150 °C, 2 MPa, and 70% water content, and liquid-to-solid ratio of 36.81 mL/g [66]. These extraction conditions were very similar to the ones used to extract carrageenan from *Kappaphycus alvarezii* by PHWE + ILs (**Table 1**).

Moreover, Machmudah et al. (2018) [67] used PHWE + DES to extract phenolic compounds from *Garcinia mangostana* Linn employing various temperatures (120-160 °C) and pressures (1-10 MPs) in static and dynamic modes and water containing DES at 10-30% (v/v). Citric acid and alanine mixed in a molar ratio 1:1 were used as starting materials to form DES. In static mode, 120 °C was the best temperature to extract phenolics by PHWE with DES addition. However, in dynamic mode, the yields of xanthones and phenolic compounds can increase when the extraction was performed at 160 °C and extraction pressures of 5 and 10 MPa with 10 and 30% of DES addition, respectively. Additionally, the antioxidant capacity was higher in static than dynamic mode due to the autohydrolysis process that took place in static mode with longer residence time favoring the formation of derived antioxidant compounds from phenolic compounds and other undesirable chemical reactions during extraction process (see section 2) [67].

405 Based on the results of these works, it could be said that the addition of ILs and DES in PHWE
406 process could accelerate hydrolysis reactions to extract bioactive compounds being a fast and
407 effective extraction process, which improved the yield of polysaccharides and phenolic
408 compounds from seaweed and plants. However, the use of ILs as "green" solvents can be
409 somewhat questionable. For instance, imidazolium- and pyridinium-based ILs are mainly
410 derived from petroleum-based constituents. Furthermore, they can display high toxicity at poor
411 levels of biodegradation, and hazardous decomposition products can be released under certain
412 conditions. Also, to carry out the synthesis of some ILs, many chemical and purification steps
413 are needed which can involve the use of several harmful solvents [83].

414 **5. PHWE coupled with drying step**

415 PHWE has been coupled to other techniques to obtain dry extracts that avoid freeze-drying
416 step. It overcomes the main drawback of using PHWE that is the difficulty to concentrate the
417 extracts since the heat of vaporization of water is relatively high compared to that of many
418 organic solvents. In the last years, novel drying methods are being developed in order to dry
419 and concentrate extracts minimizing the degradation of bioactive compounds such as radiant
420 zone drying, freeze concentration and membrane technologies (nanofiltration, reverse osmosis,
421 osmotic distillation). However, one of the main ways of drying the extracts is freeze-drying,
422 which is rather costly and time consuming, and may also lead to degradation of the bioactive
423 compounds due to heat, light and oxygen.

424 In order to address this problem, water extraction and particle formation on-line (WEPO) was
425 successfully developed in 2010 to extract antioxidant compounds from rosemary leaves [84,85]
426 and onion waste [86]. It is a process that combines PHWE with particle formation on-line using
427 supercritical CO_2 as dispersant and hot nitrogen for drying the produced fine droplet.

In the last years, Uzel and collaborators have used a practical method for isolation of phenolic compounds employing PHWE with in-site particle generation [68,69]. The scheme of the apparatus that was used to produce particles of dry extracts rich in phenolic compounds is shown in **Figure 4**. It was divided mainly into a water delivery unit (a water source, a circulating heater, an HPLC pump), an extraction unit (a pre-heater, an extraction vessel and an oven) and a crystallizing particle part (nozzle and collector) (**Figure 4**). This extraction system was handled to recover phenolic compounds from *Ganoderma lucidum* mushroom [68] and black carrot [69]. In the case of *Ganoderma lucidum,* three different extraction temperatures (100, 150, and 200 ºC) and two pressures (5 and 10 MPa) were investigated in order to study the combined effect of pre-expansion and extraction temperature and pressure on particle formation. Air pressure and water flow rate were kept constant at 0.5 kPa and 0.5 mL/min. So, the efficient temperature for extraction was 200 °C at the highest pressure (10 MPa) for 60 min and pre-expansion temperature was kept at low temperature values of 60-70 °C. At temperatures of 60 and 70 °C, the average particle size of 4 μm was obtained. On the other hand, the same research group used the same technique to recover particles of anthocyanins and phenolics from black carrot [69]. PHWE was carried out at 10 and 20 MPa and at temperatures ranging from 40 to 100 °C. They studied the use of several ratios of ethanol in water (40-80%) in PHWE. The efficiency also depended on the ethanol addition where the results were better in ethanol (80% v/v) assisted PHWE. The best conditions to extract phenolic compounds were 80 °C at 10 MPa for 120 min. It was seen that under these conditions, an average particle size of 3 μm was obtained [69]. Therefore, this combination of PHWE with particle formation on-line was useful to have dried particles with a size of 3-4 μm from the obtained extracts with the advantages expressed at the beginning of this section.

Figure 4

6. Conclusions and future trends

Water at high temperatures and pressure is an interesting solvent because it increases the mass transfer and solubility of many bioactive compounds. Taking into account the high number of PHWE applications in the last years for the extraction of bioactives, it can be predicted that new progresses in applications involving PHWE will be achieved. For instance, one important trend is the improvement of the selectivity and extraction yield using PHWE combined with ILs and DES. Articles appeared in the literature about PHWE +ILs/DES demonstrated that it is a fast and effective extraction process and might probably be a potential catalytic agent, which improves the extraction yield of bioactive compounds such as polysaccharides and phenolic compounds. However, PHWE + ILs/DES is a promising extraction technique that has not been studied in depth and more studies are needed in order to know all its advantages and disadvantages. Other trend is the on-line hyphenation of PHWE with an air drying step allowing the formation of dry micro-particles from PHWE extracts in just one step. PHWE with in-site particle generation avoids the main drawback of performing PHWE that is the difficulty to concentrate the extracts because of the relative high vaporization heat of water.

Nevertheless, due to the risk of degradation and hydrolysis reactions during PHWE, there are some doubts about this extraction technique. Therefore, the investigations in the future should be focused on what really is happening to the analytes, the undesirable compounds that have been extracted and formed and the remaining sample matrix during the extraction with respect to temperature and time/flow rate.

Even if the trend of the researchers is substituting hazardous organic solvents with water, which is considered one of the greenest solvents to extract bioactive compounds from natural sources, more studies are needed to evaluate all aspects of environmental impact assessment. For instance, life cycle assessment (LCA), which provides a detailed evaluation of emissions to the environment as well as resources used over the full life cycle of the solvent (from production and use to recycling and disposal), should be conducted in PHWE. However, in the literature

from 2015 to present was not possible to find works that employ LCA to measure how good is PHWE from the environmental point of view to carry out the extraction of bioactive compounds. In conclusion, real evaluations of the environmental impact of the extraction process are required in order to decide what extraction process is better from an environmental point of view.

7. Acknowledgements

Authors thank financial support from the Comunidad of Madrid (Spain) and European funding from FSE and FEDER Programs (project S2018/BAA-4393, AVANSECAL-II-CM).

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737

Table 1. The most remarkable applications in PHWE published in the period 2015-present.

Source	Bioactive compound (Method of analysis)	Static/ Dynamic	Tempera-ture (°C)	Pressure (MPa)	Time (N° cycles)/Flow rate	Other	Ref.
Phenolic compounds							
Purple coneflower (*Echinacea purpurea* L.)	Total phenolic content (FC) Total flavonoid content (ACM) Antioxidant capacity (TEAC)	Static	147	3.0	8.4 min	--	[12]
Arctostaphylo uva-ursi L. herbal dust filter tea	Total phenolic content (FC) Total flavonoid content (ACM) Antioxidant capacity (RPM, DPPH)	Static	151	3.0	10 min	Magnetic stirrer (1000 rpm) Modifier: HCl 1.5 % (v/v)	[13]
Crocus sativus petals	Total phenolic content (FC) Total flavonoid content (UV assay) Antioxidant capacity (DPPH, FRAP)	Static	159	0.7	54 min	Water/solid ratio of 36 mL/g	[14]
Ginger rhizome	Total phenolic content (FC) Total flavonoid content (ACM) Antioxidant capacity (DPPH, RPM, OH, LPA, ABTS, FICA, PM)	Static	150-180	5.0	1 h	Agitation rate of 3Hz	[15]
Blue mussel (*Mytilus edulis*)	Antioxidant capacity (DPPH), angiotensin-converting enzyme inhibitory, and acetylcholinesterase inhibitory activities	Static	200	--	60 min	--	[16]
Sage (*Salvia officinalis* L.)	Total phenolic content (FC) Total flavonoid content (ACM)	Static	201	20.0	15.8 min	--	[17]
Wild garlic (*Allium ursinum*).	Total phenolic content (FC) Total flavonoid content (ACM) Antioxidant capacity (DPPH, ABTS) Phenolic composition (HPLC-DAD)	Static	181	3.6	10 min	Acidifier 1.09%; Magnetic stirrer:750 rpm	[18]
Mountain germander (*Teucrium montanum* L.)	Total phenolic content (FC) Antioxidant capacity (DPPH and FRAP) Phenolic composition (HPLC-DAD)	Static	160	1.0	30 min	Agitation rate of 3Hz	[19]
Waste onion skin	Total phenolic content (FC) Total flavonoid content (ACM) Antioxidant capacity (DPPH) Flavonoids (HPLC-DAD)	Static	170-230	3.0	30 min	Mixing speed of 400 rpm pH = 10	[20]
Spent coffee grounds (*Coffea arabica* L.)	Total phenolic content (FC) Antioxidant capacity (DPPH, ABTS, online HPLC-ABTS) Phenolic compounds (HPLC-DAD-MS)	Static	179	5.0	36 min	Solid-to-liquid ratio: 14.1 g/L	[21]
Vine shoot wastes	Phenolic compounds (HPLC-DAD) Antioxidant capacity (FRAP, DPPH) Antimicrobial activity	Static	150	4.0	40 min	Frequency of the vibrational platform: 3 Hz	[22]
Sorghum (*Sorghum bicolor* L.)	Total phenolic content (FC) Phenolic compounds (HPLC-MS) Antioxidant capacity (DPPH, ABTS) Antiproliferative activity	Static	144	--	21 min	Solvent-to-solid ratio: 35 mL/g	[23]
Coriander (*Coriandrum sativum* L.) seeds	Phenolic compounds (HPLC-MS/MS) Volatile compounds (GC-MS)	Static	100	6.0	10 min	--	[24]
Pomegranate (*Punica granatum* L.) peel	Total phenolic content (FC) Total flavonoid content (ACM) Antioxidant capacity (DPPH and ABTS) Identification and quantification of phenolics (UHPLC-MS)	Static	126	3.0	18.5 min	Water/solid ratio of 54.8 mL/g	[25]
Chamomile ligulate flowers	Total phenolic content (FC) Total flavonoid content (ACM) Apigenin (HPTL-UV/Vis)	Static	115	4.5	30 min	Solvent-to-sample ratio: 1:30; Agitation rate 3 Hz	[26]

	Phenolic compounds (UHPLC-DAD-MS/MS) Antioxidant capacity (DPPH, RPM)						
Chamomile (*Matricaria chamomilla* L.)	Phenolic compounds (UHPLC-DAD-MS/MS) - Flavonoids - Phenolic acids Antioxidant capacity (LPA, DPPH, OH, ABTS, electrochemical DNA-based sensor) Enzyme inhibitory activity Cytotoxic activity	Static	 85-115 85 150 85 115	4.5	30 min	--	[27]
Buds of *Sophora japonica* L.	Rutin and Quercetin (HPLC-UV)	Static	120	--	60 min	--	[28]
Artichoke by-products	Caffeoylquinic acids and flavones glycosides (UHPLC-UV) Cellular antioxidant activity assay	Static	93	10.3	5 min (2 cycles)	Modifier: EtOH 10 % (v/v)	[29]
Carrot leaves	Total phenolic content (FC) Luteolin content (HPL-UV)	Static	210 120	4.0	113.5 min 10 min	--	[30]
Soybean flour and soybean protein isolate	Isoflavones (HPLC-UV) - Malonyl groups - Glucoside - Aglicones	Static	<160 60 80-160 >160	10.3	14 min	--	[31]
Puerariae lobata	Isoflavones (HPLC-UV)	Static	120	--	45 min	Solid-to-liquid ratio of 1:15	[32]
Grapevine by-products	Stilbenes (UHPLC-DAD; HPLC-DAD-MS/MS, NMR)	Static	160	10.0	5 min	--	[33]
Grape seeds	Resveratrol (HPLC-UV)	Static	152.32	1.0	24.89	Solid-to-solvent ratio of 1:15 g/mL	[34]
Radix Scutellariae	Flavonoids (baicalin and wogonin) (HPLC-UV)	Static	160	--	20 min	Water loadings of 4 mL and 60-100 mesh	[35]
Turmeric (*Curcuam longa* L.)	Curcumin (HPLC-UV)	Static	140	1.0	14 min	Particle size: 0.71 mm	[36]
Turmeric (*Curcuma longa* L.)	Curcumin content (GC-FID) Essential oil (GC-MS)	Dynamic	150	2.0	100 min/1 mL/min	Particle size 0.5 mm	[37]
Black tea	Epicatechins (HPLC-UV/Vis) Theophylline (HPLC-UV/Vis) Caffeine (HPLC-UV/Vis)	Dynamic	160	0.8	24.5 min/ 12 mL/min	--	[38]
Pistachio (*Pistacia vera* L.) hulls	Phenolic composition (HPLC-MS) Antioxidant capacity (DPPH, ABTS, FRAP)	Dynamic	150-170	6.9	4 mL/min (120 mL)	---	[39]
Deffated orange peel	Flavanones (hesperidin and Narirutin) (HPLC-UV) Total phenolic content (FC)	Dynamic	150	10.0	10 mL/min	--	[40]
Morus nigra L. fruits	Total phenolic content (FC) Total flavonoid content (ACM) Total anthocyanin content (pH differential method) Anthocyanin content (UHPLC-DAD-MS/MS)	Dynamic	60	15.0	60 min/2 mL/min	--	[41]
Lycium ruthenicum Murr.	Total anthocyanin content (pH differential method) Anthocyanins (HPLC-DAD, UHPLC-MS)	Dynamic	170	--	55 min/3 mL/min	--	[42]
Moringa oleifera leaves	Vitamin C, Kaempferol and Quercetin (UHPLC-DAD-MS)	Dynamic	91	--	60 min/0.3 mL/min	--	[43]
Momordica foetida	Flavonoids (UHPLC-MS)	Dynamic	250	6.9	5 mL/s (50 mL)	--	[44]
Diterpenes							
Stevia rebaudiana Bertoni leaves	Steviol glycosides (HPLC-DAD) Total phenolic content (FC) Total condensed tannins Chlorophyll A and B and total carotenoids (UV method)	Static	160	10.3	10 min (3 cycles)	--	[45]
Triterpenes							
Red ginseng (*Panax ginseng* C.A. *Meyer*)	Ginsenoides - Rg3 and Rh2, TAC, FC - Rg2 and Rh1	Static	 200 150	10.0	 10 min 20 min	--	[46]
Ginseng roots (*Panax ginseng* C.A. *Mey*)	Ginsenoides (Rh2)	Static	140-160	6.0	20 min (2 cycles)	--	[47]
Birch bark	Betulinic acid (HPLC-UV)	Static	185	--	27.4 min	Solvent-to-solid ratio: 59.60 mL/g	[48]

Hedyotis diffusa	Ursolic acid (HPLC-MS/MS)	Static	157	1.8	20 min	Particle size 80 mesh Solvent-to-solid ratio of 30 mL/g	[49]
Dry loquat leaves (*Eriobotrya japonica*)	Pentacyclic triterpenoid (HPLC-MS)	Dynamic		10.0	60 min	--	[50]
	- Corosolic acid		180		33.3 mL/min		
	- Ursolic acid		200		41.7 mL/min		
Quinoa stalks (*Chenopodium quinoa* Wild.)	Saponins (Colorimetric assay and MS)	Static	195	10.3	1 min (1 cycle)	--	[51]
Ginseng by-products	Total saponins (UV assay), Total phenolic content (FC) Antioxidant capacity (ABTS) Phenolic compounds (HPLC-UV) Antimicrobial activity Antihypertensive activity	Static	207	4.3	15 min	Solid-to-solvent ratio of 0.04 g/mL Agitation Speed of 199 rpm	[52]
Polysaccharides							
Pacific oyster *Crassostrea gigas*	Bioactive polysaccharide (TLC, HPLC, FT-IR) Antioxidant capacity (DPPH, ABTS) In vitro antidiabetic activity Antihypertensive activity	Static	125.01	--	14.9 min	Liquid-to-solid ratio 44.69:1 (mL:g)	[53]
Brazilian ginseng roots	Fructooligosaccharides	Static	120	12.0	5 min	--	[54]
	Beta-ecdysone		120		15 min		
Sacharina japonica	Fucoidan Antioxidant capacity (FRAP, TAC) Antimitotic activity Cell culture, cell viability assay	Static	127	8.0	12 min	Modifier: 0.1 % NaOH Solid-to-liquid ratio: 0.04 g/mL	[55]
Passion fruit peel	Pectic oligosaccharides (HPLC and HPSEC)	Static	150 175	--	4.5 min 5.5 min	--	[56]
Passion fruit peel	Pectic oligosaccharides (HPLC and HPSEC) Antioxidant capacity (DPPH) Total phenolic content (FC)	Static	140	--	6.9 min	Modifier: 10 % Ethanol	[57]
Green coffee beans (*Coffee arabica*)	Modified polysaccharides (FT-IR, UV spectroscopy, XRD and TGA) Antioxidant capacity (DPPH, ABTS, FRAP, RPM, ORAC, TAC)	Static	180 220	3.0 6.0	30 min 15 min	--	[58]
Spent coffee grounds	Bioactive polysaccharides	Static	179	2.0	5 min	Ultrasonic pre-treatment	[59]
Pomelo (*Citrus grandis* (L.) Osbeck) peels	Low methoxyl pectin	Dynamic	120	3.0	20 min/ 1 mL/min	--	[60]
Proteins/ Other bioactive compounds							
Sambucus nigra L. branches (elderberry)	Proteins (SDS-PAGE)	Static	50	15.0	5 min	--	[61]
Nannochloropsis salina	Total lipids content Fatty acids (GC)	Dynamic	175	2.0	60 min / 4 mL/min	Sample weight: 1g	[62]
Camellia oleifera Abel. seeds	Total saponins (Vanillin-sulfuric method)	Static	121	2.0-7.0	32.1 min	Solvent-to-solid ratio: 8.33 mL/g	[63]
	Free fatty acids (GC-MS) Antioxidant capacity (DPPH)		134		32 min	Solvent-to-solid ratio: 10.79 mL/g Agitation at 150 rpm	
PHWE with ILs/DES							
Brown seaweed *Saccharina japonica*	Total phenolic content (FC) Phenolic compounds (HPLC-UV/vis) Antioxidant capacity (ABTS, TAC, FRAP)	Static	175	5.0	5 min	Stirred at 400 rpm **IL** concentration: 0.25 M $[C_4C_1im][BF_4]$ (1-butyl-3-methylimidazolium tetrafluoroborate)	[64]
Red seaweed *Kappaphycus alvarezii*	Carrageenan Antioxidant capacity (ABTS, DPPH)	Static	150	5.0	5 min	1% BMIMAc **IL** in water (1-Butyl-3-methylimidazolium acetate) Stirred at 200 rpm Solid-liquid ration: 1:80 g/mL	[65]
Brown seaweed *Saccharina japonica*	Alginate Fucoidan	Static	150	2.0	25 min	**DES**: Choline chloride: Glycerol, ratio 1:2 (mol/mol) 70 % water content Liquid-to-solid ratio: 31.81 mL/g	[66]
Pericarps *Garcinia mangostana* Linn	Total phenolic content (FC) Antioxidant capacity (DPPH)	Dynamic	160	10.0	180 min /1 ml/min	30% DES addition **DES:** Citric acid: Alanine ratio 1:1 (mol/mol)	[67]
	Xanthone and phenolic compounds (UV-vis)	Static	120	5.0	60 min		

PHWE + Particle formation					**Dried conditions:**	
Ganoderma mushroom	Phenolic compounds (HPSEC–UV/vis and MALDI-TOF-MS)	Dynamic	200	10.0	Pre-expansion temperature: 60-70 °C Air pressure: 0.5 kPa, Water flow rate: 0.5 mL/min	[68]
Black carrot	Anthocyanins and phenolic compounds	Dynamic	80	10.0	Pre-expansion temperature: 50-60 °C Air pressure: 0.5 kPa, Water flow rate: 0.5 mL/min. 80% ethanol in water (v/v)	[69]

ABTS, 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulphonate); ACM, aluminum chloride method; DAD, diode array detector; DES, deep eutectic solvent; DPPH, α-diphenyl-β-picrylhydrazyl; FC, Folin-Ciocalteau assay; FICA, ferrous ion chelating ability; FID, fame ionization detector; FRAP, ferric reducing ability of plasma; FT-IR, Fourier-transform infrared spectroscopy; GC, gas chromatography; HPLC, high performance liquid chromatography; HPSEC, high-pressure size-exclusion chromatography; IL, Ionic liquid; LPA, lipid peroxidation assay; MALDI; Matrix-Assisted Laser Desorption/Ionization; MS, mass spectrometry; NMR, nuclear magnetic resonance; OH, hydroxyl radical scavenging activity; ORAC, oxygen radical absorbance capacity; PM, phosphomolybdenum method; RPM, reducing power assay; SDS-PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis; TAC, total antioxidant capacity; TEAC, trolox equivalent antioxidant capacity; TGA, thermogravimetric analyser; TLC, thin-layer chromatography; TOF-MS, time-of-flight mass spectrometry; UHPLC, ultra-high performance liquid chromatography; UPTLC, ultra-performance thin-layer chromatography; UV, ultraviolet; Vis, visible; XRD, x-ray diffraction;

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Figure 1. Changes of water dielectric constant as a function of the temperature at constant pressure (20 MPa). The figure presents the dielectric constant values equivalents to some common organic solvents at room temperature and pressures (25 ºC and 0.1 MPa). Reprinted with permission from [8].

Figure 2. Response surface contour and three dimensional plots of PHWE of Pacific oyster (*Crassostrea gigas*) cakes showing the interaction effects of temperature and time on the extraction yield of polysaccharides (PS Yield (%)). Reprinted with permission from [53], Elsevier.

Figure 3. HPLC chromatograms of phenolic compounds extracted from brown seaweed S*accharina japonica* by (A) SLE with water as the solvent; (B) SLE with 0.5 M [C_4C_1im][BF_4] in water as the solvent; (C) PHWE at 175 ºC; and (D) PHWE + ILs ([C_4C_1im][BF_4]) at 175 ºC. Reprinted with permission from [64], Elsevier.

Figure 4. Combined extraction/particle production apparatus: 1, distilled water reservoir; 2, HPLC pump; 3, pre-heater; 4, extraction vessel; 5, oven; 6, extract outlet pipe line; 7, ball valve; 8, collection chamber; 9, air atomizing expansion nozzle; 10, air inlet-needle valve; 11, vacuum-air exit. Reprinted with permission from [68].