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Pressurized hot water extraction of bioactives

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ABSTRACT

The purpose of this review is to give the reader a thorough background to the fundamentals and applications of pressurized hot water extraction (PHWE) for the analysis of bioactive compounds. We summarize the field in the period 2009–14, and include fundamentals of water as a solvent: equipment; method optimization; applications; coupling; and, future prospects. We highlight that solvent properties of water are tunable by changing the temperature, particularly self-ionization, dielectric constant, viscosity, diffusivity, density and surface tension. Furthermore, important aspects to consider are the risk of degradation of the analytes and other potential reactions, such as hydrolysis, caramelization and Maillard reactions that may lead to erroneous results. For the extraction of bioactive compounds, we report PHWE methods based on using water of 80–175°C and short extraction times. In conclusion, PHWE provides advantages over conventional extraction methods, such as being "greener", faster and more efficient.

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Contents

1. Why another review article on pressurized hot water extraction?

Pressurized hot water extraction (PHWE) is an extraction technique that uses liquid water as extractant (extraction solvent) at temperatures above the atmospheric boiling point of water (100°C/273 K, 0.1 MPa), but below the critical point of water (374°C/647 K, 22.1 MPa) (Fig. 1, [1]). The use of PHWE in analytical chemistry started with the work in environmental analysis by Hawthorne and colleagues in the mid-1990s [2,3], and can also be referred to as subcritical water extraction (SWE), superheated water extraction and pressurized liquid extraction (PLE) or accelerated solvent extraction (ASE) with water as a solvent. There are a few relatively recent review articles on analytical PHWE, which the reader is recommended to read [4–8].

The aim of this review article is to give a thorough background on the fundamental properties of water – an aspect that has been virtually overlooked in most review articles written so far about analytical PHWE. Hence, the first part of this review article concerns the fundamentals of chemical/physical properties of water and how these change with the increase in temperature, as well as how these affect the extraction performance both positively and negatively in different analytical applications.

The second part deals with technical solutions of PHWE and how to conduct the experiment in practice. This technical part includes discussions on using commercially available and home-built equipment. The third part includes aspects on method optimization in PHWE. The fourth part summarizes some of the key

applications and related publications mainly in the field of extraction of bioactive compounds from plants, food, biological and pharmaceutical samples, especially for the last five years (2010–14), without at all being exhaustive. The final part is a conclusion and an outlook towards the near future in terms of PHWE development with respect to equipment and methodologies in analytical chemistry.

2. Fundamentals of water from ambient to near-critical conditions

Water is perhaps the most interesting naturally occurring liquid on Earth, so necessary for life, and available to use without costly transportation. To use water as a solvent has nearly negligible environmental impact considering production and transportation. However, using water as a solvent in extraction processes might be energy demanding in those cases where water needs to be removed by evaporation. However, it is worth noting that the energy demand to heat liquid water (25°C to 250°C, 5 MPa) is almost three times less than needed to vaporize the water to create steam (25°C to 250°C, 0.1 MPa) [9].

2.1. Water at ambient conditions

Water has chemical and physical properties like no other solvent [10]. At ambient temperature, water is a polar liquid, which dissociates into the hydronium ion (H_3O^+) and the hydroxide ion (OH^-), as described by its dissociation constant (K_w), which is 1.0×10^{-14}
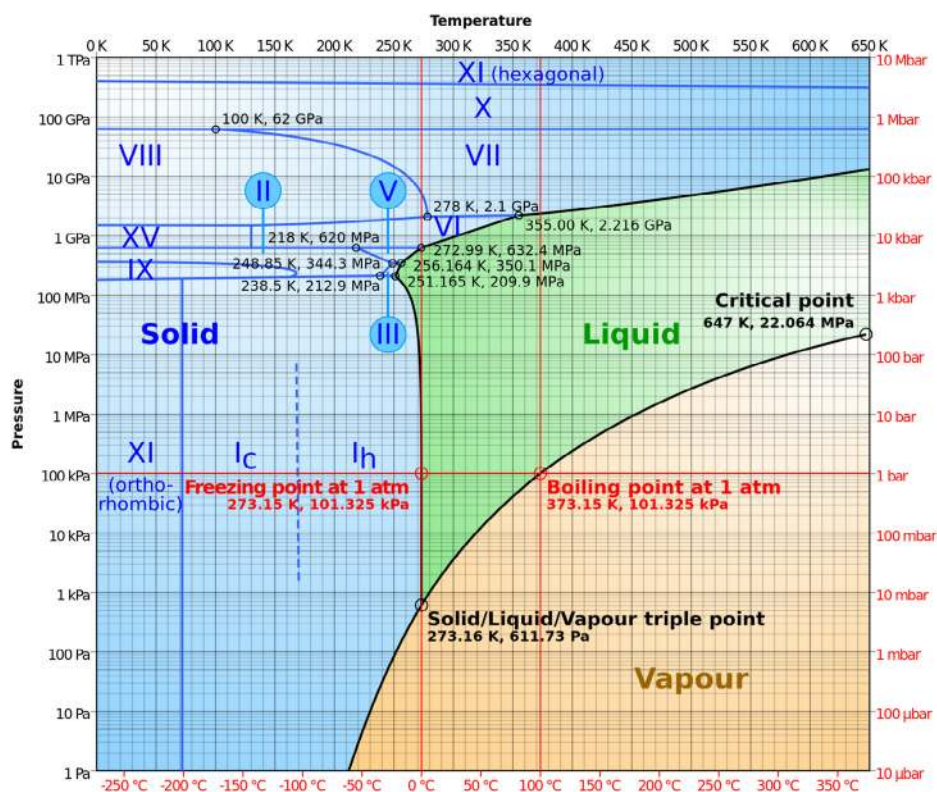

Fig. 1. Phase diagram of water [1].

Table 1
Chemical and physical properties of liquid water at different temperatures and saturation pressures

Property	T = 298 K (25°C, 0.1 MPa)	T = 373 K (100°C, 0.1 MPa)	T = 473 K (200°C, 1.5 MPa)	T = 623 K (350°C, 17 MPa)
Dissociation constant, K_w [10]	1.0×10^{-14}	5.6×10^{-13}	4.9×10^{-12}	1.2×10^{-12}
pK_w	13.99	12.25	11.31	11.92
Relative static permittivity, ε_r [13]	78.5	55.4	34.8	14.1
Dipole moment	1.85	1.85	1.85	1.85
Specific heat capacity, C_p, (J g^{-1} K^{-1}) [14]	4.18	4.22	4.51	10.1
Heat of vaporization, H_v (kJ/mol)	44.0	40.7	35.0	15.6
Density (g/cm^3) [15]	0.997	0.958	0.865	0.579
Dynamic viscosity, η (mPa s) [16]	0.891	0.282	0.134	0.067
Surface tension (dyn/cm) [16]	72.0	58.9	37.6	3.7
Self-diffusion coefficient, D (m^2/s) [17]	2.3×10^{-9}	8.6×10^{-9}	23.8×10^{-9}	N/A

at 25°C. This dissociation is commonly described as the "self-ionization of water" [11] (Equation 1):

$$K_w = \frac{[H_3O^+][OH^-]}{[H_2O]^2} \tag{1}$$

Further, water has an extremely high relative static permittivity (ε_r, also referred to as the dielectric constant) of 80 at 20°C. This means that water creates electrostatic bonds with other molecules, thereby decreasing or eliminating intermolecular interaction between surrounding ions. In other words, water dissolves salt very well. The relative static permittivity (ε_r) can be measured by a capacitor, relating the capacitance in vacuum (C_0) to the capacitance in the liquid (C_x) (Equation 2):

$$\varepsilon_r = \frac{C_0}{C_X} \tag{2}$$

ε_r is a macroscopic property related to polarisability (π^*), which is a microscopic property of how easily an induced dipole with a separated charge distribution across the molecule can be established. π^* is just like ε_r determined experimentally. Both ε_r and π^* are relatively high for liquid water at ambient conditions, and they decrease with increasing temperature as described further below.

Water with its high ε_r also has a high dipole moment (1.85 D), while n-hexane with an ε_r of 1.9 at 20°C has a dipole moment of zero D [12]. However, ε_r should not be confused with the dipole moment of the molecule. For example, dichloromethane has a relatively low ε_r of 9.1 (20°C), while its dipole moment is quite high (1.60 D), which is also true for ethyl acetate (ε_r = 6.0, 1.78 D).

The high dipole moment of water can be explained by the high electronegativity of oxygen compared to hydrogen, and the smaller angle between the O-H bonds (104.5°) compared to a typical tetrahedral angle of 109°. This charge distribution results in intermolecular attraction between the water molecules, and also with other dipolar molecules (i.e., so-called hydrogen bonding). Water has strong hydrogen bonding, which results in a very high specific heat capacity ($C_{p,m}$, 75.3 J mol^{-1} K^{-1}, isobaric, molar, at 25°C) and a high heat of vaporization (H_v, 40.7 kJ/mol at 100°C).

Table 1 lists some important chemical and physical properties of water – aspects that are important to take into consideration when using water as a solvent in extraction. Many of these properties dramatically change with variation in temperature.

2.2. Water at elevated temperature and pressure

What makes water an interesting, viable solvent in extraction is that several of its chemical and physical properties largely change when varying the temperature [17]. As shown in Table 1, one of the most dramatic changes for liquid water at saturation pressure is the ε_r, going from 78 at 25°C to 14 at 350°C [13]. Water at near critical

conditions (P and T just below the critical point) dissolves hydrophobic compounds such as PAHs and PCBs [3,18]. Salt is no longer soluble in the water (i.e., it precipitates). What happens is that the increasing temperature diminishes electrostatic interactions between the water molecules, and also between water molecules and surrounding ions and molecules (i.e. both ε_r and π^* decrease with increasing temperature). An increased movement/rotation of water molecules can also be observed at higher temperature. Hence, the use of liquid water at higher temperature and pressure allows for dissolving less polar compounds, since the intermolecular interactions involving hydrogen bonding becomes less pronounced, thereby favoring London dispersion forces (induced dipole-induced dipole forces). In other words, liquid water at elevated temperature (and pressure) renders the water a less polar of a solvent. As a comparison, liquid water at temperatures of 200–275°C and saturation pressure has ε_r similar to that of methanol and ethanol at ambient conditions [13,19].

Solvent properties can be experimentally characterized and "quantified" using the three solvatochromic Kamlet-Taft parameters [20]:

- hydrogen-bond donating ability (acidity, α);
- hydrogen-bond accepting ability (basicity, β); and,
- polarisability/polarity (π^*)

Each parameter is an index scale between 0 and 1 based on two reference solvents, one set to a value of 0 and the other to a value of 1. For example, π^* is set to 0 based on cyclohexane and DMSO is given a value of 1. Most solvents will have values between 0 and 1 on each scale. The indices are obtained experimentally using compounds that absorb visible light (e.g., 4-nitroanisole and N,N-diethyl-4-nitroaniline dyes), and, when dissolved in the solvent, result in a shift in absorbance maximum for a certain absorbance band [20]. Using this method, solvents can be compared in terms of solvent properties [20,21]. In Table 2, water is compared with some common organic solvents with respect to their solvatochromic parameters. It is obvious that liquid water at elevated temperature attains a polarisability similar to methanol at 275°C, but the hydrogen-bond accepting ability is very different from both methanol and

Table 2
Solvatochromic parameters for water and some common organic solvents; average values calculated from [20]

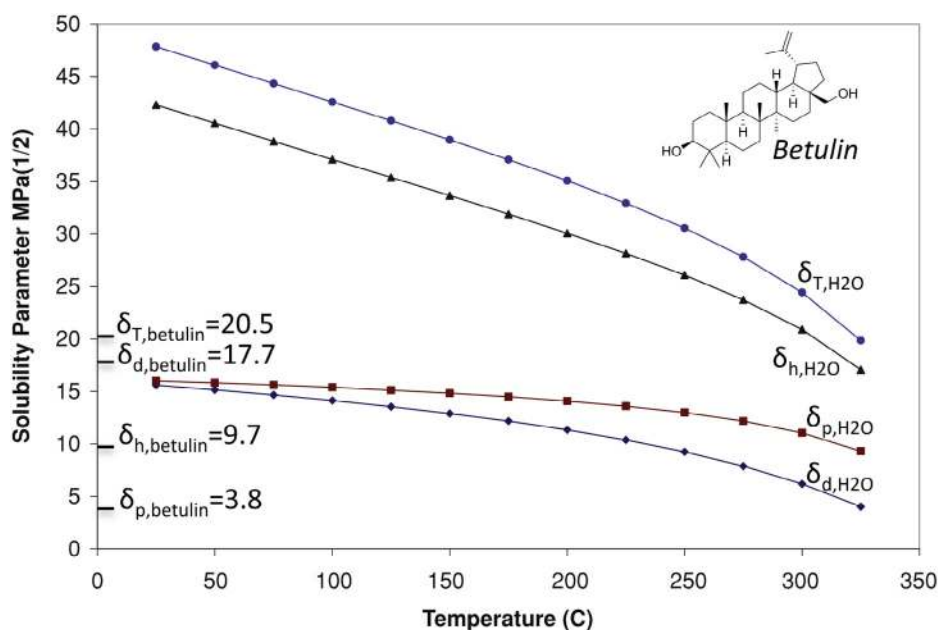| Solvent	α	β	π^*
Water, ambient	1.20	0.37	1.12
Water, 275°C	0.84	0.20	0.69
Methanol	1.01	0.71	0.59
Ethanol	0.89	0.79	0.53
Ethyl acetate	0	0.36	0.61
Cyclohexane	0	0	0

Fig. 2. Hansen solubility parameters *versus* temperature for water at saturation pressure (curves) and for betulin (marked in the left-hand side), modified from [23]. {Reprinted with permission from [23], John Wiley & Sons}.

ethanol. Hence, there is a risk in considering only polarisability or dielectric constant when discussing the tunable solvent properties of pressurized hot liquid water.

Carr et al. reviewed the solubility of organic compounds in pressurized hot liquid water, including a few bioactive compounds (e.g., d-limonene and caprylic acid) [19]. They described how the solubility of organic compounds in the water often goes through a minimum value, due to "hydrogen-bonding cages" around the solute compound, which give decreasing solubility with increasing temperature. However, at a certain temperature, the solubility instead increases with temperature since the positive heat of cavity formation dominates over the negative heat in solubility.

However, there are drawbacks of experimentally determining solubility of bioactive compounds in water. The availability of chemical standards is limited, and there is a pertinent risk of degradation of the compound during the equilibration in the solubility determination experiment. Therefore, good complements to experiments are solubility models and theoretical approximations. One such example of a theoretical tool to assess solvent properties is the Hansen Solubility Parameters (HSP), first developed by Hansen [22], which can be used to describe solvent properties or to predict the solubility of an analyte or other compound in a solvent. The method is based on three cohesion energy density (CED) parameters:

- energy from dispersion forces between molecules (δ_d);
- energy from dipolar forces between molecules (δ_p); and,
- energy from hydrogen bonds between molecules (δ_h)

These three parameters form coordinates in a three-dimensional space for the analyte with respect to the solvent molecule. The more the spheres overlap, the more soluble the analyte is in the solvent. The total solubility parameter (squared) is the sum of the square of the three CED parameters [22] (Equation 3):

$$\delta_T^2 = \delta_d^2 + \delta_p^2 + \delta_h^2 \tag{3}$$

CED parameters are calculated based on the group contribution method [22] {e.g., in Srinivas et al. [23], where the solubility

of betulin in water compared to in ethanol was explored using HSP}. Betulin is an interesting anti-fungal and anti-inflammatory compound found in large proportion in birch bark [24]. In Fig. 2 and in [23], it is demonstrated that, for water, a temperature of 250–325°C is needed to dissolve betulin. Fig. 2 also shows that the largest contributor to the total solubility parameter of water is hydrogen-bonding CED, which is also the one decreasing most with increasing temperature in line with the discussion above.

From the discussion above, it is clear that temperature has a large effect on the dielectric/solvent properties of water, but pressure does not. In the literature, pressure is commonly described as having minimal effect on the solvent strength of pressurized hot liquid water [4] or on extraction efficiency in PHWE [5]. The pressure is mainly applied to maintain water in its liquid state, although, some references state that an elevated pressure helps wet the sample matrix, resulting in improved extraction efficiency [16,25]. It is worth noting that the increasing temperature of liquid water at saturation pressure leads to completely overlapping curves of decreasing π^* and density (see Fig. 3 [26],). As shown in Table 1 and Fig. 4C, the density of liquid water at saturation pressure goes from 0.997 g/mL at 25°C
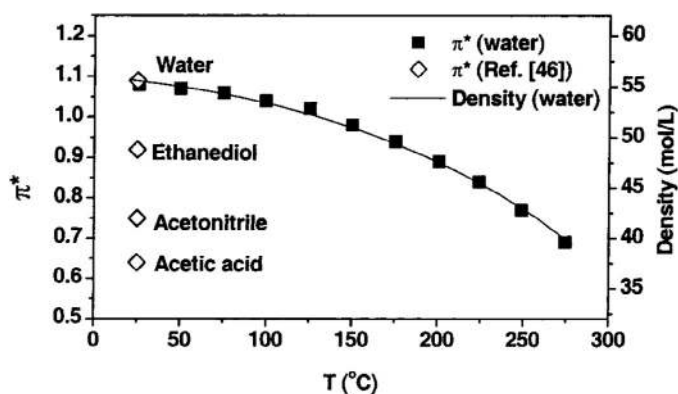

Fig. 3. Polarisability and density of liquid water as a function of temperature. {Reprinted with permission from [26], ©2002, American Chemical Society}.
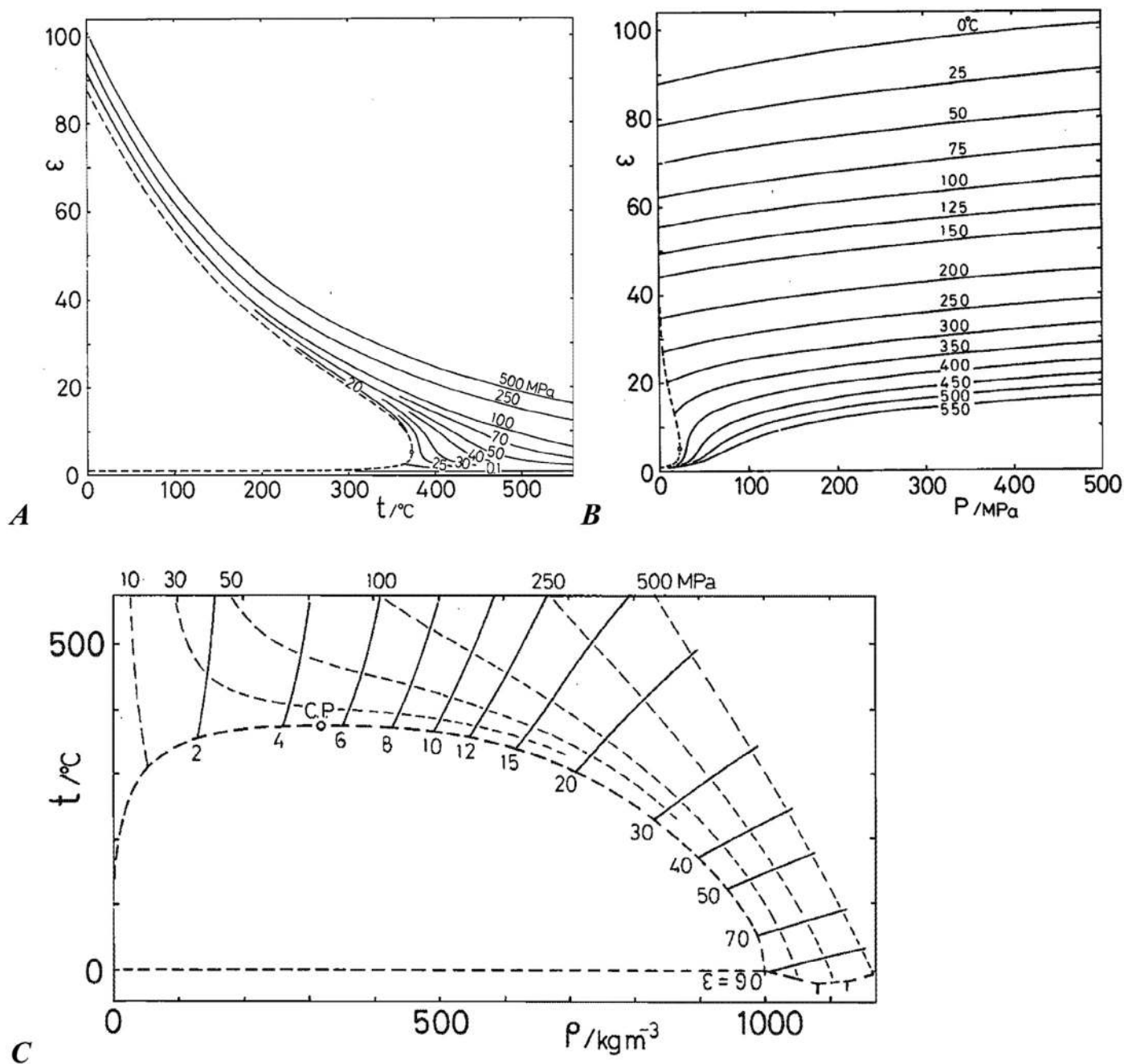
Fig. 4. Static electric permittivity of liquid water as a function of temperature (A), pressure (B) and temperature/pressure/density (C). {Reprinted with permission from [13], AIP Publishing LLC}.

to 0.579 g/mL at 350°C [15]. Pressure needed to compensate for the weakened intermolecular interactions leading to decreasing density of the water is in the range of 100 MPa or more at temperatures above 200°C (Fig. 4). Hence, it is important to realize in solubility works and experiments on extraction efficiency that the density of liquid water at elevated temperatures (and pressures) is not constant but rather significantly decreases within the experimental range of temperature values (100–374°C).

In addition to dielectric properties and density, the self-ionization properties of water also vary with temperature. Table 1 shows how the dissociation constant (K_w) of water increases with two units from 1.0×10^{-14} at 25°C to 1.2×10^{-12} at 350°C, with a maximum value of 4.9×10^{-12} at 250°C. This implies that the pH changes from about 7.0 to 5.5 (Fig. 5), and the ionic strength of hydronium and hydroxide

ions is significantly higher at 250°C than at ambient temperature. These changes may affect PHWE in several aspects. First of all, at elevated temperatures, the risk of unwanted reactions, such as hydrolysis, increases, since the ion strength is higher and the pH is lower. Hydronium ions act as catalyst in reactions [27]. Furthermore, some analytes may undergo shifts in equilibrium towards other charged forms (e.g., anthocyanins can occur in five different forms depending on pH) [28].

The chemical and physical properties described above relate mainly to thermodynamic solvent properties of pressurized hot liquid water. In addition, there are a few properties that affect the mass-transfer properties in liquid water; including viscosity, diffusivity and surface tension – and all of these are affected by the temperature of water (Table 1, Fig. 6).
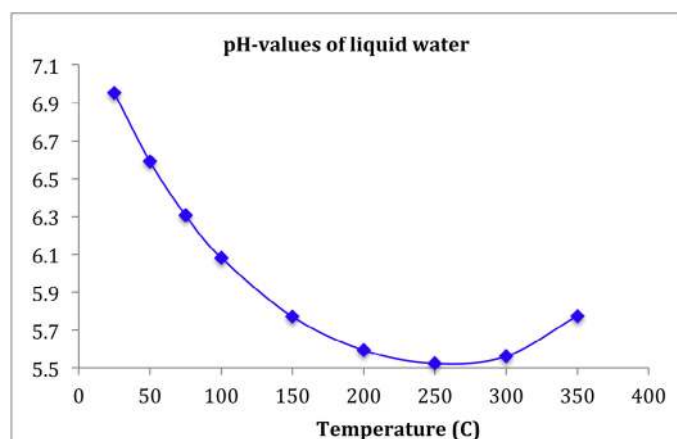
Fig. 5. Water ionization plotted as half of the negative logarithm of the dissociation constant (Kw) (i.e., pH values, as a function of temperature, at 25 MPa).

The viscosity of water as a function of its temperature can be described by the following Arrhenius-type of equation [30] (Equation 4):

$$(T) = 2.414 \times 10^{-5} \times 10^{247.8/(T-140)} \tag{4}$$

where μ is the dynamic viscosity (N·s/m²) and T is the absolute temperature (K). As shown in Equation (4), the viscosity decreases with increasing temperature.

The Stokes-Einstein relation describes the diffusion coefficient of a compound in a liquid as a function of the viscosity of the liquid (Equation 5):

$$D = \frac{k_B T}{6\pi \eta r_H} \tag{5}$$

where D is the diffusion coefficient (m²/s), η is the viscosity of the liquid (mPa·s), r_H is the hydrodynamic radius of the compound (m), T is the absolute temperature (K) and k_B is the Boltzmann constant (1.38×10^{-23} J/K). Clearly, the diffusivity increases with increasing temperature and decreasing viscosity. As shown in Table 1, the self-diffusivity of water deceases with a factor of 10 from 25 to 200°C, and, at the critical point (374°C), it is about 100 times higher than in ambient conditions. Diffusion of compounds in a liquid also
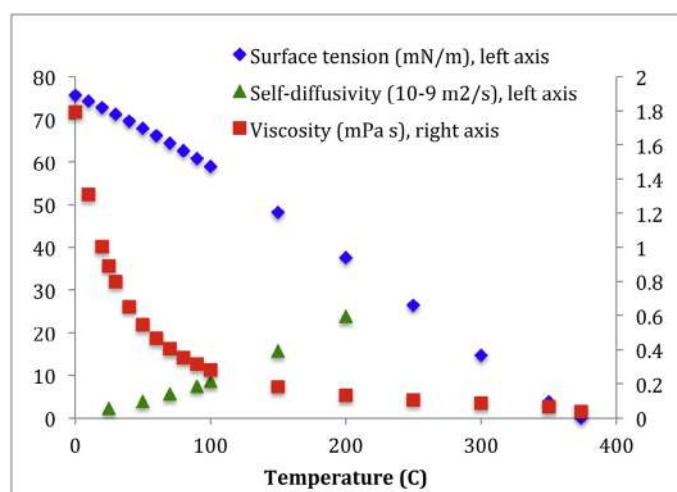

Fig. 6. Viscosity, self-diffusivity and surface tension of liquid water as a function of temperature, at saturation pressure. Data taken from [16,17,29].

depends on the concentration gradient, as described by Fick's second law. For diffusion of analyte inside spherical sample particles, Fick's second law can be written as in Equation (6) [31]:

$$\frac{\partial C}{\partial t} = D_{eff} \left(\frac{\partial^2 C}{\partial r^2} + \frac{2}{r} \frac{\partial C}{\partial r} \right) \tag{6}$$

where C is concentration (mol/m³), t is time (s), r is the radius of the sample particles (m) and D_{eff} is the effective diffusivity (m²/s).

Karacabey et al. [31] have reported that the diffusion coefficients of trans-resveratrol increased from 3.3×10^{-11} m²/s to 10.4×10^{-11} m²/s with ascending temperature from 105°C to 160°C. However, it was pointed out that it is a challenge to determine diffusivity of compounds in PHWE experimentally, since there is always a risk of chemical reactions that may give erroneous results.

Surface tension of water in contact with air decreases with increasing temperature of water (see Table 1 and Fig. 6). Comparing 25°C to 200°C, the surface tension at the latter is about 50% of that at the former. At the critical point, the surface tension is zero. A lower surface tension of the extractant (water) in PHWE will lead to an improved wetting of the sample (i.e., creating a larger contact area between the extractant and the sample matrix). This, in turn, may lead to a more complete extraction.

To summarize, liquid water at elevated temperature and pressure is a solvent of lower ε_r, π^* and density that enables faster mass transfer and improved wetting of the sample due to higher diffusivity and lower viscosity and surface tension (Figs. 3, 4 and 6). In Section 4, there is a deeper discussion on how to optimize PHWE considering the different variables described here, especially those effecting solubility and extraction kinetics. First, we describe practical aspects about PHWE equipment.

3. Equipment used for PHWE

How to perform extraction practically using liquid water as extractant? First, water, either deionized or tap water, is used as the extractant. The water should be oxygen-free in order to prevent oxidation of the analytes. The methods commonly used for achieving water degassing are ultrasound (sonication) or helium purge, of which the former is low cost if an ultrasound bath is available, if much slower than the latter. At least 60 min of degassing by sonication is necessary to ensure that most of the oxygen gas is removed from the water.

The basic equipment to carry out PHWE is uncomplicated. There are two types of equipment: dynamic (continuous-flow) systems and static (batch) systems, and combinations of the two, see Fig. 7.

3.1. Dynamic PHWE

Dynamic PHWE basically needs a pump, an extraction vessel, a heating device, a pressure restrictor and a collection vial (Fig. 7A). The pump used in the system can be an old HPLC pump, since the precision of the flow rates used in extractions is usually not as critical as in chromatography. The pump delivers water to the extraction vessel, and via the pressure restrictor to the collection vial. The pump should be able to achieve the pressure necessary to keep the water in liquid state during the extraction process (normally 3.5–20 MPa). Heating of the water is by either an oven (e.g., an old GC oven) or a heat exchanger, heating tape or heating jacket. The tubing, usually made of stainless steel, is coiled inside the heating device in order to ensure that the set temperature is also the temperature of the water prior to entering the extraction vessel. The extraction vessel is usually made of stainless steel, and should have frits at both ends in order to avoid sample losses and clogging the tubing. If moderate temperatures and pressures are used (i.e., typical extraction conditions for bioactive compounds of 100–200°C and 5–10 MPa),
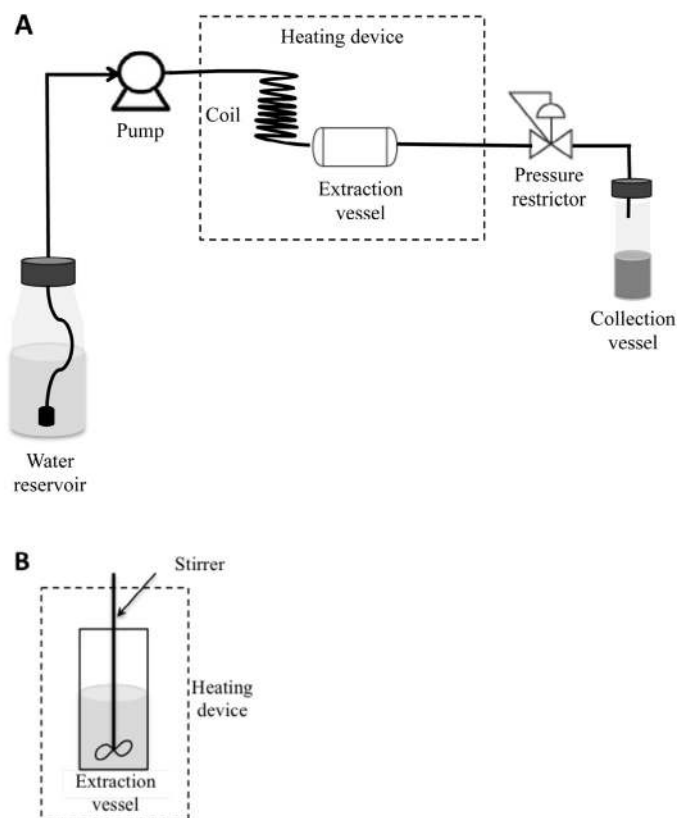
Fig. 7. The main parts of a dynamic (continuous-flow) PHWE system (A) and a static (batch) PHWE system (B).

an empty HPLC column can be used as extraction vessel. However, if temperatures near the critical point are to be used (i.e., above 250°C towards 374°C), the extraction vessel should be of a corrosion-resistant metal alloy (e.g. Hastelloy), in order to prevent corrosion of the vessel. Pressure restriction is needed to control the pressure inside the extraction vessel and to prevent boiling-off effects of water at the exit of the extraction vessel. The pressure restrictor could be a needle valve, a backpressure regulator, a thin capillary or simply short tubing with a squeezed end providing an exit small enough to maintain an adequate pressure upstream.

3.2. Static PHWE

In static PHWE (Fig. 7B), a pump is unnecessary. However, if an optional pump is used to deliver water to the extraction vessel, two valves are necessary (inlet and outlet) in order to maintain pressure inside the vessel during the extraction. With a pump, pressure can be controlled by adding an increasing amount of water to the vessel with the outlet valve closed. If a pump is not used and water is added manually to the extraction vessel, when the vessel is closed and heated, pressure builds up and extraction is conducted at the saturation pressure of the system. In static PHWE, convection is accomplished using a stirrer in order to speed up mass transfer. The extraction vessel in static PHWE is usually of the autoclave type or at least of a wider diameter than in dynamic PHWE, in order to fit in the stirrer. For heating, an oven, a heating jacket or heating tape is appropriate. A pressure restrictor is not needed, unless the speed of removal the extract from the vessel is to be controlled.

An experiment with either dynamic or static PHWE is done by first placing the sample inside the extraction vessel, in some cases mixed with glass beads or other dispersant, and then equilibrating the system to a set temperature. Thereafter, water is added manually or delivered at a certain flow rate to the vessel using a

pump, and out through the restrictor to the collection vial. The flow rate of the water is monitored at the pump or using continuous gravimetric analysis of the collected extract.

There are advantages and disadvantages of using both types of system. Static PHWE is simpler and easier to use, since a pump and a pressure restrictor are not needed. However, the residence time of the analytes in static PHWE is much longer than in dynamic PHWE, which may cause thermally labile analytes to degrade [32]. Hence, the extraction time affects both the extraction efficiency and the degree of degradation of the analytes. Furthermore, in static PHWE, equilibrium for distribution of analytes from the sample matrix to the extractant will stabilize after some time, since the volume of the extractant is constant. Hence, in this case, the efficiency of the extraction depends on the distribution ratio of the analytes into the water. However, in dynamic PHWE, the residence time of analytes in the high-temperature water is shorter, since fresh extractant is continuously being pumped through the extraction vessel and out to the collection vial. In this case, the flow rate of the extraction will control the residence time, so extraction and degradation kinetics in PHWE are easier to control using a dynamic (continuous-flow) extraction set-up. The disadvantage of dynamic PHWE is that it is more costly, and there is always a risk of clogging inside the tubing during the extraction.

Some of the commercially available PHWE instruments address the issues above. For example, even if the extraction is conducted in static mode with no flow rate through the vessel, the extractant can be regularly replaced with fresh solvent (water) in order to prevent equilibrium taking place before the extraction is complete. In such a case, the extraction involves several static extraction cycles, in which each cycle has a certain duration, and each volume of extractant is replaced to a certain degree. Another challenge mainly in dynamic PHWE is clogging of the tubing. Downstream of the extraction vessel, water with the extracted analytes is cooled to

temperatures at which some of the extracted analytes are no longer soluble, so they precipitate and block the tubing. There are two ways to avoid this problem. One is to use heating tape around the tubing from the exit of the oven to the collection vial. Another is to use an additional pump to wash the lines after the extraction vessel and before the pressure restrictor [33].

For any PHWE equipment, it is important to consider the maximum operating temperature and pressure, the material of the extraction vessel, and general safety precautions, such as burst disks, ventilation and waste lines.

4. Optimization of the PHWE method

Extraction of analytes from solid and semi-solid samples can be described by the following five steps:

(1) wetting the sample matrix with extractant;
(2) initial desorption from the sample matrix;
(3) diffusion inside the pores of the sample matrix;
(4) partitioning between the sample matrix and the extractant; and,
(5) diffusion through the stagnant extractant layer until the zone of convection is reached {modified from [34]}.

All of these steps happen more or less in parallel. In PHWE, temperature is the key parameter to optimize, since it affects the efficiency of all these five steps, as described below. In addition, extraction time and/or flow rate are important variables to optimize.

4.1. Temperature

As discussed above, higher temperature of the water leads to improved wetting of the sample matrix [step (1) above]. Further, increasing temperature also favors mass-transfer kinetics by disrupting analyte-matrix interactions, especially hydrogen bonding and other dipole-dipole forces, thereby facilitating initial desorption of the analytes from the sample matrix [step (2)]. A higher temperature also results in faster diffusivity [steps (3) and (5)] as well as altered (usually higher) solubility, the latter leading to a shift in partitioning of the analytes between the sample matrix and the extractant [step (4)]. In summary, an elevated temperature in PHWE brings several advantages in terms of improved extraction kinetics.

There are three main drawbacks in using elevated temperatures in PHWE:

- decreasing selectivity of the extraction;
- pertinent degradation of the analytes; and,
- other chemical reactions in the sample matrix.

The higher the temperature, the more unwanted compounds, so called contaminants, will be extracted, leading to lower selectivity and increasing need for further clean-up after PHWE. For example, Vergara-Salinas et al. [35] characterized two PHWE extracts from grape pomace obtained at 100°C and 200°C, respectively. The HPLC chromatograms of the extracts obtained at 200°C, measured at 280 nm and 320 nm, showed peaks absent in the extract obtained at 100°C, which may correspond to either compounds formed in thermal degradation or other reactions, or additional compounds extracted from the sample leading to lower selectivity of the extraction method. It is generally difficult to be sure which compounds are native and which are artefacts [32,36].

Degradation of the analytes and formation of new compounds are unavoidable issues at higher temperature. As shown in Table 1, the water dissociation constant (K_w) increases from 1.0×10^{-14} at ambient temperature (25°C) to 4.9×10^{-12} at 200°C. This means that, at higher temperatures, the water is a strong source of hydronium

(H_3O^+) and hydroxide (OH^-) ions, which can catalyze reactions, including hydrolysis of polysaccharides and proteins into smaller molecules (e.g., oligosaccharides, monosaccharides, peptides and amino acids) [37]. These small molecules are more susceptible to react with each other. For example, it has been demonstrated that Maillard, caramelization and thermo-oxidation reactions may occur during PHWE in glycation model systems [38] and real natural samples [39]. The occurrence of those reactions forming new compounds exhibiting different structures and chemical properties may result in erroneous analytical results. In particular, if a spectrophotometric antioxidant assay is used as the analytical method to determine antioxidant phenolic compounds in plants, the result may erroneously show that the highest temperature investigated in PHWE gives the highest antioxidant compound recovery [40]. In reality, the higher antioxidant recovery is due to the formation of new antioxidant compounds from Maillard, caramelization and thermo-oxidation reactions [38,39]. Consider, for example, the chemistry behind the total phenolic assay (Folin-Ciocalteu assay, FC), which relies on the transfer of electrons from phenolic compounds and other reducing species to molybdenum, forming blue complexes that can be detected spectrophotometrically [41]. Obviously, other reducing species that are not phenolic compounds can also transfer electrons to molybdenum [42]. As an example, Fig. 8 shows response surfaces for optimization of temperature and extraction time in PHWE of flavonols from apple byproducts, demonstrating that 120°C and 3-min extraction time using tap water as the solvent were optimal, while other antioxidant assays gave erroneous results aligned with browning at higher temperature.

In many cases, degradation of thermolabile analytes during extraction is unavoidable. For example, anthocyanins are extremely sensitive to oxidation, depending on temperature and pH of the solvent. There are several publications about degradation of phenolic compounds in pressurized hot water [43–45], demonstrating that effects of temperature on stability of the analytes should be taken into consideration in method development. For example, degradation-rate constants have been determined for anthocyanins in red onion by static PHWE using water/ethanol/formic acid (94/5/1, vol%) as a solvent at 110°C, and data obtained were used to calculate theoretical extraction curves as if there was no degradation [32,36]. Results showed that the losses during the extraction were ~21–36% for the different anthocyanin species in red onion (Fig. 9). In other words, if degradation during PHWE can be minimized, 21–36% higher recovery of the anthocyanins can be recovered from the red onion, giving more accurate analytical results.

To summarize, it is important to optimize the extraction temperature in PHWE carefully to take full advantage of enhanced solubility and improved mass transfer but still to minimize the degradation effects. This approach is more important than it is in PLE with solvents other than water [46]. Often, a properly optimized PHWE gives higher, more accurate extraction yields than conventional solvent extraction [47–49].

4.2. Flow rate and extraction time

One way to minimize chemical reactions during PHWE is to use a continuous flow system with a flow rate of the extractant that is high enough. A higher flow rate will not only decrease the residence time for the analytes in the elevated temperature water, but also enhance the extraction rate of the analytes if the kinetics is limited by the solubility in the solvent. However a flow rate of the extractant that is too high will lead to unnecessary dilution of the extract, and may necessitate a concentration step after PHWE. If the extraction kinetics is mainly limited by desorption and diffusion inside the pores of the sample matrix, then a higher flow rate will not improve the extraction rate. In Liu et al., the highest flow rate investigated (4 mL/min) gave the highest extraction yield of
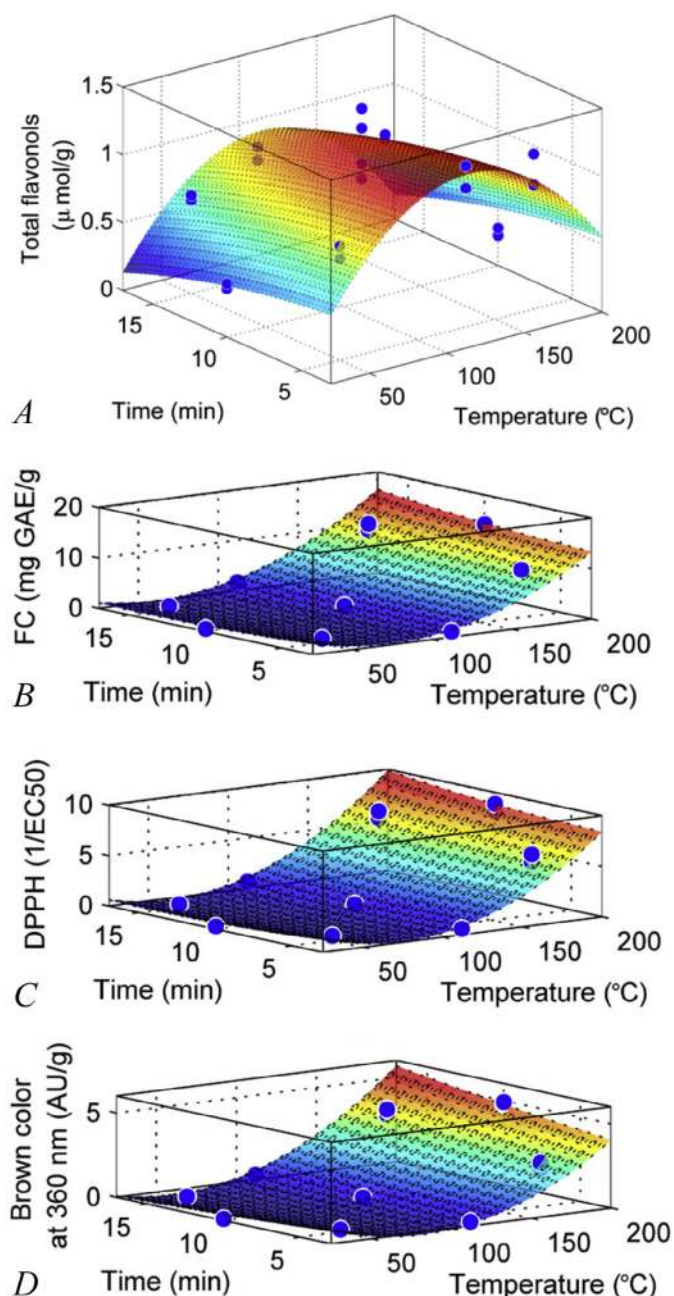
Fig. 8. Response surface plots of PHWE of apple-press cakes showing the effects of temperature and time on: the flavonol concentration (A); the total phenolic compounds as determined by the FC assay (B); the antioxidant capacity by DPPH (C); and, the formation of brown color (melanoidins) measured at 360 nm (UA) (D) [40].

anthocyanins from red onion, mainly due to issues with thermal degradation during the extraction [32]. Similarly, in static PHWE, extraction time needs to be optimized along with the solvent-to-sample ratio.

4.3. Pressure

As mentioned above, pressure has very little influence on the properties of water, as long as the water remains in the liquid state. Hence, a pressure of 5–10 MPa is usually employed unless the saturation pressure of water is used.

4.4. Other parameters

Besides the important variables described above, other parameters might also have a great influence on extraction efficiency in PHWE. For example, the addition of some organic and inorganic modifiers, surfactants and additives may increase the solubility of the analytes in the extractant, and affect the physical properties of the sample matrix and the desorption of analytes from the sample. For example, 5% ethanol and 1% formic acid in water favored the extraction of anthocyanins from red cabbage [50].

Further, the particle size of the sample influences the extraction kinetics, since a smaller particle size leads to increasing contact surface between the sample and the extractant. The particle size has to be appropriate to maximize the contact surface while avoiding channeling effects (i.e., agglomeration of particles). In some applications, dispersants (e.g., glass beads) are introduced to the sample in the extraction vessel in order to favor uniform distribution of the sample and the extractant in order to maximize the extraction yield. Agitation can also be used to avoid the formation of agglomerates.

The solvent-to-sample ratio is an important parameter in PHWE. An increase in the ratio of extractant to sample results in a larger fraction of the analytes being extracted without replacing the extractant with fresh solvent [51]. However, a higher solvent-to-sample ratio requires more water to be heated. In addition, the analyte will be less concentrated in the extract, so a concentration step of the analyte might be necessary, causing longer total analysis times and increasing the risk of the analytes degrading. It is therefore important that the solvent-to-sample ratio is as small as possible but at the same time big enough to provide the highest possible extraction yield [52].

Moisture content of the sample is another parameter that may influence the extraction yield. Some studies show that crude samples with high moisture content give better extraction yields of polyphenols than dried samples [33,53,54].

5. PHWE in chemical analysis of bioactive compounds in complex samples

There is increasing interest in ingredients of bioactive compounds from complex natural sources to be used in food and pharmaceutical products and in the search for native plants containing interesting bioactive compounds (e.g., antioxidants and anti-inflammatory compounds). PHWE is one of the most interesting techniques to isolate bioactives from plants and other complex samples, whether for analytical purposes or industry processes. In this review, we review the main applications of analytical-scale PHWE of bioactive compounds from natural sources. Table 3 offers a compilation of the literature on the extraction of bioactive compounds using PHWE.

5.1. PHWE of phenolic compounds

PHWE has been mainly used to extract relatively polar bioactive compounds (e.g., phenolic compounds). In the past decade, phenolic compounds were thoroughly studied because of their potential health benefits as antioxidants [85]. They were suggested as playing an important role in the prevention of several diseases associated to oxidative stress, such as cancer, cardiovascular and neurodegenerative diseases [86–88]. These beneficial properties are strongly related to the phenolic chemical structure. The phenolic compounds are classified in different groups (e.g., phenolic acids, flavonoids, stilbenes and lignans) as a function of the number of phenol rings and the structural elements that bind these rings to one another [89].
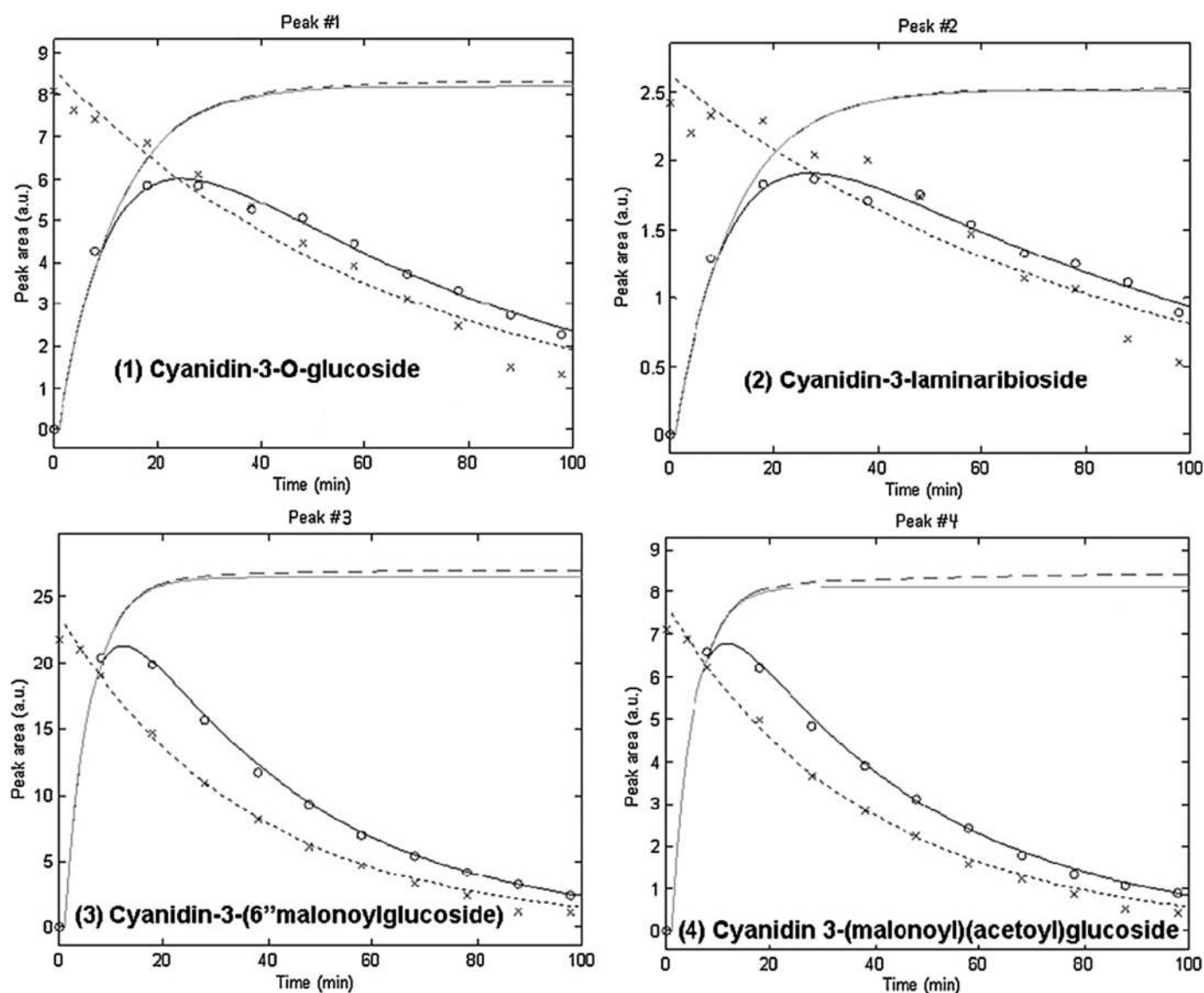
Fig. 9. Extraction curves for four major anthocyanins in red onion. Experimental values for extraction with simultaneous degradation are shown as circles (triplicates) with the model values (calculated for each minute) as an adjacent solid curve. The degradation data points are shown as crosses (triplicate means) and the corresponding model as an adjacent dashed curve. Theoretical extraction curves as if there was no degradation are calculated by two different methods (method A: upper right, dashed; method B: upper right, solid). {Reprinted with permission from [36], Elsevier}.

Phenolic compounds have been extracted by PHWE from many different sources, such as plants and food-industry byproducts. Usually, extraction temperatures of 80–150°C and extraction times of 1–60 min have been used to extract phenolic compounds by PHWE (see Table 3). In many works, a higher antioxidant capacity has been observed in the extracts obtained at temperatures of over 175°C and at longer extraction times, compared to the extracts obtained at lower temperature and shorter extraction times [39,40,55,56,60,63,90]. In many of these studies, only the total phenolic compounds and the total antioxidant capacity were measured, with drawbacks as discussed above. Instead, more advanced analytical techniques are necessary to quantify phenolic compounds as well as other bioactive compounds.

In Plaza et al., a PHWE method was optimized for the extraction of flavonols in apple byproduct using a desirability function response surface, considering maximum antioxidant capacity and minimal formation of brown color, giving an optimum of 125°C and 3 min [40]. These optimized PHWE conditions for the extraction of

flavonols are in agreement with other studies found in the literature for extracting flavonols by PHWE [61,79,91] and with thermodynamic studies carried out for quercetin by PHWE [92].

Optimal extraction conditions greatly vary, depending on the kind of phenolic compound to be extracted. For example, extremely labile polyphenols (e.g., anthocyanins), whose stability depends on pH, generally require a lower extraction temperature. For example, static PHWE was optimized for anthocyanins in red cabbage [50] and red onion [66] using water/ethanol/formic acid (94/5/1, vol%) as a solvent at 99°C, 5 MPa and 7-min extraction time.

Monrad et al. [33] designed and optimized a dynamic hot-cold extraction of polyphenols with just water as solvent from grape pomace to minimize the degradation of the compounds. Water was preheated prior to entry to the extraction vessel, where it then flowed continuously through the unheated extraction vessel. Between the extraction vessel and the back-pressure regulator, a mixing tee was placed to pump cold water at the same flow rate. The extract mixture was chilled in a cooling coil inside a water-ice bath prior to its

Table 3
Compilation of works found in the literature, 2009–14, on PHWE of bioactive compounds from natural sources

Bioactive compound	Source	Temperature (°C)	Pressure (MPa)	Static/Dynamic	Time/Flow rate	Modifier/Other	Ref.
Antioxidants							
	Cow cockle seed	175	–	Dynamic	180 min/2.0 mL/min	–	[55]
	Oregano	50	10.3	Static	20 min	–	[56]
	Olive leaves	200	10.3	Static	20 min	–	[57]
Phenolic compounds							
	Lemon balm	150	10.3	Static	10 min	Ethanol/water (70/30, vol %)	[58]
	Grape pomace	140	10.0	Static	5 cycles × 0 min each		[54]
	Potato peel	180	6.0	Static	30 min		[59]
	Marigold	220	6.0	Static	45 min		[60]
Prenylflavanoid							
Isoxanthohumol	Hop	150	10.3	Static	30 min (6 cycles × 5 min each)		[47]
Flavonols							
	Apple by-products	125	10.3	Static	3 min	–	[40]
	Grape pomace	124	10.3	Static	1 min	–	[61]
	Moringa oleifera leaf	100	–	Dynamic	20 min/1.0 mL/min		[62]
Polymethoxylated flavones							
Sinensetin, nobiletin and tangeretin	Citrus pomace	200	14.0	Static	60 min		[63]
Phenolic ketones							
6-gingerol	Ginger root	130	3.5	Static	60 min	–	[64]
6-shogaol	Ginger root	170	3.5	Static	60 min	–	[64]
Stilbenoids							
Stilbene glucosides	Spruce	180	5.0	Static	15 min (3 cycles × 5 min each)	–	[65]
Trans-resveratrol / Trans-ε-viniferin	Grape canes	85–105	5.2	Dynamic	150 min/1.0 mL/min	Ethanol/water (15/85, vol %)	[31]
Anthocyanins	Red cabbage	99	5.0	Static	7 min	Water/ethanol/formic acid (94/5/1, vol %)	[50,66]
	Red onion						
	Red onion	110	1.5	Dynamic	60 min/4.0 mL/min	Water/ethanol/formic acid (94/5/1, vol %)	[32]
	Grape pomace	80–120	6.8	Static	0 min	Ethanol in water (50–70%)	[67]
	Grape pomace	85.4	10.3	Static	1 min	Ethanol/water (80/20, vol %) with acetic or formic acid at pH 2.5	[61]
Non acylated anthocyanins	Grape pomace	100	10.2	Static	5 min	–	[68]
	Grape pomace	85.4	10.3	Static	1 min		[61]
Acylated anthocyanins	Grape pomace	85.4	10.3	Static	1 min	Ethanol/water (80/20, vol %) with acetic or formic acid at pH 2.5	[61]
Anthocyanins and procyanidins	Grape pomace	88.4	4.1	Dynamic	12 min/9.0 mL/min	–	[33]
Proanthocyanidins	Grape pomace	100	10.2	Static	5 min		[68]
Anthocyanins and flavan-3-ols	Grape pomace	140	–	Dynamic (continuous)	– min /65.0 mL/min	Shaft temperature (71°C); rotation speed (20 Hz)	[53]
Tannins and tannins–anthocyanins	Grape pomace	150	10.2	Static	5 min		[68]
Diterpenes							
Steviosides	Stevial rebaudiana leaves	100	1.1–1.3	Dynamic	15 min/1.5 mL/min	–	[69]
		125	23.0	Dynamic	45 min/4.0 mL/min	–	[70]
		100	10.3	Static	4 min	–	[49]
Polysaccharides	Lycium barbarum L.	100	5.0	Static	53 min	Ultrasonic power (160 W)	[71]
	Golden oyster mushroom	200	0.002 to 5.0	Static	60 min	–	[72]
	Chlorella vulgaris	150	10.3	Static	20 min	–	[73]
	Himanthalia elongate	100	10.3	Static	20 min	–	[74]
	Haematococcus pluvialis	100	10.3	Static	20 min	–	[75]
	Dunaliella salina	160	10.3	Static	15 min	–	[75]
Phenolic–carbohydrates	Potato peel	190	4.0	Static + dynamic	9 + 3 min/3.0 mL/min	Ethanol/water (30/70, vol %)	[76]
	Barley hull	150	15.0	Static	15 min	–	[77]
Protein and carbohydrate	Okara	240	–	Static	5 min	–	[78]
Oil and water-soluble	Sunflower seed	130	3.0	Static	30 min	–	[52]
Enzymatic reactions							
Quercetin glucosides → quercetin	Onion waste	90	5.0	Static	10 min	pH 5, Enzyme β-glucosidase TnBgl3B	[79]
	Onion waste	95	5.0	Static	5 min	pH 5, Enzyme β-glucosidase TnBgl1A_N221S	[48]
	Onion waste	84	5.0	Dynamic	90 min/3.0 mL/min	5 % Ethanol and pH 5.5, enzyme β-glucosidase TnBgl1A_N221S/P342L immobilized	[80]
WEPO						**Dried conditions**	
Antioxidants	Rosemary	200	8.0	Dynamic	20 min/0.2 mL/min	CO_2 pressure (8 MPa) and flow rate (2.5 mL/min) N_2 flow rate (0.6 mL/min)	[81–83]
Quercetin derivatives	Onion	120	8.0	Dynamic	45 min/0.3 mL/min	CO_2 pressure (8 MPa) and flow rate (10 mL/min) N_2 pressure (1.22 MPa)	[84]

collection at room temperature. The optimal PHWE conditions were found to be 88.4°C in the vessel (140°C of preheated temperature), 9-mL/min water flow and 9.9 g of sample. Under these conditions, 96% of anthocyanins and 84% of procyanidins were extracted, compared to recoveries obtained using a conventional extraction method (methanol:water:formic acid 60:37:3 v/v/v for anthocyanins and acetone:water:acetic acid 70:29.5:0.5 v/v/v for procyanidins) [33].

In general, the best PHWE conditions to extract anthocyanins are medium temperatures (80–100°C), short extraction times (in batch extractions) or high flow rates (in continuous-flow extractions) and ethanol and formic or acetic acid as additive in the extraction solvent (see Table 3) [32,33,36,50,61,66,67]. The extraction of different kinds of anthocyanins can be favored with the use of additives. For example, just water as extraction solvent in the presence or the absence of organic acids extracted a greater amount of polar non-acylated anthocyanins from grape pomace than acidified hydroethanolic mixtures [61]. The hydroethanolic mixtures (especially acidified with formic or acetic acid) extracted greater amounts of acylated anthocyanins from grape pomace [61].

Other very sensitive compounds are stilbenoids *trans*-resveratrol and trans-ε-viniferin [31]. The optimal conditions to extract them by PHWE are very similar to those for anthocyanins but without adding organic acids (see Table 3) [31]. However, higher extraction temperatures (150°C) are needed to extract less sensitive compounds by PHWE (e.g., tannins and tannin-anthocyanin adducts) [68].

PHWE can be a selective technique to extract certain phenolic compounds *versus* others. For example, PHWE at 150°C extraction temperature was an effective method to extract selectively from hop prenylflavonoid isoxanthohumol (IX) (2.34 ± 0.75 mg/g), compared to xanthohumol (XN) (0.11 ± 0.03 mg/g) (the latter was not interesting in this study because it has no anti-inflammatory properties) [47]. Moreover, PHWE was compared to a reference method, solid-liquid extraction (SLE) with dimethyl sulfoxide as a solvent with agitation at room temperature during 24 h in darkness, and to PLE with ethanol at 150°C. The SLE yield of XN was nearly one sixth of that of IX, with averages values of 0.67 mg/g and 3.93 mg/g, for IX and XN, respectively. However, use of ethanol as extractant in PLE yielded 0.67 mg/g and 1.89 mg/g of IX and XN, respectively. Then, the ratio IX/XN rises from 0.17 in the SLE and 0.35 in PLE with ethanol as solvent to 21 in PHWE, which represents a near 124-fold increase. PHWE at 150°C was therefore the more selective extraction technique to extract IX from hops [47], giving a yield more than double that of the reference method.

5.2. PHWE of diterpenes

One of the more recent applications of PHWE is the extraction of non-caloric sweetening agents from natural sources. Among the most well-known alternatives to sucrose are steviol-glycosides (steviosides) [80], which are diterpene glycosides obtained from the leaves of *Stevia rebaudiana* Bertoni (family Asteraceae). These compounds are around 300 times sweeter than sucrose [93]. The sweetening properties of the steviol-glycosides differ from one another in sweetness and quality of the taste, so the organoleptic properties of the steviosides depend a lot on the extraction technique used to extract them from stevia leaves [93].

Teo et al. [69] were the first to study the effect of the temperature (60–120°C) and time (5–50 min) on the extraction of steviosides from *Stevia rebaudiana* leaves by PHWE. The extraction was achieved at constant flow rate of 1.5 mL/min. The stevioside and rebaudioside A were extracted with an optimal temperature of 100°C and an optimal extraction time of 15 min. Yildiz-Ozturk et al. [70] also optimized the PHWE of steviosides using dynamic extraction but exploring larger extraction temperature range and higher flow rates

than Teo et al. [69]. Optimum extraction conditions were found at 125°C, 45 min and 4 mL/min flow rate [70]. In both works, not surprisingly, the most crucial extraction parameter to optimize was temperature [69,70]. Increasing temperature from 100°C to 125°C resulted in higher recovery of both stevioside and rebaudioside [70]. Although steviol glycosides are thermostable and moderately polar compounds, when the extraction temperature was increased over 150°C, the yields were considerably decreased due to degradation [70]. Teo et al. [69] observed a decrease in the extraction yield of steviosides at a lower extraction temperatures (120°C) than Yildiz-Ozturk et al. [70] (150°C), because they used a lower flow rate, which implies a longer residence time inside the vessel.

In a recent work, the extraction of steviosides in static PHWE using several extraction cycles was optimized [49]. All the investigated variables (temperature, time and number of cycles) had a significant influence on the extraction yields. Optimum extraction conditions were found to be 100°C, 4 min and 1 cycle, which yielded 91.8% ± 3.4% of total extractable steviol glycosides. An additional optimization was achieved by reducing the particle size of the leaves to under 0.5 mm, giving a final yield of 100.8% ± 3.3%. In this work, the optimized PHWE method was compared to a conventional extraction method (water at 60°C for 120 min with stirring) as well as several less conventional methods [i.e., PLE, supercritical-fluid extraction (SFE), microwave-assisted extraction, ultrasound-assisted extraction (UAE), enzymatic extraction, hot water extraction (HWE)] previously reported in the literature [69,94–99]. The conclusion was that PHWE is faster and more convenient, and does not require as much energy.

5.3. PHWE of other bioactive compounds

Polysaccharides with different bioactive properties have been extracted from different natural sources by PHWE. For example, polysaccharides from *Lycium barbarum* L. have been extracted combining UAE with PHWE [71]. The extraction technique was called ultrasound-enhanced SWE (USWE). Four variables – extraction temperature, extraction time, liquid-to-solid ratio, and ultrasonic power – were optimized. The maximum yields of polysaccharides were obtained at an extraction temperature of 100°C, an extraction time of 53 min, a liquid-to-solid ratio of 26 mL/g, and an ultrasonic electric power of 160 W. USWE was compared with other three extraction methods in terms of extraction conditions, extraction yield for polysaccharides and antioxidant capacity – PHWE (110°C for 1 h, 5 MPa, and a liquid-to-solid ratio of 25 mL/g), UAE (electric power of 140 W, a liquid-to-ratio of 25 mL/g, and 110°C for 1 h) and HWE (water at 100°C for 2 h). The polysaccharide extraction yield was 3.4% for HWE, 3.0% for UAE, 4.0% for PHWE and 4.4% for USWE. USWE gave higher yields of polysaccharides than the other extraction methods, perhaps because ultrasound helps to disrupt the cell walls of plant materials. The antioxidant capacity assay revealed significant antioxidant activities of polysaccharides, and there were few differences in the antioxidant effects of polysaccharides obtained by USWE, PHWE and HWE.

Another polysaccharide interesting to extract by PHWE was β-glucan from golden oyster mushroom [72], which has antioxidant activity [100]. The extraction temperature and time affected the β-glucan contents in the PHWE extracts. PHWE at 200°C for 60 min showed the highest concentration. However, at 250°C and 300°C, β-glucans degraded as the extraction time was increased. However, PHWE may be a feasible extraction technique in chemical analysis of β-glucans and obtaining high-quality extracts rich in β-glucan from golden oyster mushrooms.

There were works focused on the extraction of phenolic compounds and carbohydrates by PHWE from different sample matrices (e.g., potato peel and barley hull) [76,77]. The optimal conditions for complete extraction of phenolics and carbohydrates from barley

hull were 150°C, 15 MPa and 15 min of static extraction time [77], and from potato peel 190°C, 4 MPa, and 9 min of static extraction time followed by continuous extraction using a flow rate of 3 mL/min, giving total extraction time of 30 min [76]. These studies showed that higher temperatures and higher residence times produced browning color in the extracts due to the formation of Maillard and caramelization reaction products, as discussed above [76,77]. The total carbohydrate yield in potato peel, the pressure and the acidic or alkaline PHWE therefore influenced the browning reactions [76]. For example, the brown color is mainly due to fragmentation of carbohydrates in the extracts. Further, the use of acidic PHWE (pH 3) decreased the browning reactions while an increase in pH and temperature increased the brown color of the extracts. Furthermore, the increase of pressure in PHWE did not favor the extraction of carbohydrates. For example, the most carbohydrates were extracted at the lowest pressure of 40 bar (268.4 mg of glucose equivalent/g of dry potato peel) and the minimum extraction was obtained at 120 bar (147.7 mg of glucose equivalent/g of dry potato peel) [76].

PHWE can be used for simultaneous extraction of oil (free fatty acids) and water-soluble compounds (protein, carbohydrates and phenolics) from sunflower seeds [52]. The highest amount of oil was obtained at 130°C, with a maximum yield of 44.3% ± 0.3% after 30 min of extraction, giving results similar to those obtained after 4 h by Soxhlet extraction (46.2% ± 0.7%). Also, a higher yield of total carbohydrate could be observed at temperatures higher than 100°C, which indicated higher carbohydrate solubility at higher temperatures. On the contrary, a temperature higher than 100°C resulted in a significantly lower protein yield. The yield of total phenolic compounds was constant for 60–100°C. However, the concentration of water-soluble compounds obtained in the extracts decreased at temperatures higher than 130°C, probably a consequence of degradation, Maillard and caramelization reactions, as discussed above.

6. PHWE in coupling

6.1. PHWE coupled with enzymatic reaction

In chemical analysis, it may be of interest to conduct a derivatization reaction of the analytes to make them easier to analyze. One such case is phenolic compounds, which naturally occur as many glycosylated forms, making them difficult to quantify due to the lack of chemical standards and since the limit of detection might be insufficient for several low-abundant forms of the same polyphenol aglycone. In this case, it is a common practice to hydrolyze the glycosides prior to final analysis, which is usually achieved at the same time as the extraction with a mixture of hydrochloric acid, methanol and water.

The development of novel enzyme-based methods to achieve hydrolysis reactions coupled with PHWE is a recent development, which was shown to be a faster, more accurate and greener method to achieve hydrolysis compared to conventional acid-catalyzed hydrolysis [48]. In several studies [48,79,80,101,102], quercetin glycosides were extracted from onion byproducts by PHWE, followed by β-glucosidase-catalyzed conversion of quercetin glycosides to quercetin and sugars. To comply with water at elevated temperature, thermostable catalytic enzymes were used, here β-glucosidase from *Thermotoga neapolitana (TnBglA)*. These enzymes have their highest activity in water at temperatures of around 90–95°C. Enzymatic hydrolysis has been accomplished both off-line [48,79] and on-line [80] with PHWE; the former was faster while the latter more convenient and easier to automate. Variables optimized in these methods were temperature, pH, addition of low concentration of ethanol, time/flow rate and immobilization of enzyme to different support materials. A general conclusion was that the amount of hydrochloric acid needed to hydrolyze flavonoid glycosides, such as quercetin glycosides, inevitably leads to simultaneous degradation

of the compounds. The combination of PHWE and enzymatic hydrolysis therefore gives significantly higher, more accurate, concentrations of polyphenols in complex samples than conventional solvent extraction using hydrochloric acid as the catalyst [80].

6.2. PHWE with clean-up using sorbents

As discussed above, PHWE is not the most selective extraction technique. PHWE may necessitate clean-up before final analysis can be done. For example, a clean-up step can be carried out by using more or less selective adsorbents in the extraction vessel or in a separate vessel downstream of the sample. By employing different materials, such as molecular imprinted polymers (MIPs) or specially designed polymers, it is possible to isolate the analytes of interest from the PHWE extract more selectively, leading to a higher sample throughput and a better quality of analytical determination. For example, Pakade et al. [103] used MIPs with high recognition for quercetin to obtain onion extract using PHWE. The binding capacity for the MIPs was higher at 84°C than 25°C, being ~30 μmol/g at 25°C and ~120 μmol/g at 84°C. MIPs demonstrated good affinity and selectivity to the quercetin from a yellow solution of onion extract as compared to similar flavonoids, kaempferol and morin.

6.3. PHWE coupled with a drying step

The main disadvantage of using water as a solvent in extractions is the difficulty in concentrating the extracts, since the heat of vaporization of water is relatively high compared to that of many organic solvents. Furthermore, the need to concentrate the sample is often relevant, since the concentration of bioactive compound in the water extract could be low. Also, in some cases, the presence of water could decrease the stability of the extract. One way to dry the extract is by freeze-drying, which is rather costly and time consuming, and may also lead to degradation of the bioactive compounds due to heat, light and oxygen.

Trying to address this problem, an on-line process for PHWE with drying the extracts was developed in 2010 [81], based on a previous innovation by Sievers and co-workers called carbon dioxide-assisted nebulization in a bubble-dryer (CAN-BD) [104]. In this novel method, the PHWE extract is mixed continuously with supercritical carbon dioxide in a small-volume Tee junction, resulting in a spray of small-sized water droplets that are rapidly dried by a stream of hot nitrogen. The process is called water extraction and particle formation on-line (WEPO). Basically, this is a combined extraction-drying process that produces micron-sized particles of the extract containing the analytes and all other co-extracted compounds. The WEPO process was employed by Herrero et al. [81] and Rodríguez-Meizoso et al. [82,84] to obtain antioxidants from rosemary leaves. Rosemary has been studied widely due to the presence of antioxidants (e.g., carnosol, carnosic acid, rosmarinic acid and other phenolic compounds) [105,106], and PHWE methods have been optimized [107–109]. These works show that PHWE at 200°C achieved the maximum antioxidant capacity. In the WEPO set-up, the extraction temperature was set at 200°C while different flow rates (0.1–0.3 mL/min) were studied to carry out the extraction of antioxidants from rosemary. The best flow rate to give higher yields and smaller particle size was 0.2 mL/min. The parameters influencing the drying process were CO_2 pressure (8 MPa) and flow rate (2.5 mL/min), and nitrogen flow rate (0.6 mL/min) to get a fine, constant spray. With this process, it was possible to collect microparticles as a fine powder with particle-size diameters less than 93 μm.

The WEPO process was also used to obtain dried extract from onion with the same composition of quercetin derivatives as non-dried extracts [84]. The extraction temperature was set to 120°C, the water-flow rate at 0.3 mL/min, and the pressure of the extraction was 8 MPa. Parameters influencing the drying process were CO_2

pressure (8 MPa), nitrogen pressure (1.22 MPa) and compressed CO_2 flow rate (10 mL/min). A fine powder with spherical particles of 0.25–4-µm diameter was obtained.

Finally, it was demonstrated that the WEPO process, compared to SFE with vacuum drying and PHWE with freeze-drying, resulted in the smallest environmental impact based on a gate-to-gate life-cycle assessment [82].

7. The future of PHWE

Water at elevated temperature and pressure is without doubt an interesting solvent, providing efficient mass transfer and high solubility for many bioactive compounds. However, there is still no commercial analytical system devoted to PHWE (i.e., the PLE systems available on the market are designed for organic solvents, limited in temperature a maximum 200°C, and tubing and vessels are most often made of stainless-steel type 316). A system especially designed for PHWE should be made with a higher quality steel alloy, improved pre-heating of the solvent, the possibility of varying the flow rate of the extractant, and a temperature and pressure range going to the critical point of water (i.e., 374°C, 22.1 MPa). However, there is a preparative-scale system available, designed for PHWE at temperatures up to 300°C and a flow rate of 100 mL/min. Further, an appropriate degassing system would be beneficial. Another aspect is the risk of clogging of tubing because of caramelization reactions, an issue that a commercial PHWE system would need to address.

In terms of future development in methodologies involving PHWE in chemical analysis, a trend is to combine extraction with clean-up. Novel polymer-based sorbent materials suitable for use with pressurized hot liquid water as extractant are a likely development, which also includes MIPs. Also, more efforts will be seen in the coupling PHWE to chromatography, including recent development in chromatography techniques (mobile phases operated at higher temperature and pressure, and mobile phases mainly consisting of liquid or supercritical carbon dioxide). Perhaps, with the development of more robust stationary phases in chromatography in combination with miniaturization efforts, PHWE coupled to pressurized hot water chromatography will be realized for bioactive compounds. So far, "superheated water chromatography" has mainly been explored for thermally stable compounds, without widespread success.

There are still doubts about PHWE replacing other more established extraction techniques based on the use of organic solvents as extractant, mainly due to the risk of hydrolysis reaction and other degradation reactions during the extraction. Hence, a future development should involve more research on what happens during the extraction to the analytes, the other unwanted extractable compounds and the remaining sample matrix, with respect to water properties and time/flow rate. Such information would be valuable in method development, but also in process development on a larger scale.

Acknowledgements

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