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Developing novel one-step processes for obtaining food-grade O/W emulsions from pressurized fluid extracts: processes description, state of the art and perspectives

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Abstract

In this work, a novel on-line process for production of food-grade emulsions containing oily extracts, i.e. oil-in-water (O/W) emulsions, in only one step is presented. This process has been called ESFE, Emulsions from Supercritical Fluid Extraction. With this process, emulsions containing supercritical fluid extracts can be obtained directly from plant materials. The aim in the conception of this process is to propose a new rapid way to obtain emulsions from supercritical fluid extracts. Nowadays the conventional emulsion formulation method is a two-step procedure, i.e. first supercritical fluid extraction for obtaining an extract; secondly emulsion formulation using another device. Other variation of the process was tested and successfully validated originating a new acronymed process: EPFE (Emulsions from Pressurized Fluid Extractions). Both processes exploit the supercritical CO2-essential oils miscibility, in addition, EPFE process exploits the emulsification properties of saponin-rich pressurized aqueous plant extracts. The feasibility of this latter process was demonstrated using Pfaffia glomerata roots as source of saponin-rich extract, water as extracting solvent and clove essential oil, directly extracted using supercritical CO2, as a model dispersed phase. In addition, examples of pressurized fluid-based coupled processes applied for adding value to food bioactive compounds developed in the past five years are reviewed.

Keywords: supercritical fluids; food bioactive compounds; hyphenated processes; emulsification.

Practical Application: Pressurized fluid technology should be considered during the entire food ingredients processing.

1 Introduction

Sub and supercritical fluids are commonly applied to extract different food bioactive compounds. Bioactive compounds have been identified from diverse sources and their therapeutic benefits, nutritional value and protective effects in human and animal healthcare have fostered their application as functional food ingredients. Due to the complexity of many natural product matrices and the desire to concentrate specific food bioactive compound newer developments have been investigated. Different technologies, such as, fractionation and chromatographic techniques followed by encapsulation using sub and supercritical fluids were developed, thereby extending the application of a pressurized fluids processing (King & Srinivas, 2009).

Recently, pressurized fluid technology is not being considered as an alternative to a single extraction step only; rather it has been developed as an integrated process for processing (Temelli, 2009). Without such an integrated approach, the full potential of this technology cannot be realized. So, we point out here several options for applying pressurized fluids into natural products processing, noted in the literature or investigated in our laboratory.

In general, for food bioactive compounds extraction purposes, the use of supercritical CO_2 has been employed for non-polar to moderately polar solutes, and subcritical water or polar organic solvents for more polar solutes. The supercritical state of carbon dioxide is achieved at moderate pressures and temperatures (31 °C and 7.38 MPa, respectively), which is suitable for most applications. Ethanol, a naturally derivable sustainable solvent, is suggested as a preferred co-solvent to be paired with carbon dioxide. The amount of added co-solvent depends on the matrix composition and on the polarity of the compounds to be extracted and can usually vary from 1 to 90% (w/w), this latter forming a gas-expanded liquid (King & Srinivas, 2009; Pereira & Meireles, 2010). To obtain a more concentrated and/or stable extract from the latter process, the addition of new unit processes might be necessary. The use of pressurized-based technologies to achieve these requirements will be discussed, as well as, the possibility and perspectives of coupling these unit processes. It is less frequent to find all the steps already combined, once researchers, in general, investigate and optimize an individual unit process separately. Examples of already coupled processes will be given during the text and two case studies including the development of integrated processes for extraction and direct emulsification of oily extracts will be presented. For all cases, the pressurized fluid flows through a fixed bed of milled and roasted natural source, dissolves the extractable components of the solid to further directly be fed into the subsequent step.

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2 Literature review

2.1 Coupling of separators to pressurized fluid extractors for fractionation purposes

Fractional separation of the extracts is a well-known concept that can be useful to improve the process selectivity. In several cases, it is possible to perform an extraction in successive steps employing different solvents or increasing solvent pressure and/or temperature, to obtain the fractional extraction of the soluble compounds contained in the natural matrix. In order to achieve a more concentrated extract, the addition of new unit processes is commonly also required. Several technologies have been on-line coupled with pressurized fluids successfully. In this section some of them will be described.

Supercritical Fluid Extraction (SFE) consists basically of two steps: (1) extraction of the soluble substances from the natural source by the supercritical solvent, normally CO_2, and (2) separation of these compounds from the supercritical solvent after the expansion. Since many supercritical fluids are gases at room temperature, CO_2 for example, the recovery of the extracted compounds is simplified and can be removed by decompression. For extract fractionation, the separation step (2) can be done during extraction procedure coupling some separators in series operating at different conditions. Commercially, some companies provide at different scales SFE equipments that can promote this on-line fractionation. The solute-solvent mixture is separated in the separators by rapidly reducing the pressure, increasing the temperature, or both, inducing the selective precipitation of different compound families as a function of their different saturation conditions in the supercritical fluid (Pereira & Meireles, 2010).

The Professor Reverchon's group in Italy has provided a comprehensive review on the sequential fractionation of supercritical extract (Reverchon & Marco, 2006). In this review, the authors summarized some the successfully cases that different compounds were fractionated. The most successful case is for essential oil. In this case, the extraction temperature and pressure were set to achieve a high CO_2 density to extract the maximum amount of solutes. In the separators the solvent power of CO_2 reduces to near zero, which allows the complete elimination of gaseous CO_2 from the extract. It takes advantage of the different solubilities exhibited by waxes and essential oil compounds at temperatures of around 0 °C in liquid CO_2. At these conditions, the solubility of waxes is near to zero. In contrast, essential oils compounds are completely miscible at these conditions. Therefore, waxes solubilized during supercritical extraction, can be precipitated in the first separator set at about 0 °C whereas essential oil compounds will be collected in the second separator. The large pressure reduction induces the passage of CO_2, to the gaseous state, where occurs the complete elimination of gaseous CO_2 from the extract, which can be recycled to the process (Reverchon, 1997).

In the mid-1970s, different process implementations have been attempted starting from the case of single-stage extraction and separation of bioactive compounds from natural sources, while the first proposal for practical application was made in 1943 in the use of supercritical hydrocarbons for the deasphalting of petroleum oils (Messmore, 1943). With the first studies being published demonstrating that both temperature and pressure during SFE have a significant effect on the equilibrium solubility, several researches started thinking about how take advantage of this for fractionation purposes. Small changes of temperature and/or pressure, especially in the region near the critical point of the solvent, demonstrated affect equilibrium solubility, meanwhile, in liquid extraction, only temperature has a strong effect (Vitzthum et al., 1975). Taking advantage of the different solubilities of extractable compounds in the supercritical solution, the possibility of coupling separators after the extractor started to be studied. Then, it started the construction of more sophisticated extraction schemes containing two or more separators, which allow fractionation of the extract in two or more fractions (Figure 1).

One of the first studies that studied the extraction and subsequent on-line fractionation of different food bioactive compounds from natural sources was carried out by Hubert & Vitzthum (1978). The researchers using hops from the brewing industry obtained an SFE green extract, which was lighter in colour than that obtained using dichloromethane. Additionally, SFE was evaluated for the enrichment and fractionation of the essential oil and the bitter principles of hops (*Humulus lupulus*), both of which contribute to the flavor of beer. Sequential fractionation of the extract by stagewise reduction in the pressure produced two different extracts (green and yellow extracts).

Since then, the use of SFE coupled with on-line fractionation of bioactive compounds from natural sources has increased considerably and the arrangement of this latter unit process started to be the key step towards successful SFE process implementation. Some important reviews were published in the past five years by our research group based on the senior author's 30 years of experience in extraction, fractionation and identification of food bioactive compounds from natural sources mostly obtained using supercritical technology (Santos & Meireles, 2011; Pereira & Meireles, 2010; Rosa & Meireles, 2009).

Besides by setting opportune temperatures and pressures in the separators, on-line fractionation development can be done successfully employing other unit operations. An emerging technology in this regard, which may prove lucrative for the separation of structurally similar bioactive compounds extracted

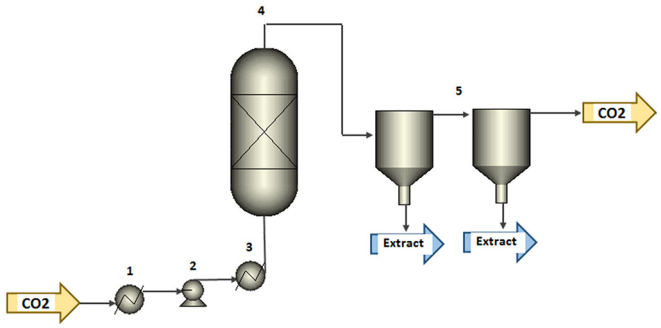

Figure 1. Scheme of the supercritical fluid extraction (SFE) process coupled with separators. 1. Heat exchanger for cooling, 2. Pump, 3. Heat exchanger for heating, 4. Extractor, 5. Separators.

from natural sources, is adsorptive separations using sub and supercritical fluids.

2.2 Coupling of adsorptive columns to pressurized fluid extractors for fractionation purposes

After SFE extraction, the extract can be separated from the supercritical fluid in different ways. As discussed previously, two methods are based on precipitating the components from the extract by reduction of the fluid density, i.e., via (i) pressure reduction or (ii) temperature increase, which can be done in one step or two or more steps employing separators in series for fractionation purposes. The third technique is based on the adsorption of the extracted solutes on an appropriate adsorbent. Initially, developed for relatively large scale chemical processing applications, this on-line coupling option has been also attracted attention for analytical purposes. In order to decrease the losses of SFE extract components (more volatile components for instance) during decompression step the use of adsorbents was tested. Good results were obtained and other advantages were additionally observed: i) the possibility of selective removal of some co-extracted compounds using a specific adsorbent; ii) the compatibility with on-line methods of analysis. In 1990, Vannoort et al. published a comprehensive review of the coupling of supercritical fluid extraction with chromatographic techniques up to date. Examples of SFE combined to chromatographic techniques such as thin-layer chromatography (TLC), high-performance liquid chromatography (HPLC), gas chromatography (GC) and supercritical fluid chromatography (SFC) where presented for on-line extraction, fractionation and analysis (Vannoort et al., 1990).

The use of supercritical fluids as a mobile phase in chromatography was first described in 1962. Due to the physicochemical properties of supercritical fluids, i.e., a lower viscosity and higher diffusion coefficients than liquids, combined with higher solubility than in the vapour phase, they offer a number of advantages. Concentration and purification of the extracts are necessary in most instances. Thermal methods are very sensitive, but limited either by the thermal stability of the solute or by the adsorbents used for pre-concentration (Lohleit & Bachmann, 1990).

The on-line coupling of SFE to analytical methods had experiencing rapid growth. In these combined processes, the compounds to be separated are in a supercritical solvent, such as carbon dioxide, and passed over a packed-bed adsorption column. The desired compounds are or concentrated in the column by adsorbing on the adsorbent or concentrated in the effluent. The adsorbent would then be regenerated by the pure solvent. This process is a separation technique based on different adsorptive interactions of the species with the adsorbent combined with adsorption column hydrodynamics and mass-transfer characteristics. SFE combined with an adequate analytical instrument is especially useful if complex samples have to be analysed and helps to improve both selectivity and sensitivity (Cross & Akgerman, 1998).

SFE-SFC seems to be the most favorable system for combined extraction, fractionation, identification and quantification of food bioactive compounds from natural sources, because the dissolved extracts and the chromatographic carrier are in the same physical state. Many researchers have reported applications of SFE-SFC for various compounds from different matrixes. Sato et al. to analyze capsaicinoids in the placentas of Capsicum fruits rapidly and safely, performed efficiently a directly connection of supercritical fluid extraction and supercritical fluid chromatography (SFE-SFC) (Sato et al., 1999). The Professor King's group in USA has extensively studied this combined process, but with an approach focused on large scale production of functional food ingredient from natural sources. Figure 2 shows a scheme of this coupled process. Since the purpose is not identification of compounds no detectors are inserted in the system. SFE extract stream is directed through the pressure-reducing regulator prior to its deposition onto the head of the chromatographic column. This allows the pressure to be reduced, allowing the extract to be concentrated at the top of the column without initially eluting any of the extract from the column. In addition, this gradual reduction of the pressure avoids freezing of the regulator from the Joule–Thompson expansion effect. After a set volume of CO_2 is passed, the extraction is terminated and the extraction cell is bypassed. The CO_2 stream is then directed into the column for fractionation of the SFE extract. SFC is performed followed by adsorbent bed reconditioning between runs. During the SFC steps ethanol or other solvents can be used as co-solvent for CO_2 (Taylor & King, 2002).

In theory, on-line fractionation by adsorptive processes also can be performed using solvents, such as water, ethanol, ethyl acetate and other organic solvents in the subcritical state as extracting solvent. Otherwise, few reports can be found, being an interesting research theme to be further studied. Sometimes referred to as subcritical solvent extraction, pressurized solvent extraction (PSE) and accelerated solvent extraction (ASE), Pressurized Liquid Extraction (PLE) has been successfully used for the extraction of several bioactive compounds from different plants (Petersson et al., 2010). A major advantage of PLE over conventional solvent extraction methods conducted at atmospheric pressure is that pressurized solvents remain in a liquid state well above their boiling points, allowing for high-temperature

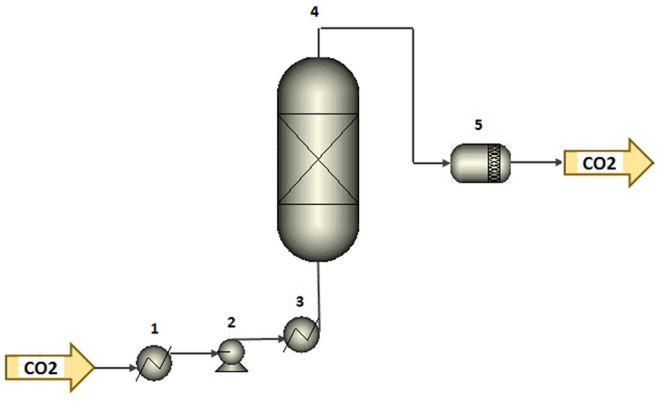

Figure 2. Scheme of the supercritical fluid extraction (SFE) process coupled with an adsorptive column. 1. Heat exchanger for cooling, 2. Pump, 3. Heat exchanger for heating, 4. Extractor, 5. Adsorptive column.

extraction. These conditions improve analyte solubility and the kinetics of desorption from matrices (Richter et al., 1997). The use of a PLE technique is an attractive alternative because it allows for fast extraction and reduced solvent consumption. PLE enables the rapid extraction (less than 30 min) of analytes in a closed and inert environment under high pressures [no higher than 20 MPa] and temperatures (25–200 °C). Hence, extracting solvents that are inefficient in extracting at low temperatures, may be much more efficient at the elevated temperatures used in PLE (Ju & Howard, 2003).

2.3 Coupling of enzymatic hydrolysis columns to pressurized fluid extractors for food bioactive compounds quantification purposes

In PLE, the organic solvent can in some cases be replaced by water, being called this process as pressurized hot water extraction (PHWE). In such case, by increasing the temperature, the dielectric constant of water decreases and the solubility of the bioactive compounds increases. However, the choice of temperature has to be selected with respect to the compound of interest. Specifically for quercetin another task should be overcome. After obtaining a quercetin-rich extract, since these compounds are mainly present in glucosylated forms, a further hydrolysis step is required to simplify the qualitative and quantitative analysis. Hydrolysis will remove the glucosylation, thus increasing the yield of the quercetin aglycone. Thus, in 2013 a continuous flow system was developed by Charlotta Turner's group in Sweden, combining PHWE with enzymatic hydrolysis on-line for quantitative extraction and hydrolysis of quercetin glucosides from raw onion samples (Lindahl et al., 2013). Figure 3 shows a scheme of this coupled process. The developed on-line extraction and hydrolysis method can be applicable for other plant matrices containing polyphenolic glucosides, in which the conjugated sugar is glucose. Otherwise other thermostable enzymes would be needed, which requires further investigation in finding the optimal conditions. Compared to conventional extraction with acid-catalyzed hydrolysis, the new method is milder and thereby more accurate. Furthermore, a hydrolysis step converts all quercetin glucosides to quercetin, which means that quercetin is quantified without the need for a large number of flavonoid standards and challenges with not low enough limit of detection (Lindahl et al., 2013).

2.4 Coupling of anti-solvent precipitation vessels to pressurized fluid extractors for fractionation and/or encapsulation purposes

The development of integrated processes for combining food bioactive compound extraction to on-line particle formation using anti-solvent precipitation techniques is a recent trend and only few reports are available on that subject. There is a report of the development of an on-line process to obtain dried powders of extracts from natural sources in one single operation performed also by the aid of Charlotta Turner in 2009 coupling to the pressurized hot water extraction (PHWE) step an anti-solvent precipitation vessel (Ibanez et al., 2009). This process was defined by the authors as Water Extraction and Particle formation On-line (WEPO). As the name implies, this process employs water as the extracting solvent. In this case, supercritical CO_2 is not suitable for the elimination of the extraction solvent due to the low solubility of water in CO_2, thus in this second stage supercritical CO_2 is used as a dispersion medium and a hot N_2 stream is used as the drying agent. Referred to as carbon dioxide-assisted nebulization with a bubble dryer (CAN-BD) this latter technique when coupled originated the patented hyphenated process PHWE-CAN-BD, which combines the pressurized hot water extraction (PHWE) and the drying of the extract by the CAN-BD process (Ibanez et al., 2009). Figure 4 shows a scheme of this coupled process. A similar on-line process developed by our research group in 2012, where PLE and particle formation are coupled, was also reported using organic solvents as extracting solvent instead of water (Santos et al., 2012a). Due to the similarities with the WEPO process, this process was defined as Organic solvent Extraction and Particle formation On-line (OEPO). Differently from the WEPO process, the OEPO process allows also the encapsulation of the extract

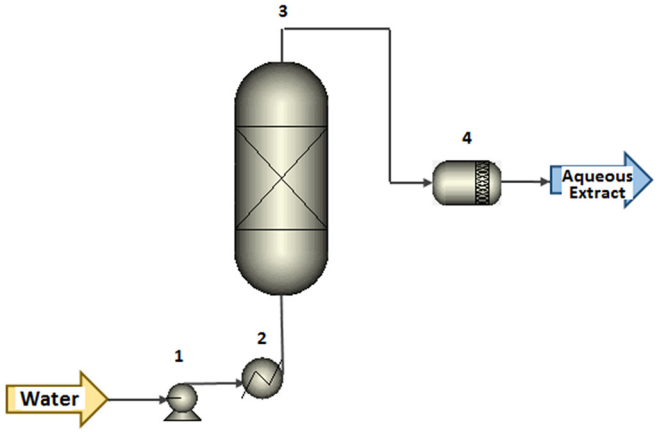

Figure 3. Scheme of the pressurized liquid extraction (PLE) process using water as extracting solvent named also by pressurized hot water extraction (PHWE) coupled with an enzymatic hydrolysis column. 1. Heat exchanger for heating, 2. Pump, 3. Extractor, 4. Enzymatic hydrolysis column.

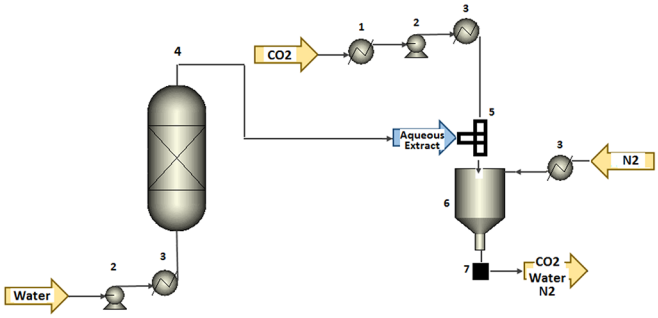

Figure 4. Scheme of the pressurized liquid extraction (PLE) process using water as extracting solvent named also by pressurized hot water extraction (PHWE) coupled with carbon dioxide-assisted nebulization with a bubble dryer (CAN-BD) process. 1. Heat exchanger for cooling, 2. Pump, 3. Heat exchanger for heating, 4. Extractor, 5. T-mixer, 6. Precipitation vessels, 7. Filter.

immediately after its production by the co-precipitation with a coating material. Indeed, the OEPO process consists of coupled PLE-SAS (Supercritical Anti-Solvent) precipitation, PLE-SAS co-precipitation and PLE-SFEE (Supercritical Fluid Extraction from Emulsions). Figure 5 shows a scheme of this coupled process. The results of this novel process using Brazilian ginseng roots and Annatto seeds as a natural source of bioactive compounds showed that the OEPO process as developed can be considered as a suitable and promising process to obtain, in only one step, different products (precipitated extract, co-precipitated extract or encapsulated extract in suspension) with desired particle size directly from the plant material.

In SAS and SFEE processes, respectively, a solution or an emulsion containing the extracted food bioactive compounds is sprayed continuously into a chamber through a nozzle with the supercritical CO_2. The high pressure CO_2 acts as an anti-solvent, decreasing the solubility of the solutes in the solvent mixture. Therefore, a fast supersaturation takes place, leading to nucleation and formation of micro or nanoparticles (Sosa et al., 2011). If a coating material is also employed encapsulates are formed by co-precipitation with the solute (Bahrami & Ranjbarian, 2007; Cocero et al., 2009). These processes have several figures of merit, including lower operating temperature than that used in conventional process such as spray drying, and lower residual solvent in the final product. Also, mean particle size, particle size distribution and morphology can be controlled by changing process parameters such as pressure and temperature (Guha et al., 2011).

2.5 Coupling of mixers to pressurized fluid extractors for emulsification purposes

As demonstrated previously sometimes the required final product is not the crude plant extract, so further steps are needed, which can be in a continuous flow way or not. Besides fractionation, quantification and encapsulation purposes the development of continuous flow systems can be extended to produce as a final product oil-in-water (O/W) emulsions directly from the plant materials. Towards this direction, our research group has developed in 2013 a novel on-line process for production of food-grade emulsions in one step containing essential oils from saponin-rich pressurized aqueous plant extracts (Santos et al., 2013). Named by our research group as Emulsions from Pressurized Liquid Extraction (EPLE), this process combines the dynamic PLE process using water as extracting solvent and the on-line emulsification of essential oils in water. Thus, extraction and emulsification steps take place in the same system with a small time delay between these two processes. Figure 6 shows a scheme of this coupled process. For process validation, *Pfaffia glomerata* roots was used as source of saponin-rich extract and clove essential oil as a model dispersed phase. From the pressurized fluid extraction cell, the aqueous extract solution is led to a T-mixer where it is mixed with essential oil stream. The essential oil was pumped by another high pressure pump. The contact of both streams in the T-mixer causes the emulsification. Then, the effluent (emulsion) is collected. Different essential oil flow rates can be employed in order to have different emulsion droplet size by adjusting the ratio between essential

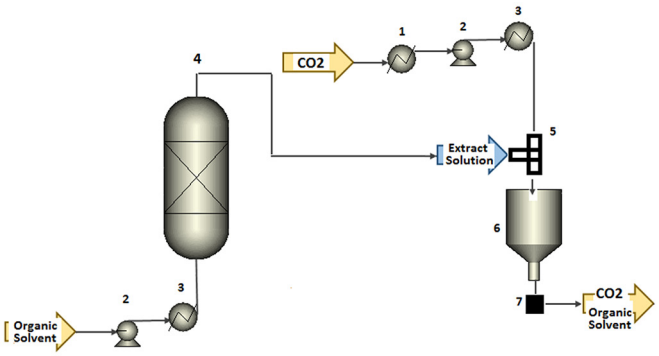

Figure 5. Scheme of the pressurized liquid extraction (PLE) process using organic solvent as extracting solvent coupled with supercritical antisolvent (SAS) precipitation process; this represents the hyphenated process PLE-SAS precipitation, one of the several variation of the Organic solvent Extraction and Particle formation On-line (OEPO) process. 1. Heat exchanger for cooling, 2. Pump, 3. Heat exchanger for heating, 4. Extractor, 5. T-mixer, 6. Precipitation vessels, 7. Filter.

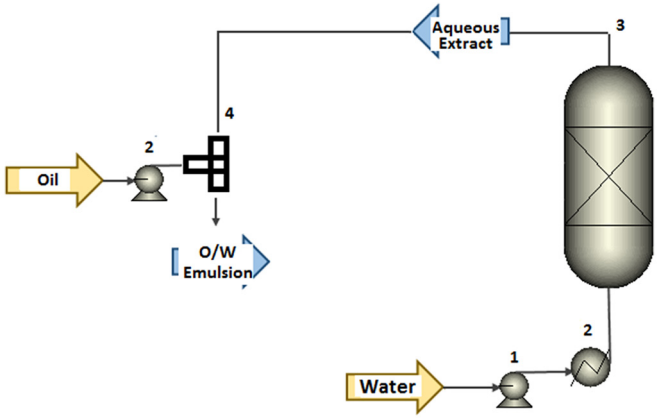

Figure 6. Scheme of the Emulsions from Pressurized Liquid Extraction (EPLE) process; this process uses water as extracting solvent. 1. Pump, 2. Heat exchanger for heating, 3. Extractor, 4. T-mixer.

oil to aqueous solution flow rates (Santos et al., 2013). In the following section, the description of the development of novel similar processes is presented for the first time.

3 Processes development

Two novel on-line processes for production of food-grade emulsions containing oily extracts in only one step were developed. Both processes exploit the supercritical CO2-essential oils miscibility, being that (Emulsions from Pressurized Fluid Extractions) EPFE process exploits the emulsification properties of saponin-rich pressurized aqueous plant extracts similarly to the previously developed process EPLE (Emulsions from Pressurized Liquid Extraction) (Santos et al., 2013). Meanwhile, the other novel process ESFE (Emulsions from Supercritical Fluid Extraction) obtains emulsions only by using the supercritical fluid extraction effluent. The aim in the conception of these two novel processes is to propose a new rapid way to obtain food-grade emulsions from supercritical fluid extracts. Nowadays the conventional

emulsion formulation method is a two-step procedure, i.e. first supercritical fluid extraction for obtaining an extract; secondly emulsion formulation using another device. The feasibility of these processes was demonstrated using clove buds to obtain the supercritical CO2-essential oils flow, as a model dispersed phase. For the development of the EPFE process Pfaffia glomerata roots and water were used as source of saponin-rich extract and extracting solvent, respectively.

3.1 Materials

Clove (*Eugenia caryophyllus*) buds were obtained from a local market in Campinas, Brazil. The clove was comminuted in a knife mill (Marconi, MA-340, Piracicaba, Brazil). The milled buds (8.60% moisture) were stored in the dark in a domestic freezer (Metalfrio, model DA 420, São Paulo, Brazil) at –10 °C until extraction.

Brazilian ginseng roots (*Pfaffia glomerata*) were cultivated in the experimental field of CPQBA (Campinas, Brazil), where they were collected being 3 years old. They were washed and dried in a forced air circulation dryer at 40 °C for 5 days. The dried roots were then comminuted in a in the same mill used for clove buds. Next, the particles of higher size were milled again, this time using a knife mill (Tecnal, model TE 631, Piracicaba, Brazil) for 2 s at 18,000 rpm. The dried and milled roots (8.89% moisture) were stored as done for clove buds.

N-octenyl succinic anhydride (OSA)-modified starch, kindly provided by National Starch Food Innovation (Hamburg, Germany), was used as surfactant alternatively to *Pfaffia glomerata* extracts.

Dry Carbon dioxide (CO_2), 99.9% purity (Gama Gases Especiais Ltda., Campinas, Brazil) used as the extracting solvent was supplied in the liquid phase. Distilled water was used as extracting solvent and to prepare OSA-modified starch solutions.

3.2 Emulsions from Supercritical Fluid Extraction (ESFE) process description

Emulsions from Supercritical Fluid Extraction (ESFE) process combines the dynamic Supercritical Fluid Extration (SFE) process using carbon dioxide as extracting solvent and the on-line emulsification of the effluent flow in water by the aid of a surfactant. Thus, extraction and emulsification take place in the same system with a small time delay between these two processes. The SFE system was designed and assembled at LASEFI/DEA/FEA (School of Food Engineering)/UNICAMP (University of Campinas). Figure 7 shows a scheme of the ESFE process. From the SFE cell, the extract solution is led to a T-mixer where it is mixed with OSA-modified starch solution stream. The surfactant solution was pumped by a HPLC pump (Thermoseparation Products, Model ConstaMetric 3200 P/F, San Jose, USA). The contact of both streams in the T-mixer causes the emulsification. Then, the effluent (emulsion) is collected. Different essential oil flow rates can be employed in order to have different emulsion droplet size by adjusting the ratio between SFE to surfactant solution flow rates. All connections used in the ESFE system were made using stainless steel tubes (1/16" and 1/8").

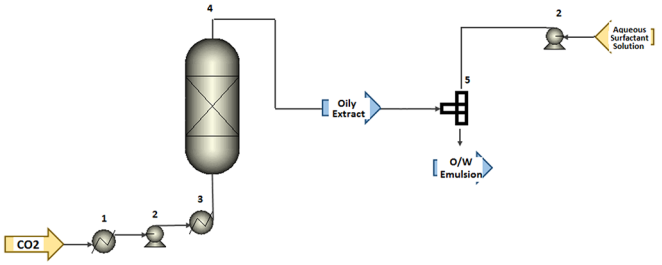

Figure 7. Scheme of the Emulsions from Supercritical Fluid Extraction (ESFE) process. 1. Heat exchanger for cooling, 2. Pump, 3. Heat exchanger for heating, 4. Extractor, 5. T-mixer.

Operational SFE conditions selected were 40 °C /15 MPa since these conditions resulted in optimum extraction yields (Moraes et al., 2015). Teflon column and glass beads (mean diameter of 0.005 m) were used to fill part of the 415 cm^3 SFE vessel volume to reduce the empty space to place the milled clove buds to be extracted. A thermostatically controlled bath is used for heating the SFE extractor. In order to develop the ESFE process, SFE process should be only performed during the constant extraction rate period, when the SFE extract flow has constant rate. The composition of this essential oil is presented elsewhere (Moraes et al., 2015).

3.3 Emulsions from Pressurized Fluid Extractions (EPFE) process description

The pressurized liquid extraction (PLE) system was designed and assembled at LASEFI/DEA/FEA (School of Food Engineering)/UNICAMP (University of Campinas). The solvent was pumped by a HPLC pump (Thermoseparation Products, Model ConstaMetric 3200 P/F, Fremoni, USA) into the PLE cell, which was placed in an electrical heating jacket at a desired temperature, until the required pressure was obtained.

Dried and milled pieces of Brazilian ginseng roots were placed in a 6.57-cm^3 extraction cell (Thar Designs, Pittsburg, USA) containing a sintered metal filter at the bottom and upper parts. The cell containing the sample was heated, filled with extraction solvent (distilled water) and then pressurized. The sample was placed in the heating system for 6.5 min to ensure that the extraction cell would be at the desired temperature during the filling and pressurization procedure. After pressurization, the sample with pressurized solvent was kept statically at the desired pressure for the desired time (static extraction time). The pressure of the PLE extraction was set in all experiments to 15 MPa, the same of the SFE process, to be possible their coupling. Thereafter, the back pressure regulator (BPR) valve (Model #26–1761-24–161, Tesco, Elk River, USA) was carefully opened, keeping the pressure at an appropriate level for the desired flow (1.0 cm^3/min), to rinse the extraction cell with fresh extracting solvent for dynamic extraction time. From the PLE cell, the aqueous extract solution is led to a T-mixer where it is mixed with SFE extract stream. The contact of both streams in the T-mixer causes the emulsification. Then, the effluent (emulsion) is collected. Figure 8 shows a scheme of the ESFE process. Different essential oil flow rates can be employed in

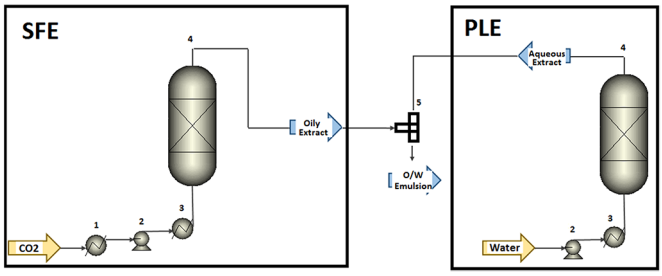

Figure 8. Scheme of the Pressurized Fluid Extractions (EPFE) process; this process represents a variation of the Emulsions from Pressurized Liquid Extraction (EPLE) process described for the first time in Santos et al. (2013) by coupling a Supercritical Fluid Extraction (SFE) process producing directly essential oil. 1. Heat exchanger for cooling, 2. Pump, 3. Heat exchanger for heating, 4. Extractor, 5. T-mixer.

order to have different emulsion droplet size by adjusting the ratio between SFE effluent to PLE effluent flow rates. All connections used in the EPFE system were made using stainless steel tubes (1/16" and 1/8").

Operational PLE conditions selected were 120 °C since these conditions resulted in optimum extract emulsification properties (Santos et al., 2013). In order to develop the EPFE process, PLE and SFE processes should be only performed during their constant extraction rate periods, when the extracts flows have constant rates.

4 Perspectives

Essential oils are typically formed by a mixture of terpenoids that are volatile, thus, they rapidly evaporate. In the food industry there is strong interest in creating new products based on essential oil systems in which emulsions can be used as delivery systems. The poor water solubility of essential oils is a crucial technical barrier for these delivery systems which led to the development of complex surfactant systems. However, this complex surfactant systems that has been employed are, in general, potentially toxic and unstable (Bilbao-Sáinz et al., 2012). The choice of the surfactant and method used to produce emulsions is also crucial. The formation of an emulsion includes the mixing of the immiscible liquids and the time for surfactant molecules to organize at the interface of the two phases (Cucheval &. Chow, 2008).

Saponins are natural surface-active substances (surfactants) present in more than 500 plant species. Due to the presence of a lipid-soluble aglycone and water soluble sugar chain(s) in their amphiphilic structure, saponins are surface active compounds with detergent, wetting, emulsifying and foaming properties (Stanimirova et al., 2011). Recently, it was demonstrated that plant extracts containing saponins can be used as surfactant and Brazilian ginseng roots (*Pfaffia glomerata*) are a great source of these compounds. To the best of our knowledge saponins are currently used in various food industry applications, on the other hand none of these products use Brazilian ginseng roots as saponin source.

Saponins have been used in foods as natural surfactants; they serve as preservatives to control the microbial spoilage of food. Because of consumer preferences for natural substance, saponins have more recently been used as a natural small molecule surfactant in beverage emulsions to replace synthetic surfactants such as Tweens (Cheok et al., 2014).

There are a number of mechanisms available to produce emulsions. According to the literature, emulsions can be prepared by high- and low-energy emulsification methods. The high-energy methods generally employ mechanical or ultrasound devices that generate shearing (rotor stator) or pressure differences (a high-pressure homogenizer or power ultrasound) to decompose the emulsion structures (Yang et al., 2012). As it was developed we can indicate that the developed processes are based on low-energy emulsification principles, otherwise, by the inclusion of ultrasound irradiation in this stage we can convert our processes into high-energy-based emulsification processes. From the developed experiments until now, it was obtained oil-in-water (O/W) emulsions with medium droplet size 300-800 nm by performing the experiments as described in the previously sections. Obviously, better emulsion, i.e. with smaller droplet size, could be obtained with the optimization of this point during both developed process. Further experiments will be done in this direction. We investigated the influence of the ratio of supercritical fluid essential oil obtaining flow rate to Brazilian ginseng roots aqueous extract solution flow rate on the emulsion droplet size (Sauter diameter, d_{32}) by laser light scattering method using Mastersizer 2000 (Malvern Instruments, Worcestershire, United Kingdom). This ratio was varied maintaining a constant flow of clove essential oil extract solution and using variable flows of Brazilian ginseng roots aqueous extract solution due to simplicity. As expected, the essential oil/aqueous solution ratio had a strong influence on the emulsion droplet size, and as this ratio increased, the d_{32} increased as well. This can be due to the formation of an emulsion with a larger droplet size after the mixing step, which results in the formation of bigger particles inside the droplets of the emulsion. Similar results were also reported in a detailed study that aimed the production of stable oil-in-water emulsions of β-carotene using a simple T-mixer as emulsifying device. As a general conclusion, the ratio between the oil to water flow rates appears as the most important parameter for controlling the product characteristics (Paz et al., 2012).

Evidently, these developed processes can be applied for other plant materials. Clove buts can be changed to another essential oil-rich source and Brazilian ginseng roots to another saponin-rich source. With regard to the choice of the surfactant material needed during ESFE, their biocompatibility and lack of toxicity are of course important considerations as well their low solubility in the supercritical fluid. OSA-modified starch surfactant achieve both needs. It has been expansively used because they are suitable for food and nutraceutical applications and they are also capable of providing a double functionality as a surfactant for the emulsion stabilization and as a carrier material in the final product (Santos et al., 2012b).

The fabrication of O/W emulsions is a process with widespread applications in formulation engineering. The idea to create the emulsion on-line and operate the process in a continuous mode is not new, existing several commercial on-line dispersing

technologies already available (Brocart et al., 2002). On the other hand, this is the first report describing the development of novel processes that combines the use of supercritical fluid extraction method to obtain emulsions immediately. The developed on-line processes do not only combine extraction with emulsification in one step, which greatly reduces the laboratory work, but also reduces the environmental impact by using environmentally friendly substances, i.e. carbon dioxide, water and alternative biosurfactant obtained from Brazilian ginseng roots.

5 Conclusions

Emulsions from Supercritical Fluid Extraction (ESFE) and Emulsions from Pressurized Fluid Extractions (EPFE) processes were described in detail and successfully developed. Both processes exploit the supercritical CO2-essential oils miscibility, additionally, EPFE process exploits the emulsification properties of saponin-rich pressurized aqueous plant extracts. The feasibility of this latter one-step process was demonstrated using Pfaffia glomerata roots as source of saponin-rich extract, water as extracting solvent. Clove essential oil, directly extracted using supercritical CO2, was used as a model dispersed phase during the development of both processes.

In addition, examples of pressurized fluid-based coupled processes applied for adding value to food bioactive compounds from diverse sources previously developed by us and other research groups were reviewed in order to provide some insight into future potential of this emerging research topic. It was discussed the recent trends on coupling of separators and adsorptive columns to pressurized fluid extractors for fractionation purposes, coupling of enzymatic hydrolysis columns to pressurized fluid extractors for bioactive compounds quantification purposes, coupling of anti-solvent precipitation vessels to pressurized fluid extractors for fractionation and/or encapsulation purposes and finally coupling of mixers to pressurized fluid extractors for emulsification purposes.

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