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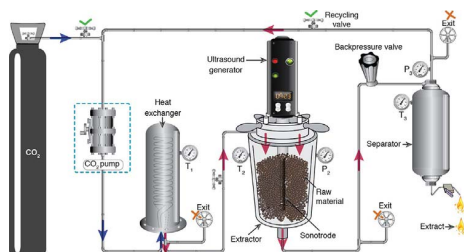
# Recovery of valuable components and inactivating microorganisms in the agro-food industry with ultrasound-assisted supercritical fluid technology

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## GRAPHICAL ABSTRACT



## ABSTRACT

Food industry is increasingly interested in replacing conventional processes of plant extraction, as well as microbial inactivation, by alternative, greener and cheaper techniques. In many cases, chemical extraction processes are hindered by several drawbacks such as the use of chemical solvents, which is associated with many health and environmental issues. Furthermore, the use of high temperatures to improve and/or accelerate the processes, which may affect and degrade the thermolabile compounds, is usually required. Among the most promising techniques that could be competitive to the current extraction methods of molecules from plant matrices and microbial inactivation of food products, ultrasound assisted supercritical fluid extraction has taken great interest. This non-exhaustive review, covering the last 20 years, gives a critical commentary on the main published results using this emerging technology. Promising prospects opened up by new applications are described, without omitting the main limitations still to be overcome for a harmonious industrial development of these technologies.

## 1. Introduction

Traditionally, the recovery of valuable components (e.g., polyphenols, sterols, colorants, seed oils, etc.) from plant matrices is mainly based on pressing and/or liquid solid extraction (e.g., maceration, percolation, decoction, infusion, etc.), which consists of using hot water and/or organic solvents such as hexane, ethanol, etc. However, although reaching high extraction yields in some cases, undesirable

consequences such as quality deterioration and degradation of valuable compounds may occur [1,2]. For instance, oil extraction from canola seeds is industrially based on several pre processing steps followed by pressing, expelling most of the oil but also giving meal with  $\approx 20\%$  wt oil, which is then extracted meaning percolation with hexane. The recovered oil is then refined via a sequential process including i) degumming, ii) acidification and neutralisation, iii) washing, iv) bleaching, v) winterization, and vi) deodorisation. Besides the use of

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toxic solvent (hexane) that is associated with health, environment, and economic issues, 1) the whole process requires high energy consumption (approximately 40 and 10 kWh/ton of canola seed processed, for oil extraction and oil refining, respectively) [3], and 2) oil recovered by hexane is of poor quality due to the degradation of thermolabile compounds, and the low solubility of high added value compounds (i.e. polyphenols) in hexane. This example and others have incited both researchers and food industries to investigate and evaluate novel extraction or valorization processes that meet the requirements of a green process concept. This concept aims to avoid/minimize the use of organic solvents, along with other benefits such as shortening the treatment time, decreasing the processing temperature, intensifying the mass transfer process, increasing the extraction yields, preserving high extract quality, and reducing the energy consumption [4,5]. These novel extraction processes have been classified as emerging, alternatives, innovative, or non conventional technologies and are including a wide range of techniques (e.g., ultrasound assisted extraction, supercritical fluid extraction, microwave assisted extraction, high pressure processing, pulsed electric fields, high voltage electrical discharges, gas assisted mechanical expression, etc.) [6-8]. Besides the features cited above dealing with the green extraction concept, some of the non conventional technologies allow pasteurising or sterilising the extraction medium and provide safer products by inactivating the microbial burden. For instance, it was widely known that high pressure processing [8-10], pulsed electric fields [11-13], ultrasounds [14], supercritical fluids [15], and other technologies are able to inactivate contaminating microorganisms including in some cases viruses [16,17]. This ability allows further reducing the heating steps (e.g., for sterilization, pasteurization), which minimizes the energy consumption and preserves the most thermolabile substances from degradation. Combining simultaneously a conventional process with a non conventional one for further efficiency has been investigated through different research works. For instance, gas assisted mechanical expression (GAME) involving the combination of mechanical expression and supercritical fluid extraction has been investigated, and demonstrated interesting results compared to individually applied processes [18-20]. Other works have been focused on combining simultaneously two non conventional extraction processes, such as the use of ultrasound technology assisted by supercritical carbon dioxide (sc CO<sub>2</sub>) as solvent. This technique called ultrasound or ultrasonic assisted supercritical fluid extraction (US SFE) was successfully applied to extract seed oils, antioxidants and high added value compounds, as well as to inactivate microorganisms. In fact, the use of sc CO<sub>2</sub> as solvent instead of other organic solvents (e.g., hexane) contributes to limit and minimize the pollution hazards, although in the case of polar molecules extraction, adding co solvents to CO<sub>2</sub> (i.e., ethanol) could be required. Ultrasounds, on the other hand, allow the extraction of both polar and non polar molecules. Therefore, combining these two technologies is a key point to further enhance the mass transfer of targeted compounds from solid matrix to sc CO<sub>2</sub> [21]. This review is thus devoted to discuss for the first time 1) the current insights into the use of US SFE as emerging technology for the recovery of intracellular components from plant matrices, and its potential to efficiently inactivate microorganisms, in this latter case the process will be noted US SFI and 2) the limits to be overcome towards the industrialization of this technology.

## 2. Current state of ultrasound assisted supercritical fluid extraction (US-SFE)

Ultrasound assisted extraction (UAE) is an advantageous alternative to conventional extraction techniques (liquid/liquid extraction (LLE), solid phase extraction (SPE), and Soxhlet). When a large negative pressure is applied to a liquid, sonochemical phenomenon associated with acoustic cavitation and the formation of micro bubbles occurs (Fig. 1), constituting thus the base of UAE [22]. When growing to unstable size, the created bubbles collapse violently, which is associated

with the release of an intense local energy with important chemical (i.e., free radicals) and mechanical effects (i.e., micro jets) [23]. When bubble collapse phenomenon occurs, increased temperatures and pressures to several thousand degrees and more than 1000 atmospheres, respectively, are generated. Many parameters are affecting the extraction efficiency, including the effects of solution, power, frequency, sonication time, and the type of treated material. These parameters were recently reviewed [24].

UAE is a promising technique, with various benefits, that was widely applied to extract valuable compounds from plant matrices [25]. UAE is harmless for operators, safe to use, and with low maintenance costs. Therefore, it has been acquired by several industries to replace the conventional extraction techniques (i.e., LLE...), as it could be operated rapidly in a broad range of solvents for large scale preparations suited for industrial purposes [26,27]. For instance, industrial scale up extraction of polyphenols from apple pomace was reported in the literature, demonstrating relevance and more sustainable technology to current conventional methods [28,29].

UAE is usually using liquid solvents (e.g., water, ethanol, methanol, DMSO, etc.) [30-33]. However, during the last decade, the use of supercritical fluids as solvent for UAE experiments has drawn more attention [21,34-36]. The use of supercritical fluids instead of liquid solvents allows obtaining pure extract without subsequent removal of the extracting solvent, and often enhances the extraction yield due to the supplementary effect of pressure on the solute solubility. The most widely supercritical fluid used is carbon dioxide (sc CO<sub>2</sub>). This fluid takes its advantages from the relatively low critical point of CO<sub>2</sub> (critical temperature 304.2 K; critical pressure 7.38 MPa), making it suitable to extract thermo sensitive compounds [37]. Furthermore, the non flammability, non toxicity, low cost, and availability at good purity, make it preferred supercritical fluid for industrial applications. Generally, sc CO<sub>2</sub> is used to extract hydrophobic or slightly hydrophilic compounds. However, co solvents or modifiers (e.g., ethanol, methanol, ethyl acetate, etc.) could be added when the extraction of more hydrophilic compounds is targeted. Many industrial applications for the extraction of valuable compounds using sc CO<sub>2</sub> currently exist [38]. Among them the extraction of nicotine from tobacco as well as caffeine from coffee grains and tea leaves are the most commonly known [39].

The use of pressurized water (either sub or supercritical) has also been reported for the extraction of several molecules from plants (e.g., phenolic and antioxidant compounds [38,40]). It can also be used to produce biofuels from agri food residues or non food plants [41,42].

### 2.1. Extraction of oils and fats using US SFE

Mechanical expression and/or hexane extraction is nowadays the most commonly used process to extract oil from oilseeds (soybean, canola, sunflower, cotton, linseed, etc.). At industrial or semi industrial scales, these processes could be combined (i.e., continuous mechanical pressing (expelling) with continuous solvent extraction, and batch hydraulic pressing followed by solvent extraction) [43,44]. After total defatting, the resulting meal is generally used for animal feed or aquaculture production [45]. The need to minimize the use of toxic hexane for oil extraction has led both food scientists and food industry to develop and evaluate novel "clean" extraction processes using alternative solvents with the numerous advantages cited above. As alternative to conventional processes based on mechanical expression and/or hexane extraction of vegetable oils, US SFE was evaluated. Fig. 2 shows a schematic illustration of US SFE equipment used at laboratory scale for the extraction of intracellular compounds.

Many works have been cited in the literature to discuss the feasibility of using US SFE for vegetable oil extraction, and some of them are summarized in Table 1.

As shown in Table 1, different vegetable materials were used for investigation such as particle almonds [35], passion fruit (*Passiflora edulis* sp.) seeds [47], etc. The effects of operating conditions

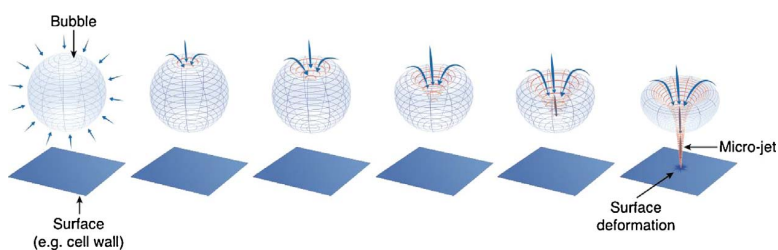


Fig. 1. Creation of a micro-jet through collapsing acoustic cavitation bubbles.

(temperature, pressure, time, CO<sub>2</sub> flow rate, frequency and US power) on extraction efficiency and oil quality were studied. The optimal conditions were determined and the results were compared to those obtained with conventional extraction methods. Among others, these works have shown the efficiency of US SFE process to replace conventional processes.

Therefore, scaling up becomes a major concern and a research work investigated this opportunity by implementing a high power ultrasonic coupled with sc CO<sub>2</sub> extraction [48]. The authors developed and validated an innovative, robust, stable, reliable and efficient ultrasonic system at pilot scale to assist sc CO<sub>2</sub> extraction of oils from different matrices (Fig. 3).

It was shown that ultrasonic energy used in the extraction process represents an efficient way to produce deep agitation in the medium, which enhances the mass transfer processes, due to the presence of some mechanisms (e.g., radiation pressure, streaming, agitation, high amplitude vibrations, etc.). Equipment was designed to fulfill the industrial requirements and was successfully validated for SFE of oil from different vegetable matrices. However, further reading in ultrasonic reactor design could be found in the review published by Luo et al. [49]. It was reported that many parameters must be considered in the design of ultrasonic reactors such as reactor type (e.g., bath, probe, flat plate or tube), reactor geometry, transducer design and arrangement, and volume or scale of feedstocks for practical systems. The authors presented and compared different sonochemical reactors (Liquid whistle, Ultrasonic bath, Probe reactor, Cup horn reactor, Resonating tubular reactor (Sonitube), Reverberative flow reactor and Polygonal Reactor) that are commonly used in ultrasound assisted biomass reactions. The advantages, disadvantages, applications and the possibilities of scale up were presented and discussed.

## 2.2. Extraction of high added value compounds using US SFE

The process of US SFE was not only applied to extract oil from plant

matrices but also tested and evaluated to extract high added value compounds as summarized in Table 2.

US SFE process has been used for the extraction of many molecules from different vegetable matrices: gingerols from ginger, lutein from marigold, caffeine from green tea, triterpenic acids (oleanolic and ursolic acids) from *Scutellaria barbata*, *Hedyotis diffusa* and *Hedyotis corymbosa*, antioxidant compounds from blackberry (*Rubus sp.*) and ba gasse (industrial by product), capsaicinoids from malagueta pepper (*Capsicum frutescens* L., etc.). These studies were performed mainly at the laboratory scale using batch equipment. Most of these studies showed increased extraction rate and yield with US assistance. The authors ascribed this behaviour to the impact of US in disrupting the cell structures, which increases the accessibility of the solvent to the intracellular compartments, and enhances the intra particle diffusivity. It was shown that the optimal operating conditions depend on the target molecule and the properties of the used vegetable material (particle size, moisture content, etc.). Scanning Electron Microscopy images showed that the plant matrix was highly disturbed by the presence of ultrasound, which could be the origin of enhanced release of extractable materials. The effects of ultrasound on SFE were more pronounced from larger particles.

## 3. Current state of inactivation of microorganisms using ultrasound-assisted supercritical fluid (US-SFI) technology

In addition to the extraction of valuable substances from plant matrices, the inactivation of microorganisms is another important domain of application of coupling ultrasound and supercritical fluids. There are numerous reports demonstrating that pressurized gases are effective against living microorganisms as is acoustic cavitation generated by ultrasound.

Food products are inherently perishable; bacteria, molds and yeasts being responsible for most of the food spoilage. Foodborne diseases cause the illness of several millions of people around the world. In

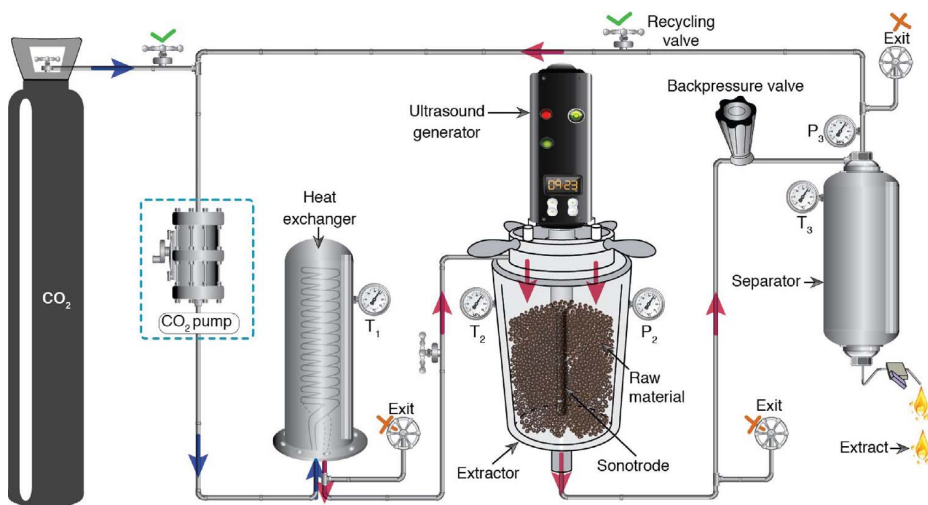


Fig. 2. Schematic representation of US-SFE experimental set-up used at laboratory scale for the extraction of oil from seeds. T, P: temperature and pressure sensors, respectively.

**Table 1**  
Application of ultrasound-assisted supercritical fluid technology for the recovery of vegetable oil.

Description of the study	Process conditions	Major findings	References
Extraction of oil from particulate almonds	Pressure of 28 MPa; flow rate of 20 kg/h, temperature of 55 °C, and US power of 50 W ( $\approx$ 20 kHz)	Enhancement of the extraction kinetics and yields with an average extraction time of 8.5 h	[35]
Extraction of oil and coixenolide from adlay seeds ( <i>Coix lachrymal-jobi</i> L. var. Adlay)	Temperature of 40 °C, Pressure of 20 MPa, CO <sub>2</sub> flow rate of 3.0 L/h, and extraction duration of 3.5 h	Enhancing the extraction yield by 14%.	[34]
Oil extraction from cumbaru almonds ( <i>Dipteryx alata</i> Vogel)	Optimal conditions of temperature at 50 °C, and pressure at 35 MPa,	Higher initial rate of extraction but no effect in the final global oil extraction yield	[46]
Oil extraction oil from passion fruit ( <i>Passiflora edulis</i> sp.) seeds	Temperature at 40 °C and pressure at 16 MPa. US power at 160 W	Increase of the oil extraction yield by 29%.	[47]

industrialized countries, up to 30% of the population suffers each year from foodborne diseases [55]. Some foodborne diseases, although well studied and identified, are considered as emergent because of their common occurrence. Among them, *Campylobacter* spp., *Salmonella* spp., *Listeria* spp., and *Escherichia coli* have been identified as the major causes for foodborne illness [56–58].

The main traditional inactivation processes in food industries include heat pasteurization and sterilization. These methods are very effective, if correctly designed and applied, in inactivating pathogenic microorganisms and ensuring shelf stable products. However, due to the use of high temperatures, the most thermo sensitive compounds present in the treated food could be affected and lead to undesirable textural and sensory changes in the final product. In order to avoid these issues and to reduce the high energy consumed during the conventional processes (pasteurization and sterilization), numerous non conventional technologies (e.g., high pressure processing, pulsed electric fields, etc.) have been evaluated and developed.

It has been demonstrated that pressurized gases (CO<sub>2</sub>, N<sub>2</sub>O, propane, etc.) have a bactericidal effect. In his review, Perrut [16] points out that the use of CO<sub>2</sub> at the relatively low pressure of 0.6 MPa could divide the time of heat pasteurization by two. This effect is different from the simple hydrostatic pressure effect since it can be obtained at much lower pressures. It involves specific interactions between the microbial cells – and especially the cytoplasmic membrane – and the gas molecules. Both gas dissolution inside the cell and specific decay of the double layer of phospholipidic membranes have been cited as possible mechanisms. Spilimbergo and co workers have published a series of papers demonstrating the possible effect of sc CO<sub>2</sub> on the survival of bacterial populations [59–61].

To enhance the effect of carbon dioxide, several techniques have been proposed such as

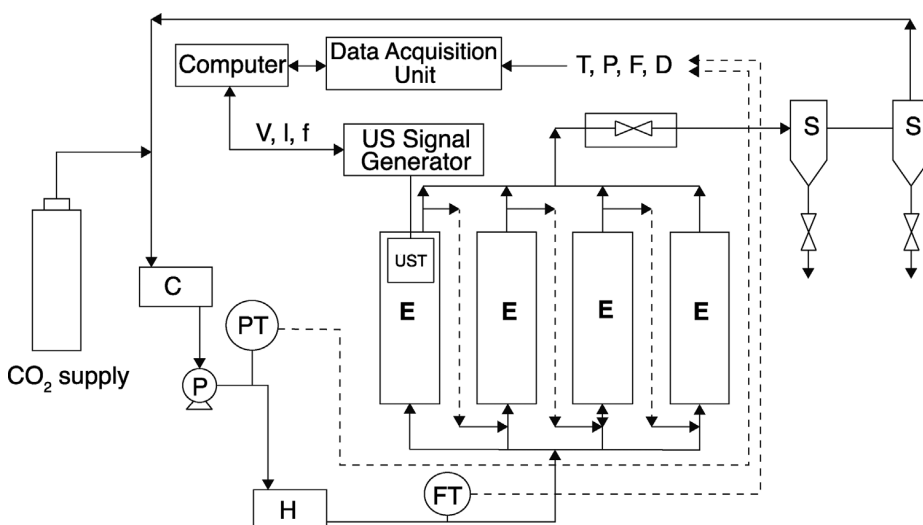
- 1) the application of successive cycles of pressurization/

depressurization to promote cell lysis, 2) the addition of strong oxidants such as hydrogen peroxide or peracetic acid, and 3) the use of pulsed electric fields combined with CO<sub>2</sub> treatment.

In this review, we focus on the coupling of ultrasound technology with sc CO<sub>2</sub>, for the inactivation (US SFI) of the most common food borne pathogens (Table 3) described above.

Ultrasound provokes acoustic cavitation with the periodic formation of micro bubbles followed by their violent collapse that are responsible for damages caused to cell walls and membranes of bacteria or yeasts leading to their inactivation.

The impact of sc CO<sub>2</sub> assisted by ultrasound was evaluated to inactivate microorganisms (*E. coli* and *S. cerevisiae*) in liquid and solid matrices [62]. In this work, it has been reported that using only sc CO<sub>2</sub>, without ultrasound assistance, leads to the complete inactivation (7–8 log cycles) of *E. coli* and *S. cerevisiae* after 25 and 140 min of CO<sub>2</sub> (35 MPa, 36 °C) treatment, respectively, which is considered relatively long and energy consuming. However, combining both processes of sc CO<sub>2</sub> and ultrasound was efficient to reduce this time to 1–2 min for culture media and  $\approx$  4.9 min for juices (Fig. 4). On the other hand, for solid samples (chicken, turkey ham and dry cured pork cured ham), it has been reported that the inactivation rate of *E. coli* increased with temperature. Moreover, this rate was accelerated when using US SFI, and was more pronounced in treatments with isotonic solution surrounding the solid food samples. The authors ascribed this result to the use of ultrasound that enhances the inactivation process by sc CO<sub>2</sub>. In fact, ultrasound process results in vigorous agitation that facilitated the solubilization of CO<sub>2</sub> and enhanced the mass transfer process. In addition, the cavitation generated by ultrasound is able to cause substantial damage to the cell walls, which accelerates the extraction of vital constituents and leads to microbial death. The authors concluded that this combined process represents a great potential for reasonable alternative method at industrial scale that could result in the reduction of



**Fig. 3.** Scheme of the SFE pilot plant assisted by power ultrasound. Units: (E) extractors, (S) separators, (C) cooler, (P) high pressure pump, (H) heater, (PT) pressure meter, (FT) flow meter, (UST) ultrasonic transducer. Electrical parameters: (V) voltage, (I) current, (f) frequency. Extraction parameters: (T) temperature, (P) pressure, (F) CO<sub>2</sub> flow rate, (D) density. Reprinted from [48] with permission.

**Table 2**  
Application of ultrasound-assisted supercritical fluid technology for the recovery of bioactive compounds.

Description of the study	Process conditions	Major findings	References
Extraction of curcumin from turmeric ( <i>Curcuma longa</i> L.)	Temperature of 50 °C, pressure of 25 MPa, extraction time of 90 min, CO <sub>2</sub> flow rate of 3 mL/min with 10% cosolvent	Higher curcumin content in extraction yield and shorter extraction time	[76]
Extraction of pungent compounds from a typical herb (ginger)	Pressure of 160 bar, temperature of 40 °C, US power of 500 W (20 kHz)	Extraction rate and yield were increased with US assistance.	[21]
Lutein extraction from marigold ( <i>Tagetes erecta</i> L.)	Particle size of 0.245-0.350 mm, pressure of 32.5 MPa, temperature of 55 °C, CO <sub>2</sub> flow rate of 10 kg/h, ultrasonic power of 400 W, ultrasonic frequency of 25 kHz and ultrasonic irradiation time/interval of 6/9 s.	The obtained yield of lutein esters was 690 mg/100 g.	[50]
Extraction and removal of caffeine from green tea.	Pressure of 30 MPa, temperature of 55 °C, extraction duration of 4 h, moisture content of 30%, and ultrasound power of 100 W	The removal of caffeine from green tea without damaging the structure of the active ingredients.	[51]
Extraction of oleanolic acid and ursolic acid from <i>Scutellaria barbata</i> D. Don.	Particle size of 0.355 mm, pressure of 27.6 MPa, temperature of 55 °C, CO <sub>2</sub> flow rate of 2.1 mL/min, extraction duration of 50 min and 14.1% (v/v) aqueous ethanol (ethanol/water = 80/20, v/v) as modifier.	Similar extraction yield as for heat reflux extraction (HRE). However, the extraction time was reduced from 180 min to 50 min, for HRE and US-SFE, respectively.	[52]
Extraction of triterpenic acids (oleanolic and ursolic acids) from <i>Hedyotis diffusa</i> and <i>Hedyotis corymbosa</i> .	Extraction pressure at 10.4-28.0 MPa, extraction temperature at 30-70 °C, extraction time of 10-140 min, mean particle size at 0.096-0.925 mm and CO <sub>2</sub> flow rate at 0.9-2.3 mL/min. US power of 185 W (40 kHz) and duty cycle of 79%.	Better extraction yields, reducing the extraction time, the solvent consumption and allowing milder operating conditions.	[36]
Extraction of antioxidant compounds from blackberry ( <i>Rubus sp.</i> ) bagasse (industrial by-product).	Temperature (40, 50 and 60 °C), pressure (15, 20 and 25 MPa), and ultrasound power (0, 200 and 400 W).	Extraction rate increased at the beginning of the process. Higher antioxidant activity and phenolic contents were obtained with higher temperatures.	[53]
Extraction of capsaicinoids from malagueta pepper ( <i>Capsicum frutescens</i> L.).	Pressure of 15 MPa, temperature of 40 °C, US power of 360 W and extraction duration of 60 min	Increasing the extraction yield from 58% to 77%.	[54]

**Table 3**  
Application of ultrasound-assisted supercritical fluid technology for the inactivation of microorganisms.

Description of the study	Process conditions	Major findings	References
Inactivation of <i>E. coli</i> and <i>S. cerevisiae</i> in liquid and solid matrices.	Pressure of 35 MPa and temperature of 36 °C	Combining both processes of sc-CO <sub>2</sub> and ultrasound was efficient to reduce the inactivation time from up to 140 min to 1-2 min for culture media and ≈ 4.9 min for juices	[62]
Inactivation of <i>E. coli</i> in nutrient broth, apple and orange juices.	Pressures of 10, 22.5, 29 and 35 MPa; 36 °C, and temperatures of 31 °C, 36 °C and 41 °C; 22.5 MPa.	When combining ultrasound with sc-CO <sub>2</sub> , the time needed to reach the same inactivation level (than sc-CO <sub>2</sub> alone) was reduced by 95%.	[63]
Inactivation of <i>E. coli</i> , <i>S. cerevisiae</i> and pectin methylesterase (PME) in orange juice.	Temperature of 31-41 °C; 22.5 MPa and pressure of 10-35 MPa; 36 °C.	The inactivation rate of <i>E. coli</i> , <i>S. cerevisiae</i> and PME increased proportionally to pressure and temperature during treatments.	[64]
Inactivation of <i>E. coli</i> and <i>S. cerevisiae</i> in apple juice.	Pressures of 10-35 MPa at 36 °C and temperatures of 31-41 °C at 22.5 MPa.	Shorter treatment times were needed to reach a total inactivation of <i>S. cerevisiae</i> (2-6 min) than that of <i>E. coli</i> (7 min).	[65]
Pasteurization of fresh-cut coconut spiked with <i>Salmonella enterica</i> Typhimurium.	Pressure of 12 MPa and US power of 10 W, delivered every 2 min of treatment, as a function of temperature (from 24 to 50 °C) and treatment time (from 5 up to 30 min).	Microbial inactivation was more efficient using US-SFI, compared to CO <sub>2</sub> applied alone.	[67]
Inactivation of <i>L. monocytogenes</i> in dry cured ham.	Pressures (6, 8 and 12 MPa) of CO <sub>2</sub> , temperatures (22, 35, 45 °C), and treatment times (0.5-30 min).	The initial microbial load of <i>L. monocytogenes</i> (10 <sup>9</sup> CFU/g) was reduced below the limit of detection.	[75]

cost, with minimal nutritional and organoleptic changes. The damages generated by US SFI treatment were strong enough to avoid a possible regrowth of cells during post treatment storage during 6 weeks at 4 °C.

Similar study was conducted for the inactivation of *E. coli* in nutrient broth, apple and orange juices [63]. Different combinations of pressures and temperatures (see Table 3) were investigated. Results demonstrated that using sc CO<sub>2</sub> individually without ultrasound assistance for microbial inactivation in LB broth was proportionally increasing to the applied pressure or temperature. The average time needed to achieve a reduction of 8 log cycles was 50 min. However, when combining ultrasound with sc CO<sub>2</sub>, the time needed to reach the same inactivation level was reduced by 95%, with non significant effect of pressure and temperature; this reduction being achieved after only 2 min at 31 °C and 22.5 MPa. The authors concluded that US SFI allowed reducing the treatment time, regardless of the treatment medium, which prevents the inhibitory effect on the sc CO<sub>2</sub> inactivation, generated by the juice solutes.

The same research group investigated the feasibility of using US SFI for the inactivation of *E. coli*, *S. cerevisiae* and pectin methylesterase

(PME) in orange juice [64]. The idea was to propose a model that can predict the inactivation behaviour based on the process conditions. Different parameters were studied including temperature and pressure. Results showed that the inactivation rate of *E. coli*, *S. cerevisiae* and PME increased proportionally to pressure and temperature during treatments. The biphasic, Peleg type B, and the fractional models were respectively used to propose models for temperature, pressure and treatment time as variables. The authors concluded that the batch system of the combined US SFI process allows the use of mild process conditions and shorter treatment times than those of continuous sc CO<sub>2</sub> systems.

Similar work was performed for the inactivation of *E. coli* and *S. cerevisiae* in apple juice [65]. In this work, different pressures and temperatures were studied (Table 3). Results demonstrated that in general, shorter treatment times were needed to reach a total inactivation of *S. cerevisiae* (2-6 min) than that of *E. coli* (7 min). It has been also reported that, when considering temperature and pressure as model parameters, the inactivation kinetics of *E. coli* and *S. cerevisiae* were satisfactorily described by the Peleg type A and the Weibull

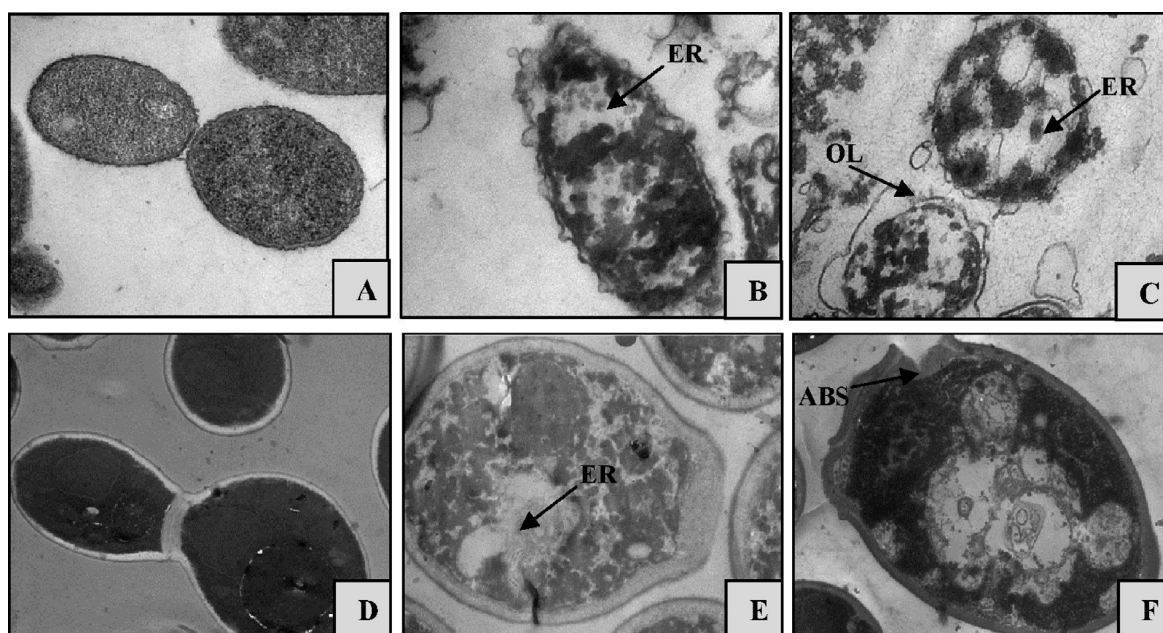


Fig. 4. Transmission electron microscopy micrographs by ultrathin sectioning of *E. coli* (A-C) and *S. cerevisiae* (D-F). A, D: Untreated; B, E: SC CO<sub>2</sub>-treated cells; C, F: US-SFI-treated. ER: empty regions; OL: cell wall-outer layer; ABS: abnormal bud scars. Reprinted from [62] with permission.

model, respectively. Microscopic images revealed that after 5 min of SC CO<sub>2</sub> treatment alone (35 MPa, 36 °C), minor morphological modifications were observed on cells, without any inactivation. However, when treating the cells under the same conditions with assistance of ultrasound (40 W), total inactivation was observed for both microorganisms. The treatment revealed the degradation of the internal cell content and the disruption of the cell plasmalemma, which is effective to prevent the regrowth of the cells during chilled storage.

*Salmonella* is the second most common cause of food poisoning after *Campylobacter*. The cause of salmonellosis is mainly attributed to the ingestion of *Salmonella spp.* After consuming contaminated foods, the symptoms (diarrhea, abdominal cramps, nausea, vomiting, fever, headache, and blood in the feces [66]) usually appear after 12–72 h and last for 4–7 days. Its inactivation is required to ensure microbiologically safe food products. As emerging technology, US SFI was evaluated for the pasteurization of fresh cut coconut [67]. Samples were first spiked with *Salmonella enterica* Typhimurium, and then processed for inactivation. The optimal conditions for inactivating both natural microbiota and the spiked ones were found at 12 MPa and 10 W, delivered every 2 min of treatment, as a function of temperature (from 24 to 50 °C) and treatment time (from 5 up to 30 min). Physicochemical analyses (total acidity, pH, color, texture, dry matter, fat content, enzymatic activity, antioxidant capacity, phenols, flavonoids, and phenolic acids) were performed to study the impact of the process on the product quality following the treatment and during chilled storage of 4 weeks. The authors reported that microbial inactivation was more efficient using US SFI, compared to CO<sub>2</sub> applied alone. In fact, 8 log reduction of *S. Typhimurium* was achieved when using US SFI at 12 MPa, 40 °C, and 20 min, whereas only 4 log reduction was achieved using CO<sub>2</sub> sole. Similar results were observed for natural microbiota, reducing their counts below the limit of detection, even for mesophilic microorganisms, which are very resistant. It has been also reported that the treatment did not lead to any change in total acidity during the total storage of 4 weeks, while minor changes were observed for the other physicochemical parameters. The authors concluded that US SFI represents a promising potential for the industrial pasteurization of fresh cut fruits.

Another major pathogen that is also drawing attention for its inactivation is *Listeria monocytogenes*. This microorganism represents the

ability to grow under aerobic and anaerobic conditions [68], at a wide range of temperature (0.4 °C to 45 °C) [69,70], over a wide pH range from 4.0 to 9.6 [71], in a modified atmosphere packaging [72], and even at relatively low water activity levels [73]. These features make *Listeria* a serious concern in food industry that requires strong controls along the food chain [74].

For instance, dry cured ham spiked with *L. monocytogenes* were treated by US SFI and the impact of this combined technology in inactivating the microorganism was evaluated [75]. Different pressures (6, 8 and 12 MPa) of CO<sub>2</sub>, temperatures (22, 35, 45 °C), and treatment times (0.5–30 min) were studied, and their impact on *L. monocytogenes* inactivation was compared to that of thermal and US SFI treatments. Following the inactivation process, different parameters including color, pH, and acidity changes were evaluated. A sensory analysis was also performed for the treated samples, and the shelf life was determined by a storage study for 4 weeks at 4 °C. Results demonstrated that applying ultrasound alone was not sufficient for microbial inactivation. However, applying US SFI achieved a certain level of inactivation, depending on the process temperature used. The efficiency of inactivation was reaching a maximum at 35 °C rather than 22 °C and no further increase was observed at 45 °C when compared to 35 °C. It has been also reported that when processing the samples at 12 MPa, 35 °C, at 10 W for 5 min, the initial microbial load of *L. monocytogenes* (10<sup>9</sup> CFU/g) was reduced below the limit of detection. No significant changes in acidity, pH, color, and sensory attributes were noticed between the treated and the untreated dry cured ham samples. In addition to these results, it has been also reported that a full microbial and quality shelf life was assured for 4 weeks at 4 °C. The authors concluded that US SFI represents the potential to be used as alternative technology to inactivate microorganisms at industrial scale, in particular to treat ham type or meat products.

#### 4. Current challenges of US-SF treatments: knowledge gaps, limitations, future directions, and industrial perspectives

Combined US SF (both US SFE and US SFI) green technology represents a great potential for many purposes including vegetable oil extraction, recovery of high added value compounds, and inactivation of microorganisms. Most of the works cited in this review point out the

efficiency of this method to be used at industrial scale, and the possibility to upgrade the current sc CO<sub>2</sub> installations used in food industry with ultrasound probes and high frequency generators.

#### 4.1. Extraction of valuable substances

The combined method of US SFE demonstrated its ability to reduce the processing time, the temperature, and the use of organic solvents, without significantly affecting the sensory and textural properties of the final product. In addition to these benefits, the use of sc CO<sub>2</sub> allows a fast and complete separation of the extract (oil and bioactives) by simply reducing the pressure and leaving no residue at all in the final product. Consequently, less purification steps for extract/solvent separation are required, although this separation might be sometimes needed when using co solvents in addition to sc CO<sub>2</sub>. Another major advantage lies in the quality of the obtained product. In most – if not all cases, supercritical extract is considered as the highest possible quality product.

Despite of all of these benefits, the use of this new technology requires more investigations before it becomes a common practice in the food industry. First of all, no global and precise energy balance of this emerging technique has been reported yet in the literature, which is one of the keys to scale up. It is known that using sc CO<sub>2</sub> alone was faced to the high consumed volumes of CO<sub>2</sub>, requiring further energy for pumping, and thus could only be applied to products with high added value. This drawback might however be attenuated by the fact that CO<sub>2</sub> is usually pumped in its liquid incompressible state which consumes rather little energy. In addition, CO<sub>2</sub> can be recycled reducing drastically the quantities used. Another disadvantage that is often cited is the relatively high investment cost, which is linked to the use of high pressures. To determine the profitability of any new technology or process to be implemented at industrial scale, it is necessary to calculate the added value obtained by this new system, which is highly variable and in many cases very difficult to quantify as it is related to how much the consumer is willing to pay, and the economic cost associated with the use of this new technology. Moreover, most of the works available in the literature were performed in the laboratory scale using batch equipments. Some studies showed that the efficiency of ultrasound techniques drops dramatically for larger volumes. The origins of this efficiency drop are unknown and should be studied. Therefore, the development of equipment coupling US and SFE and operating continuously should be carefully investigated. This task will not be easy because many constraints (gas sealing, cavitation erosion, etc.) must be taken into account in the design. In addition, global life cycle assessment of the product must be performed to determine the impact of technology on the environment of all the new stages of the product manufacture process. Equipping the current SFE installations with US probes may significantly increase the cost of the product without improving its quality. Thus, a compromise should be taken into account between the total investment, the final value of the product, and the additional extraction yield generated by ultrasound. The industrial future of this technology will probably lies in the design of continuous processing equipment, which is quite challenging. The sequential/multistage use of US SFE with other conventional or non conventional technologies will open new perspectives to improve the efficiency and to shorten the extraction times of oils and bioactive compounds. The second point that should be taken into consideration is that the proposed technique of US SFE for seed oil extraction should be compared to other non conventional technologies such as gas assisted mechanical expression, which was successfully applied to extract seed oils. The comparison should consider the total yield (for oils/bioactives), the consumed energy, the treatment time, and the amount of materials that could be processed per day. The required investment should also be considered for evaluation.

#### 4.2. Inactivation of microorganisms

Inactivating microorganisms using US SFI for food preservation is an interesting non thermal technology. As compared to thermal technologies, it offers many advantages to the food processor, including the preservation of quality, minimization of loss of flavors and nutrients, fresh like taste, and energy savings. Moreover, US SFI should be compared to other recent technologies (pulsed electric field, arc discharge processing, ultraviolet light, hydrostatic pressure processing, etc.). For instance, high hydrostatic pressure processing is currently more and more used in food industry as an alternative to heat sterilization and pasteurization. The use of irradiation is also an efficient method for food sterilization. Its social acceptability may however vary depending on the country and therefore impair its broad dissemination.

In addition to these economic features, more fundamental studies should be conducted to better understand the mechanisms of microbial inactivation during US SFI. For example, it is commonly accepted that gram negative bacteria (i.e. with a thin and mono layered cell wall) are more sensitive to inactivation than gram positive bacteria (thick and multi layered cell wall) but the link between the efficiency of US SFI and the type of cell wall has never been elucidated nor even studied. The presence – or absence – of a capsule around the cell wall is another example of parameter to be studied. When ultrasound is used alone, the higher the intensity, the more inactivated the bacteria, since more energy is released at higher intensity. But the way the synergy with supercritical CO<sub>2</sub> takes place is completely unknown. This is true not only with intensity but also with other parameters (US power, frequency, etc), which may have been studied for the ultrasonication of foods but not in association with compressed gases such as carbon dioxide.

To sum up the main challenges to overcome in this new coupled technology of inactivation of microorganisms are: (i) to get more studies with very precise description of experimental procedures in order to make possible some comparison (ii) to better understand and elucidate the phenomena of inactivation by acoustic cavitation, by pressurized carbon dioxide and above all by the two combined technologies. Moreover, the combination of high CO<sub>2</sub> pressure (> 30 MPa) and high US power (> 300 W) should be tested, as no study has been devoted to it yet. In addition, few studies have focused on the impact of this technology on the organoleptic properties of the processed foods and this point should be explored in depth. Finally, there is a lack in the literature for works performed at either pilot or industrial scale to justify if this process is economically viable and if the achievements at laboratory could be scaled up.

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