Multiple unit processing using sub- and supercritical fluids

Jerry W. King*, Keerthi Srinivas

Department of Chemical Engineering, 3202 Bell Engineering Center, University of Arkansas, Fayetteville, AR 72701, USA

**Abstract**

The past twenty years has seen an expansion of the “critical fluid” technology platform with respect to using or combining multiple types of unit operations and compressed fluids in both their sub- and supercritical states. Although supercritical fluid extraction (SFE) utilizing supercritical carbon dioxide (SC-CO2) has seen considerable industrial use, the high capitalization cost of building and running such production plants promotes expanding their use and functionality with respect to multi-unit operations and multiple fluids. An evaluation of the current status of implementing such an approach is provided, and a perspective of future needs suggested based on the senior author’s 40 years of experience in critical fluid processing of agriculturally-derived materials and natural products. The use of hot compressed water is no longer limited to just supercritical water processing, but can be advantageously exploited over a wide range of temperatures and appropriate pressures to include extraction and reaction of targeted substrates, including bioactive natural products, biomass conversion for renewable fuels, and synthesis of chemicals. As noted previously, combining SFE with fractionation methods using critical fluids, and/or reaction chemistry in critical fluid media can produce a variety of extracts or products [J.W. King, Sub- and supercritical fluid processing of agrimaterials: extraction, fractionation and reaction modes, in: E. Kiran, P.G. Debenedetti, C.J. Peters (Eds.), Supercritical Fluids: Fundamentals and Applications, Kluwer Publishers, Dordrecht, The Netherlands, 2000, pp. 451–488.] however the modification of processing equipment such as expellers, extruders, and particle production devices extend the use of the critical fluid technology platform. Sequential unit processing using multiple fluids also has been reported although demonstrated to a limited degree—however combining both compressed CO2 and water in a unit process can also impart some unique processing possibilities. The concept of multiple unit and fluid processing supports an environmentally benign or “green” production platform and is consistent with processing sustainable materials. Integration of critical fluids into existing processing concepts such as biomass conversion, biorefineries, and synthesis based on methyl ester intermediates are discussed.

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1. Introduction

Supercritical fluids and their liquefied analogues have been traditionally used in single unit operations, i.e. extraction, fractionation, using neat SC-CO2 or with appropriate modifiers [2]. Since the 1980s, almost 38% of the supercritical fluid extraction processes have been devoted to extraction of food and natural products [3]. Beginning in the mid-1980s, columnar and chromatographic techniques followed by reactions in supercritical fluids were developed to facilitate supercritical fluid derived extracts or products [4], thereby extending the application of a critical fluids processing platform beyond SFE. These newer developments were investigated in part due to the complexity of many natural product matrices and the desire to concentrate specific target components for food and other industrial uses, as illustrated in Fig. 1. This approach has been summarized by the senior author and others in previous publications [1,5,6]. The perspective we offer here is somewhat biased towards past and current studies which focus on advancing multiple unit and/or fluid processing of agriculturally-derived materials or natural products.

Despite this focus, the perspective we offer opens other options for applying critical fluids in general by embracing the idea of tandem processing using different compressed fluids. Commensurate with the goal of environmentally benign processing is the use of liquefied or supercritical CO2 for non-polar to moderately polar solutes, and compressed water between its boiling and critical points for more polar solutes and reactants. Ethanol, a naturally derivable sustainable solvent, is suggested as a preferred co-solvent to be paired with compressed water or carbon dioxide. This simple, renewable compressed fluids platform has many advantages and can be used to achieve multiple results, particularly when used sequentially, or in tandem with multiple unit processes.

* Corresponding author. Tel.: +1 479 575 5979; fax: +1 479 575 7926.
E-mail address: jwking1@uark.edu (J.W. King).

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We have found that the generic solvation properties of the two principal critical fluids, CO2 and water, to be explained by an extended solubility parameter approach [7,8]. Hence by adjustment of pressure and temperature for CO2, or temperature in the case of water, one can optimize the solubility of solutes or reactants in these media, or predict their miscibility, by comparing their relative solubility parameters as a function of temperature and pressure. Such an approach has a practical value considering the molecular complexity of many solute types processed in critical fluids. Their solubility parameters or solute–fluid interactions can be explained by using the Hansen three-dimensional solubility concept which allows the application of functional group contribution methods for calculating requisite physical property data as well as solute or solvent solubility parameters as recently reported by Srinivas et al. [9]. As indicated by Fig. 2, the reduction in water’s total solubility parameter with increasing temperature is largely due to a reduction in the hydrogen-bonding propensity as reflected by its hydrogen-bonding solubility parameter component, \( \delta_H \) [10]. Water does not attain the solvation properties of solvents like ethanol or methanol until quite elevated temperatures which is in contrast to the often cited dielectric constant concept which is invoked to explain the solvent properties of subcritical water [11].

The above solvent properties of water as described by the solubility parameter concept has some implications with regard to its use as a “green” solvent and as a substitute for ethanol in hydroethanolic-based extractions which are GRAS (Generally Regarded as Safe)-approved food processing solvents. Its substitution for ethanol as a processing medium is highly desired to save on processing costs, its separation from water in solvent recycle schemes, and oversight by revenue authorities. These are some of the factors which accelerate research in the use of subcritical water for the extraction of natural products and nutraceutical food components. This aids in considering the widespread utilization of water and carbon dioxide as a part of the overall critical technology platform [1].

Traditional oleochemical processing operations such as fat splitting or hydrogenation are often conducted under either subcritical or supercritical processes. Fat-splitting processes such as the Twitchell process [12] or Colgate-Emery synthesis [13] utilize temperatures and pressures in excess of the boiling point of water under the appropriate pressure, but below the critical point of water to facilitate the hydrolysis of triglycerides to fatty acids. However, it should be noted that these processes were frequently interpreted as steam-based hydrolysis rather than hydrolysis using subcritical water. Hydrogenations using binary mixtures of CO2–H2 or propane–H2 are supercritical with respect to the pure component critical constants, but reaction conditions are conducted under less dense conditions due to the high temperatures involved, and hence the cited rapid kinetics associated with hydrogenations conducted under these conditions [14] refers more to the accelerated mass transfer effects as opposed to reactant solubility enhancement. Enzymatic-based synthesis under supercritical conditions has been largely confined to laboratory-scale studies [15–17] and the sensitivity of enzymes to operational parameters and their attendant high cost suggests that their application maybe limited to the synthesis of high cost products.

The advantages of coupling processing options using critical fluids are illustrated in Fig. 3. Hence by combining different unit processes and sequencing them with the use of multiple fluids held at operational densities by the application of different temperatures and pressures, one can obtain multiple products and optimize the extraction or reaction process. Several specific options are illustrated for the case of processing essential oils as noted previously in Table 1 [18] using pressurized fluids. Here six discrete unit processes are listed which include standard SFE with SC-CO2,

![Fig. 2. Three-dimensional solubility parameters of water as a function of temperature.](image)

![Fig. 3. Coupled processing options for critical fluids.](image)

<table>
<thead>
<tr>
<th>Process</th>
<th>Processing combinations</th>
</tr>
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<tbody>
<tr>
<td>(1) SFE (SC-CO2)</td>
<td>(1)+(2)</td>
</tr>
<tr>
<td>(2) SFF (SC-CO2)</td>
<td>(1)+(2)+(4)</td>
</tr>
<tr>
<td>(3) SFF (SC-CO2)</td>
<td>(1)+(5)</td>
</tr>
<tr>
<td>(4) SFC (SC-CO2/co-solvent)</td>
<td>(1)+(5)+(6)</td>
</tr>
<tr>
<td>(5) SFF—(subcritical H2O deterpenation)</td>
<td></td>
</tr>
<tr>
<td>(6) SFM—(aqueous extract/SC-CO2)</td>
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SFF (supercritical fluid fractionation) employing stage-wise pressure reduction. SFF as practiced using column-based deterpenation [19], supercritical fluid chromatography (SFC), another variant of SFF called subcritical water deterpenation [20], and utilization of a SC-CO₂, or LCO₂ with a perm selective membrane described by Towsley et al. [21]. As shown in Table 1, combinations of these processes can allow a total processing scheme to be conducted using critical fluids. For example, a combination of processes (1) and (2) in Table 1 could be combined to yield a more specific composition in the final extract. Unit process (1) if conducted by sequentially increasing the extraction density when coupled with a sequence of let down pressures (unit process 2) can amplify the SFF effect. Likewise, by combining unit process (1) using SC-CO₂ followed by application of unit process (2) utilizing subcritical H₂O to deterpinate the extract from unit process (1), will yield a more specific final product from the starting citrus oil. To obtain a more enriched and/or concentrated product from the latter process, one could add unit process (6), a supercritical fluid membrane-based separation of the aqueous extract/ractions from unit process (5) as indicated in Table 1.

We shall discuss several other combinations of unit processes and fluids below based on both our research and those of others cited in the literature. Many of these examples are focused on the processing of complex natural product mixtures, lipids and oleochemicals, and more recently the processing of biomass for value-added extractives and conversion to fuel substrates, since we are most familiar in applying critical fluids in those areas.

2. Multiple critical fluid processing platforms

In the broadest sense, multiple critical fluid processing involves the integration of two or more fluids held under pressure applied as either mixtures or in a sequential manner for one or more unit processes. Listed in Table 2 are the most prevalent combinations that have been utilized or have potential application in process development. Solubility of solutes and reactants in SC-CO₂ has been extensively studied and a recent tome has assembled much of the available data [22]. Likewise there is a fair understanding as to the subcritical water extraction solvents under external compression above their boiling points [28–30]. Analytical methods developed with the use of pressurized solvents essentially use subcritical fluids above their boiling point—the pressure applied frequently is far in excess of what is required by inspection of the V–L curves for these fluids [31]. Unfortunately researchers in these disparate areas using water as a common compressed fluid have not always recognized its generic utility as a universal compressed fluid medium as well as “green” complimentary solvent to compressed CO₂ [7].

Recently we have attempted to explain the solvent characteristics of water using an extension of the solubility parameter concept, i.e., a three-dimensional solubility parameter approach, by applying it to pressurized water [8,9]. Using the Hansen three-dimensional solubility parameter approach coupled with SPHERE and Hsp3D [32] software programs, we have studied the interaction between subcritical water and complex organic solutes, including biopolymers, as a function of temperature. Water under compression has the ability by adjustment of the applied temperature and pressure to serve as an extraction solvent as well as a reaction medium depending on what is desired. Residence time of the solute (reactant) in the aqueous medium thus becomes a critical parameter in conducting extractions above the boiling point of water and for optimizing reaction conditions “higher up” the V–L curve for water. There appears in our opinion the lack of rationale design for choosing reaction conditions in subcritical water, although the semi-empirical “severity” parameter often-cited in biomass conversion studies is one attempt to quantify the required hydrolytic conditions [33]. Using a simple solubility parameter approach, as depicted in Fig. 4, in which the solubility parameter for water as a function of temperature is plotted along with the solubility parameters for cellulose oligomers (n = 1–10), it can be seen that the intercept between the solvent and the cellulose oligomers corresponds to the chosen conditions for depolymerizing the carbohydrate polymers [7]. This confirms that conditions are commensurate with those in which the biopolymer is dissolved or miscible in the subcritical water medium. We have also used this approach for other biopolymers treated in subcritical water such as hemicellulose and chitin and rationalized the difficulty in dissolving lignin-type polymers in subcritical water [7]. A similar approach also has utility in understanding the subcritical water extraction of cellulose oligomers.

Table 2

<table>
<thead>
<tr>
<th>Mode</th>
<th>Example</th>
</tr>
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<tbody>
<tr>
<td>Single fluid</td>
<td>Carbon dioxide, water</td>
</tr>
<tr>
<td>Single fluid + co-solvent</td>
<td>Carbon dioxide + ethanol</td>
</tr>
<tr>
<td>Binary gases above their Tc</td>
<td>Carbon dioxide + hydrogen</td>
</tr>
<tr>
<td>Single fluid + dissolved gas</td>
<td>Water + carbon dioxide</td>
</tr>
<tr>
<td>Sequential fluids</td>
<td>Carbon dioxide, then water</td>
</tr>
</tbody>
</table>

Fig. 4. Solubility parameter variation for subcritical water at different reduced pressures (p) and cellulose oligomers (n = 1–10) with temperature.
Variation of Hansen three-dimensional solubility parameter sphere of betulin with subcritical water and ethanol at different temperatures.

The Hansen three-dimensional sphere method noted above can also be used in choosing a subcritical fluid as an extraction solvent and optimizing the extraction conditions. The three-dimensional solubility parameters of water and ethanol at a reference temperature (25 °C) are obtained from Hansen [32] and their temperature-dependence calculated using the approach of Williams [34]. The Hansen spheres were then generated using the Hsp3D program in a Matlab platform. The inverted white triangles which appear in Figs. 5 and 6 below indicate that the targeted solute is within the solubility sphere thereby ensuring high miscibility with the subcritical fluid, while the dark triangles indicate immiscibility between the solute and the subcritical fluid referring to the compounds outside the Hansen solubility sphere. The inverted white triangle on the other side of the center of the mass of the sphere is the solute itself.

One study we have contributed to involves the extraction of betulin from birch bark extracts using subcritical water or ethanol as solvents. The experiments were conducted using subcritical water maintained at 40–180 °C and 5 MPa using an analytical-based extraction system. It was found that a limited amount of betulin was extracted into subcritical water even at temperatures as high as 180 °C. However, similar studies conducted with subcritical ethanol yielded high amounts of betulin at 90 °C and 5 MPa [35]. The three-dimensional solubility parameter Hansen sphere plots for betulin in water and ethanol respectively are shown in Fig. 5. Here the radius of the Hansen sphere for the betulin–ethanol system is far less than that observed for the betulin–water system. This is indicative of
The fact that subcritical ethanol is a far better solvent under the stated conditions than compressed hot water. A further study of the relative energy difference (RED) values for the two solute–solvent systems indicates miscibility–solubility between 50 and 125 °C in the betulin–ethanol system with a maximum betulin solubility occurring at 100 °C [9].

Similarly in Fig. 6 are the Hansen spheres for the anthocyanin, malvidin-3-O-glucoside, in water and ethanol, respectively. The sphere plots indicate a greater miscibility of malvidin-3-O-glucoside in ethanol since the Hansen sphere has a lower radius (7.23 MPa^{1/2}) in the temperature range of 25–75 °C. For the water–malvidin-3-O-glucoside system, the corresponding solubility sphere occurs over a different temperature range and a RED radius of 10.35 MPa^{1/2}, higher than that for ethanol [8]. While both subcritical solvents dissolve the target anthocyanin at different conditions, clearly ethanol would be the preferred subcritical solvent with respect to anthocyanin miscibility and solubility.

The three-dimensional solubility parameter sphere approach can also be used to explain experimental results we have obtained for the solubility and extraction of malvidin-3-O-glucoside in compressed-hydroethanolic solvent medium. The Hansen solubility spheres were plotted for water–ethanol mixtures and their corresponding interaction radii versus the composition of subcritical water–ethanol mixture is shown in Fig. 7. The plot shows an initial substantial decrease in the interaction radius between the anthocyanin and hydroethanolic mixture upon addition of 10% ethanol to water (this is due to the substantial decrease in the hydrogen bonding solubility parameter with the addition of ethanol to water). This is consistent with the enhanced extraction recorded for the flavonoid upon addition of ethanol to the extraction medium. Note that although in Fig. 7 the minimum interaction radius occurs at 80% ethanol concentration, there is not a significant difference with that recorded at 10% ethanol content. This has important implications for designing the best extraction conditions and minimizing the amount of co-solvent (ethanol) required in extracting the target flavonoid.

The use of compressed gases at conditions far above their critical temperatures as diluents in a highly compressed supercritical fluid medium in biomass conversion processes is far superior to SC-CO2. The solubility of petroleum reservoirs, analytes absorbed in sample matrices, and in the continuous extraction of vegetable oils from seed meals.

Recently, compressed gases in their supercritical fluid state; particularly SC-CO2, dissolved in liquids and subcritical liquids have become of interest as in situ catalysts or modifiers for reaction and extraction unit processing. The use of SC-CO2 as a replacement for metallic catalysts in glycerolysis reactions was reported by Temelli et al. [44] and a review of its use in synthetic organic reaction chemistry has been published by Rayner et al. [45]. Dissolving SC-CO2 under pressure in pressurized water creates a versatile medium with respect to acidic-based extraction chemistry and reactions due to the inherent carbonic acid equilibrium that is pressure dependent as studied by Toews et al. [46]. We and others have found that if sufficient CO2 under pressure is applied to aqueous solutions, that pH's between 2.0 and 2.5 can be achieved. The basis of this enhanced dissolution can be seen from the literature data that we have plotted in Fig. 8 [47–50]. Intuitively, increasing the temperature of water should decrease the amount of gas dissolved in water as borne out by the data taken at lower pressures and temperatures shown in Fig. 8, however application of greater pressure as applied to SC-CO2 lowers the pH of the solution due to an increase in the amount of dissolved gas in water (Fig. 8). Our research group and others have recently exploiting this trend to assist in the conversion of various types of carbohydrate-laden biomass by converting the constituent carbohydrate polymers to lower oligomers for eventual conversion to biofuels. Similarly control of solution pH by dissolution of SC-CO2 can also affect the equilibrium-based species that is extracted when using subcritical water as is the case for anthocyanins and similar flavonoid-based solutes [51]. This technique offers definite advantages with respect to avoiding the use of mineral acids in extraction and reaction chemistry since the dissolved SC-CO2 can be jettisoned to the atmosphere or recycled by a reduction in pressure. Studies using supercritical carbon dioxide as reaction solvent especially in catalytic hydrolysis as described above have also shown good product separation characteristics by increasing the pressure from 20 bar to as high as 120 bar. Approximately 90% of the hexanes were successfully separated from the hydrolytic mixture dissolved in supercritical carbon dioxide [52].

It was remarked previously (Table 2) that it should be possible to use one critical fluid at different temperatures and pressures to perform multiple unit operations. For SC-CO2, it is well documented that changes in the fluid density can be used as a basis for the supercritical fluid-based fractionation (SFF) of a number of complex mixtures. This is also possible in the case of subcritical water, but usually through the adjustment of temperature. As the authors have previously noted, hot compressed water is a versatile medium in both its sub- and supercritical regions by using it over a range of reduced temperatures (T_r) and pressures (P_r) depending on the unit processing result that is desired. High values of T_r (1.02–2.5) and P_r (1.2–1.8) are used for destructive schemes, such as supercritical water oxidation, while a lower range of T_r’s and P_r’s are employed for selective molecular transformations such as biomass conversion via specific reaction pathways. Biomass conversion in hot compressed aqueous media embrace

Fig. 7. Variation in the Hansen sphere radius (MPa^{1/2}) versus %ethanol in compressed water–ethanol extraction solvent.
Fig. 8. Mole fraction solubility of CO₂ in water as a function of temperature and pressure.

operational parameters (T₀ = 0.65–1.05, P₀ = 0.35–2.0) in both the sub- and supercritical phases for water. The use of subcritical water for extractions depends on the physical properties of the dissolved solutes and their tendency to degrade under the chosen extraction conditions. For example, T₀’s and P₀’s are typically in the range of 0.50–0.80 and 0.02–0.35, respectively, for the extraction of natural products.

Fig. 9 shows a hypothetical multi-unit processing scheme based entirely on subcritical water for the treatment of a potential biomass substrate [7]. Here the target substrate is pretreated with pressurized water to prepare it for eventual extraction or reaction using subcritical water. The pretreatment with pressurized water can be used to swell the substrate or comminute it for more effective extraction or reaction. Using the above criterion, an extraction can be performed using pressurized water above its boiling point to recover high value botanical extracts from the substrate prior to using subcritical water as a reaction solvent. Post-extraction treatment is then performed over a higher temperature range (150–300 °C) to hydrolyze the remaining biomass for further conversion to a lower molecular weight hydrolyzate suitable for fermentation to a liquid fuel. Partial realization of this scheme will be described further in Section 4. The advantage of the described process is that it can be potentially conducted in one reactor of integrated processing plant therefore saving on capitalization costs.

3. Multiple unit processing: concepts and possibilities

The generic concept of using critical fluids in more than one unit process utilizing critical fluids was discussed in Section 1 and a pertinent example given. In this section we wish to elaborate on this concept in two ways: (1) coupling critical fluids with an existing processing device and (2) coupling unit processes to achieve a final product or end goal. The senior author has noted previously that processing with supercritical fluids can consist of an extraction mode (SFE), fractionating using supercritical fluids (SFF), conducting a reaction(s) in critical fluid media (SFR or CFR), and the formation of fine particles in critical fluid media, or CPT. Advances in processing using critical fluids can frequently be achieved by modifying an existing device or concept, such as an expeller or extruder to provide a continuous processing method, or utilize equipment to concentrate or fractionate an initial extract or reaction mixture.

Here we shall consider two significant advancements utilizing compressed critical fluids with two devices: an expeller with a focus on seed oil processing and the use of membranes coupled with critical fluids.

The concept of continuous processing of oils from seeds and meals goes back to the mid-1980s with the description of the operation of an Auger-type screw press by Eggers [53]. Here a supercritical fluid such as SC-CO₂ is used to assist in the removal of oil from crushed seeds or meals which may have been partially pre-extracted. The physicochemical basis of the process is still not well understood, but involves the addition of liquefied CO₂ to the seeds or meal inside an expeller barrel to aid in the oil extraction process. The hydraulic compression on the seed meal creates considerable pressure and heat on seed matrix resulting in the conversion of the added CO₂ to its supercritical state. The hot compressed carbon dioxide partially solvates the seed oil akin to what occurs when SFE is performed with SC-CO₂, but more importantly dilutes or expands the expressed oil enhancing its removal from the seed or meal bed. Methods to achieve this goal have been described in the patent literature most notably by Foidl [54] whose process
has been partially commercialized and applied to the processing of soybeans. This CO$_2$-assisted expression process has been commercialized by Crown Iron Works in Minneapolis, Minnesota under the trademark of HIPLEX process and CO$_2$ expression demonstrated on a Harburg Freudenberger expeller having a 25 ton per day capacity. The process is in commercial operation at SafeSoy Technologies in Ellsworth, Iowa. The ratio of oil to CO$_2$ is 3:1 which reduces the vegetable oil viscosity by 1/10 resulting in between 80 and 90% vegetable oil recovery for soybeans and over 90% recovery of canola oil. Such solvent-free oils and meals are superior in quality to solvent extracted products. A similar device would be welcomed for subcritical water extraction of natural and food-related products as well as for the conversion of biomass substrates on a continuous basis (see Section 4) in which the substrate to be extracted or treated with pressurized water would be contacting as a slurry with the pressurized water. This could be in principle applied to such diverse matrices as grape pomace, cocoa beans, and herbal substances provided the residence times above the boiling point of water are minimized. Current systems for affecting such pressurized water extractions are staged as semi-continuous batch systems or by combining the substrate to be processed as aqueous slurry with water before passage through a heated extraction vessel. It should be noted that critical fluid-based expeller processes compete with similar unit processing done with the aid of extruders [55]. Although extruders have shown promise in the processing of finished food products [56] their attendant expense and lower throughputs make them less attractive than the expeller-based processes described above.

The second development which we feel is important is the coupling of critical fluids with membrane technologies. This can be applied in a number of very diverse applications ranging from concentrating extracts, providing additional fractionation on extracts derived from SFE using SC-CO$_2$, and as an alternative post-SFE separation process to avoid the recompression costs associated with phase-based separation methods involving recycle of the supercritical fluid after extraction. In the latter case, Brunner [57] has noted that since the molecular weight of supercritical fluid is considerably less than the extracted solutes, application of a permselective membrane that is compatible with operation at high pressure should be feasible. Coupling of supercritical fluid extraction processes with membrane technology for supercritical fluid recovery and product purification can decrease the energy requirements and provide fractionation of the extracts or reactants from an extraction and/or reaction processes. Sarrade et al. [58] has provided a comprehensive review on the integration of these two technologies.

Studies have also been conducted to study the performance of different types of reverse osmosis (RO) membranes in recovery of supercritical carbon dioxide after the extraction of essential oils from natural products like lemongrass, orange and nutmegs. Essential oil retention by the membranes was reduced with increase in the trans-membrane pressure drop but there was no effect observed when the oil feed concentration was varied. Recovery of the essential oils was approximately 90% via this coupled process [59]. RO membranes were also used for the recovery of carbon dioxide from limonene extracted from essential oil yielding matrices. The process compared the performance of one nanofiltration and three RO membranes at identical upstream and trans-membrane pressures slightly above the $T_c$ of CO$_2$

Integrating critical fluid technology with membranes has permitted the separation of low and high molecular weight compounds obtained from the SC-CO$_2$ extraction of lipids from foodstuffs such as butter or fish oil using nanofiltration membranes [60]. Similarly, it has been reported that is possible to extract polyphenols from cocoa seeds using neat SC-CO$_2$ and with ethanol as a co-solvent, and then concentrate the extract using polymeric nanofiltration or reverse osmosis membranes. This system operated at a pilot scale between 8 and 15 MPa and at 40 °C resulted in a maximum yield of polyphenols of 43% when the pressure was optimized at 8 MPa using ethanol. The study also indicated a high performance of all the membranes when the trans-membrane pressure was maintained in excess of 1 MPa [61]. The ability to concentrate extracted polyphenols using SC-CO$_2$ extraction paired with membranes suggest that a similar tandem involving subcritical water–membrane coupling would be advantageous since extraction with subcritical water results in a diluted extract. This concept was first advanced by King [62] and noted in a US patent issued to Wai and Lang [63]. They suggested that SFE could be implemented on a natural product matrix followed by subcritical water extraction sequentially on the same matrix and then followed by a membrane separator to yield a concentrate of the aqueous extract. The authors view the actual implementation of such tandem technologies as critical to the further development of the application of subcritical water extraction for processing food, nutraceutical, and natural products.

Countercurrent contacting of a liquid or SC-CO$_2$ stream on one side of a permselective hollow fiber membrane to enrich aroma components from aqueous solutions may be a way of approaching the above problem. Sims has demonstrated such a process and patented it as the POROCRIT process, and also applied it to the sterilization of citrus juices among other applications [64]. Research involving the use of compressed solvents such as carbon dioxide to extract fermentation products like ethanol and acetone within a hollow fiber membrane reactor employ a similar approach. In this study, a membrane fiber made of microporous hydrophobic polypropylene was used in a single fiber membrane contactor and operating conditions maintained at 0.02–0.07 MPa trans-membrane pressure at ambient temperature. For aqueous binary feed solutions containing 10:10 wt% ethanol: acetone or 5:5 wt% ethanol: acetone solution. Recovery of ethanol ranged between 5 and 14% while that for acetone ranged between 68 and 96% depending on the CO$_2$ flow rate using molar solvent to feed ratios between 3 and 10 [65].

Combing various unit processing options using critical fluids to achieve an end goal involves looking at a matrix of possibilities and achieving optimization for all of the combined processes. In our approach over the past two decades, we and others have investigated and optimized the individual unit processes with an overall final objective of producing a final result/product—it is less frequent to find the discrete steps already combined, but examples of both will be given in this section and Section 4. Several reviews have been published by the senior author regarding coupled processing [18], its application for nutraceutical [5] and lipid processing [66], and

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**Diagram:**

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Soybean Flakes
       SC-CO$_2$ Extraction
       ↓
Defatted Soybean Flakes
       SC-CO$_2$/E/0H Extraction
Exhausted Oil-cake
       PPL-enriched fraction
       (Lecithin - $31.00/lb)
       SC-CO$_2$/E/0H/H$_2$O
Pure Phospholipid
       Chromatography
       (PC/PS - $1800/lb)
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**Fig. 10.** Phospholipid enrichment/fractionation using coupled supercritical fluid-based processes.
the use of reaction chemistry for conversion of agricultural materials [1]. Today, production plants and toll refiners in addition to SFE offer one or more other unit processes for the refining of materials, although there is room for more expansion using multiple unit processing. Perrut [67] has recently reported a pilot plant designed for multi-fluid extraction and reactions using either SC-CO₂ or subcritical water. Similarly, we have been able to alter bench scale extractor units using booster-type pumps, originally configured for SC-CO₂, to allow extractions and reactions to be conducted with subcritical water [68].

Laboratory investigations of potential multiple unit processing with critical fluids is frequently done by investigating one discrete process followed by another, and realizing the potential for arranging these in a tandem manner to achieve the desired end product. For example, based on several studies focused on the enrichment of a lecithin-phospholipid (PL)-containing fractions from seed oil extracts [69–72], we have postulated an entire process based on SC-CO₂ with and without co-solvent addition employing SFE followed by displacement supercritical fluid chromatography (SFC) [73] as shown in Fig. 10. Here soybean flakes are initially extracted with SC-CO₂ to remove the oil from flakes, followed by extraction of the PLs from the de-oiled flakes with a SC-CO₂/ethanol mixture. The second extraction step produces an extract enriched in PLs since PLs are not appreciably soluble in neat SC-CO₂, but can be selectively removed from the flake matrix with the aid of ethanol as a co-solvent. As shown in Table 3, the second SFE using SC-CO₂/ethanol produces an extract containing a total 43.7% by weight of PLs. This is a considerable enrichment relative to the concentration of the PLs in the starting oil or seed matrix. Further PL enrichment is facilitated as noted above, by transferring this extract enriched in PLs to an alumina preparative SFC column, where SC-CO₂ modified with a 5–30 vol% 9:1 ethanol: water eluent is used to elute and fractionate the PLs. In the case of the SFC enrichment step, eluent fractions can be collected as a function of time and their PL content quantitated. As indicated by the data given in Table 3, collection of discrete fractions during the SFC process can produce purities in excess of 75% for the individual PLs, phosphatidylinositol and phosphatidylethanolamin. It should be noted in the described process that in the SFC steps, only GRAS solvents are being used for the enrichment process. Recently, a similar SFE/SFC process has been suggested for isolating sterols and phytosterol esters from corn bran and fiber [74].

To justify the above process we have quantified the degree of PL enrichment in terms of the product selling price after each unit process. Therefore starting from crude soybean oil at US$ 25–35/lb we can obtain a PL-lecithin-enriched fraction after SFE using ethanol as a co-solvent worth US$ 31.00/lb, and subsequently with SFC produce pure phospholipid extracts that retail for US$ 1800/lb. Progress toward the above has been partially realized by the construction of commercial plants to produce deoiled-lecithin by Uhde and Thar Technologies.

The above process is a SFE–SFC binary unit processing scheme. We have on a lab scale also demonstrated the feasibility of a SFR–SFR sequential set of reactions to make fatty alcohol mixtures. The generation of fatty acid methyl esters (FAMES) in this case was based on studies involving the enzymatic synthesis of FAMES directly from vegetable oils dissolved in SC-CO₂ [75,76]. Combining this transesterification reaction with a hydrogenation reaction using consecutively coupled packed bed reactors allowed the production of FAMES in either a SC-CO₂ or SC-C₂H₄ stream followed by exhaustive hydrogenation of the FAMES to fatty alcohols as shown in Fig. 11a. In this process, a non-Cr catalyst was used to successfully convert the FAMES to the C₁₆ + C₁₈ saturated alcohols at 250 °C and 25 mol% H₂ in SC-CO₂. This is an excellent example of how a two-step synthesis process can be conducted in supercritical fluid media that is environmentally benign by permitting the reuse of the critical fluid media as well as the reaction by-product from the hydrogenation step, methanol (Fig. 11b).

It is impossible to separate in the multiple unit and fluid processing platform the role of fluid interchange with tandem unit processing. Toward this end, the choice of solute or substrate modification and/or fluid medium can enhance the opportunity to utilize the various combinations of fluids or unit processes. Two will be cited here: (1) formation of methyl esters of lipid-type solutes such as fatty acids (FAMES), and (2) use of water primary for hydrolysis of complex naturally occurring substrates. FAMES are an extremely versatile modification for the critical fluid processing of fats/oils and their oleo chemical derivatives. Aside from the formation of FAMES for conversion to biodiesel via enzymatic synthesis [77] or in sub- and supercritical methanol [78], FAMES or similar esters can be used advantageously in SFE [79], columnar modes of SFF [80], SFC [81], and as noted above in SFR. This versatility is due to one or more of the following factors relative to the non-methylated analogs: (1) enhancement of solute volatility or solubility, (2) improvement of separation factor (α), (3) intermediate formation for downstream synthesis, and analytically useful derivatives [15]. Formation of FAMES before utilizing multi-unit processing can allow easier SFF of fatty acids [82], selective SFE and SFR of fatty acids from tall oil [83] for subsequent conversion to biodiesel, and to fractionate soapstock [84] or deodorizer distillate [85]. For example, counter-current multistage processing of edible oils using critical fluids has been shown to be capable of producing fatty acid esters, tocopherols, squalene, sterols, and triglycerides. Approximately 70% of the fatty acid methyl esters in deodorizer distillates plus tocopherols and sterols can be extracted with SC-CO₂ [86]. Tocopherols

<table>
<thead>
<tr>
<th>Phospholipid type</th>
<th>SFE stage</th>
<th>SFC stage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phosphatidylethanolamine</td>
<td>16.1</td>
<td>74.9</td>
</tr>
<tr>
<td>Phosphatidylinositol</td>
<td>9.2</td>
<td>20.8</td>
</tr>
<tr>
<td>Phosphatic acid</td>
<td>2.8</td>
<td>55.8</td>
</tr>
<tr>
<td>Phosphatidylcholine</td>
<td>15.6</td>
<td>76.8</td>
</tr>
</tbody>
</table>

Table 3: Percentage amounts of PLs in extracts derived from SFE and SFC processing stages

* Relative to other eluting constituents (oil and unidentified peaks).

Fig. 11. Production of fatty alcohol mixtures using a (a) SFR–SFR sequential reaction scheme, (b) feedback of methanol into the SFR process and its inherent “greenness”.
Treatment of Biomass with Subcritical Water

**Biomass Fractions:**
- Oils/Fats – Lignins  →  Oil  →  Free Fatty Acids (FFA)
- Proteins  →  Hydrolyzates  →  Peptides  →  Amino Acids
- Carbohydrates  →  Hydrolyzates  →  Monomers (Glucose)

**Deoiled Biomass Fraction:**
- Proteins - Carbohydrate  →  Amino Acids, Organic Acids, Sugars

**Unfractionated Biomass:**
- Oils/Fat - Protein-Carbohydrate  →  Oil, FFA, Protein Hydrolyzate, Sugars
- Oils/Fat - Protein-Carbohydrate  →  FFA, Amino Acids, Sugars

Fig. 12. Treatment of various types of biomass and mixtures with subcritical water and the resultant products.

and sterols in the resultant extract can then be separated from the FAMES by columnar SFF by countercurrent fractioning using SC-CO2.

Illustrated in Fig. 12 are a number of potential sequences for applying subcritical water to pre-fractionated and unfractionated biomass substrates for the production of fuels and chemicals. Hydrolysis as illustrated can produce oils or lipids by subcritical water extraction followed by SFR to produce free fatty acids (FFA). Likewise, subcritical water on isolated protein fractions (perhaps by subcritical water extraction) can be used as a time-dependent reaction medium to produce protein hydrolyzates, an extended SFR to produce peptide mixtures, etc. If one takes unfractionated biomass mixtures, such as a deoiled-protein/carbohydrate fraction, and treats it using SFR with subcritical water, mixtures of amino acids, organic acids, and sugars results. While the resultant mixed hydrolyzate might not seem useful it is in actuality a seed mixture for the eventual fermentation production of biomethane, since the resultant hydrolyzate can be treated with methanogenic bacteria to produce a mixture of CO2 and CH4 [87].

4. Examples of integrated critical fluid processing

There are numerous examples of multi-fluid and -unit processing but they can be crudely classified into several generic groups. Processing using one critical fluid offers the opportunity to mix SFE, SFF and SFR processes as has already been amply illustrated. The most versatile combination seems to be using two fluids, namely SC-CO2 and pressurized water. Using subcritical water, Smith et al. [88] have shown that the “green” processing of cashew nut shells is possible; SC-CO2 being used for the recovery of the cashew nut shell oil containing the pharmacological-active alkyl phenolic compounds. The cashew nut shells were initially treated with carbon dioxide at 40 MPa and 100 °C resulted in removal of the essential oil and partial hydrolysis steps to accelerate to remove 95% of the cashew nut shell liquid. After SC-CO2 extraction, the residual shell biomass can be rapidly dissolved (5 min) in pressurized high temperature water (410 °C) suggesting that hydrolytic-based gasification of remaining shell material would be suitable for fuel purposes.

Numerous research groups are now practicing tandem SC-CO2–subcritical water extraction using different approaches. “Hybrid” SFE with CO2 and water has been applied to a wide variety of natural product matrices [89] such as the separation of pesticides from ginseng using an upward flow of CO2 while ginseng extracts are concentrated in a downward flow of water using the same SFE vessel. Wai and coworkers [90] have sequentially applied SC-CO2 and then subcritical water for fractionating bioactive components from St. John’s wort. The active components hyperforin and adhyperforin were partitioned into CO2 at 30 °C and 8 MPa resulting in 93–99% recoveries, while hypercin and pseudohypercin were subsequently recovered via pressurized water extraction at neutral pH at 80–93% yield. Similarly, the potential and problems of sequential extraction of the South American plant, boldo, first using SC-CO2 with and without ethanolic co-solvent, and then batch-pressurized water extraction were reported by del Valle et al. [91]. The extraction selectivity for the plant essential oil and the boldine alkaloid paralleled their relative solubility in non-polar SC-CO2 and enhancement in the solubility of more polar solutes in CO2 + ethanol mixtures as well as in subcritical water. This study and others involving polyphenolic target solutes, indicate that extractions with subcritical liquids are effective up to a certain extraction temperature, but beyond this temperature, pressurized solvent processing can lead to solute degradation or molecular transformation [92–94]. In the latter cases, this reactivity of the solute in the subcritical liquid maybe viewed as desirable since the free radical scavenging capacity of the resultant extracts increase. An example of this trend was reported by the Ibanez group in Spain [95,96] for the extraction of rosemary by either SC-CO2 or subcritical water. Carbon dioxide fractions collected at 10–40 MPa and 40–60 °C resulted in removal of the essential oil and partial fractionation of the antioxidants from the rosemary matrix, while subcritical water favored extraction of the more polar bioactive compounds such as carnosic acid or resultant fractions having high antioxidant capacity.

The above typical results of employing CO2 extraction in tandem with pressurized liquid fluids suggest an interesting option and current trend in employing this mixed pressurized fluid matrix as both extraction and reaction media. As noted previously, the incorporation of pressurized CO2 into subcritical water, i.e., a gas-expanded liquid, makes for an interesting extraction and reaction medium. The Meireles group in Brazil [97,98] have utilized this principle in the processing of ginger bagasse both as a pre-treatment step and to degrade bagasse to sugars for potential fermentation. Pretreatment with SC-CO2 seemed to yield somewhat ambiguous results since the authors state that non-treated bagasse was hydrolyzed more effectively then SC2O pretreated bagasse [99]; the latter process was hypothesized to degrade the oleoresinous materials in the bagasse matrix. Their results for matrix pretreatment with SC-CO2 are in stark contrast with other reports in the literature that indicate SC-CO2 pretreatment is an effective procedure preceding biomass degradation [100]. It should be noted that the above results are somewhat different then using the previously mentioned carbonated water to hydrolyze carbohydrate polymers; also SC-CO2 is effective in removing high value components from the biomass matrix prior to hydrolyzing the biomass matrix [99]. Since reported SC-CO2 pretreatment methods exist and carbonated water hydrolysis has been shown by us [101] and others [102] to be an effective hydrolysis medium, the above ambiguity may be due to the varying recalcitrance of the target biomass matrix to hydrolytic degradation. The hydrolytic action patterns of carbonated water can vary quite significantly depending on the matrix being hydrolyzed although the hydrolysis temperature and residence time can be varied to produce optimal depolymerization of the carbohydrate polymers inherent in the biomass.

One aspect of our current research focuses on the application of critical fluids for processing grapes and grape by-products and similar natural antioxidant-containing matrices. These matrices and target solutes are a fruitful area in which to apply combinations
of mixed critical fluid and unit processing steps. One of the seminal questions is whether SC-CO₂ and co-solvent combinations or a hot pressurized fluid such as water or ethanol – or combinations thereof – are most appropriate for extracting and fractionating the targeted solutes. There is a considerable literature in the application of SC-CO₂ for extracting grape seed oil [103,104] as well as further fractionating the extract to enrich certain polyphenolic constituents. Recovery of solutes such as gallic acid, catechin, epicatechin, etc. via a SC-CO₂-based method almost always require the use of methanol or ethanol as co-solvents [105].

Other studies have utilized subcritical water to extract procyanidin compounds and catechins from grape processing wastes [106]. Extractions conducted at approximately 10 MPa and in the temperature range of 50–150 °C were adequate to recover and fractionate gallic acid, procyanidin dimers, and the corresponding oligomers from the grape pomace using an analytical scale pressurized fluid extractor (ASE). Aside from water and hydroethanolic pressurized fluid extraction, sulfured water has also been proven effective for the extraction of anthocyanins and procyanidins from grape pomace [94]. This parallels similar work by the senior author in processing berry substrates using both ASE and a batch continuous subcritical water extractor. As noted in the patent issuance on this process [107], residence time of the extracted solute in the hot pressurized water must be minimized to prevent degradation of the anthocyanin moieties or their possible reaction with sugars to other products.

It is unknown at this time whether such side reactions in pressurized water could be generating antioxidant moieties, but the potential ability to control the ratio of polyphenolic stereoisomers and to depolymerize or repolymerize biologically active antioxidant oligomers in pressurized fluid media could be a significant area for future research—particularly if they come from cheap and renewable natural resources [8].

In concluding this section it is worth considering far more ranging applications of the critical fluid technology platform that embrace the multi-fluid and -unit processing as discussed above for the industrial production of chemicals, fuels, etc. The authors have previously noted the considerable potential for applying critical fluids in biorefineries, etc. [7] but several existing examples are worth citing. Kusdiana and Saka [108] have demonstrated a two-step process for the production of biodiesel (see Fig. 13a) based on the Saka—supercritical methanol process (Fig. 13a) for converting both fats/oils and free fatty acids to biodiesel. The Saka-Dadan process uses subcritical water in front of the Saka process for the hydrolysis of fats/oils to free fatty acids followed by a more benign supercritical methanolysis of the resultant free fatty acids to FAMES. A pilot scale unit of this process is in operation in Fuji City, Japan. This overall biodiesel production platform is an excellent example of having a critical fluid-based SFR–SFR integrated process.

More recently Brunner and colleagues [109,110] have demonstrated an integrated critical fluid-based process for the production of several chemicals from rice bran. Although studied in discrete steps, the foresight of this effort towards developing an integrated critical fluid processing scheme is impressive. Rice bran is initially deoiled using SC-CO₂ at the appropriate extraction conditions resulting in defatted rice bran. This substrate is then subjected to subcritical water hydrolysis at approximately 250 °C to partially pretreat and depolymerize the rice carbohydrate lignocellulosic polymers to lower oligomers. Final conversion of the subcritical water hydrolyzate to glucose and xylose is accomplished with a mixed enzyme cocktail for subsequent fermentation conversion to bioethanol. The resultant 5–10% aqueous ethanol mixture from fermentation is then multi-staged contacted counter currently with SC-CO₂ to yield 99.8% pure ethanol. It is envisioned that non-fermentable residues left over from the hydrolysis process could be further treated with the aid of sub- and supercritical fluids to produce biogas.

Baig et al. [111] have recently reported on the combined critical fluid treatment of sunflower oil to yield value-added substances as well as a model for the “critical fluid biorefinery”. This concept can be achieved by coupling two or more reaction processes into one continuous flow system; namely the subcritical water hydrolysis of sunflower oil triglycerides to free fatty acids followed by esterification of the free fatty acids to FAMES in SC-CO₂ using lipase catalysis. The subcritical water extractor conditions were maintained at 250–390 °C with pressures as high as 10–20 MPa using oil:water ratios of 50:50 and 80:20 (v/v). The supercritical fluid enzymatic-based esterification process was operated at temperatures 40–60 °C using a Novozyme enzyme catalyst. The subcritical water studies indicated a high rate of conversion at higher temperatures (330 °C) followed by possible degradation of the free fatty acids when exposed to longer residence times. A yield of approximately 90% hydrolyzed free fatty acids was achieved in 25 min at 330 °C or for 45 min at 310 °C. The esterification process yielded between 60 and 70% FAMES at a pressure of 20 MPa and 60 °C with low enzyme concentrations.

5. Concluding remarks

In this document we have attempted to offer our perspective of the current state of integrated critical fluid processing. It is the author’s opinion that a positive future exists for combining our existing knowledge and studies involving discrete fluids and
unit processes done under critical fluid conditions. This will partly require “thinking outside the box” in terms of how industrial high pressure processing facilities are constructed and by realizing that the costs inherent in the core pumping, liquefaction, and compression facility can serve more than just one purpose, i.e. SFE, and can be applied with more than just one critical fluid medium.

Over the past twenty years the senior author has on more than one occasion been queried on the possibility of constructing a SFE plant in close proximity to an alcoholic fermentation facility that produces high purity CO2 as a by-product. This would seem logical since the opportunity to apply SFE for vegetable or specialty oil extraction could be facilitated with this source of CO2 as well as any of the above mentioned CO2-based unit processes. The production also of ethanol at such a site facilitates a preferred co-solvent for coupling with CO2 as documented previously. Today in the renewable bioenergy field it is envisioned to build coexisting bioethanol and biodiesel production capabilities at the same site. Hence based on our discussion above, this suggests the possibility of extending the application of critical fluids platform for the production of these two renewable fuels as documented above. Similarly, it was noted above that SC-CO2 could be mixed advantageously with pressurized water for extraction and reaction chemistry.

Fig. 14 shows a hypothetical process flow diagram for an integrated carbonated water pretreatment process for bioethanol production. Here the process flow diagram illustrates not only the sequence of operations associated with a modified carbonated water pretreatment—bioethanol production plant, but also the elimination of several processing steps required in current bioethanol production practice. Also illustrated are the feedback loops from the CO2 production from the fermentor as well as decompression of CO2 after the carbonated pretreatment stage. Note that the CO2 can be recovered and fed back to the CO2 storage vessels, then recombined with water and pressurized to provide a high pressure aqueous medium for pretreatment and hydrolysis. The pictured process flow diagram is similar to that reported for current bioethanol production however the addition of acid for hydrolysis has been eliminated as well as the corresponding neutralization agent and the attendant use of gypsum as a neutralizing agent. Note that in Fig. 14, lignin is separated and used as boiler fuel. However it should be possible to treat lignin at higher pressures and temperatures with sub- or supercritical fluid water reaction to also produce valuable carbochemicals [7]. Such a critical fluid-based processing concept supports the use of renewable resources, a sustainability platform, and does so in a “green” environmentally benign manner.

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References


J.W. King, R. Srinivas, J. of Supercritical Fluids 47 (2009) 598–610


