Application of Supercritical Fluids in the Conservation of Bioactive Compounds: A Review

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Abstract   The precipitation from supercritical antisolvents (SASs) of natural compounds solutions has become an excellent alternative to improve the solubility and availability of poorly water-soluble compounds. In this technique, CO_2 is used as a supercritical fluid that marks the processing as a green technology and is ideal for use in the food industry due to its nontoxicity, economy and easy separation of solutes. This article reviews the application of the SAS technique in the food industry and highlights the influential factors in the generation of micro- and nanoparticles.

Keywords   Micronization, Natural Compounds, SAS Technique, Coprecipitation

1. Introduction

Fruits, vegetables, and plants produce many secondary metabolites responsible for flavor, aroma, and color, which have shown beneficial effects for human health against cancer and other chronic diseases such as cardiovascular disease, diabetes, and aging [1]. Currently, some plants or extracts from them are commercially available as infusions, tablets or extracts [2], in this way the essential oils or secondary metabolites present, they could be degraded easily (by oxidation, volatilization, heating, light) if they are not protected of external factors because they are unstable and fragile volatile compounds [3]. To improve the availability and quality of natural compounds, powder formation has been currently used as a protective mechanism for these substances, for example, micronization or encapsulation techniques using supercritical fluids [4].

The application of supercritical antisolvent techniques or the use of supercritical carbon dioxide (CO_2) as an antisolvent can yield highly concentrated plant extracts. This approach is an alternative for the food industry because it is considered an environmentally friendly process and allows good control over product properties, especially particle size, particle size distribution, crystal habit and polymorphic form [5]. Moreover, avoiding the degradation and decomposition of active compounds by operating at reduced temperatures in light and in the absence of oxygen

as well as the subsequent processing of extracts is not necessary to separate the organic solvents [6].

The aim of this review is to summarize topics that are involved in the application of the Supercritical Antisolvent (SAS) process.

2. Natural Compounds

Plants have a secondary metabolism that allows them to produce and accumulate compounds of diverse chemical nature, called natural compounds, which are grouped into four main classes: terpenes, phenolic compounds, glycosides and alkaloids [7]. Notably, not all secondary metabolites are found in all plants. Furthermore, they are found in small quantities and vary according to the genus, family or plant species; most of these natural compounds are found in a mixture of alcohols, ketones, aldehydes and one or more secondary metabolites called essential oils.

According to Martelli and Giacomini [8], essential oils are complex volatile mixtures that composed of between 20 to 60 compounds at different some concentrations where two or three major components are present in relatively high concentrations (20 to 70%) compared to others. On the other hand, Xiao et al. [9] considered essential oils as containing two types of major components such as terpenes and aromatics, which are known for their high volatility and low residual generation. Examples of these compounds are trans-anethole and estragole, which are widely used as flavorings in the food and alcoholic beverages industry and have been found in a variety of aromatic species, including basil, fennel, anise and star anise [10]. Currently, essential oils are products of great interest to the cosmetic, pharmaceutical and food industries due to their potential properties that have been characterized as antioxidants,

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antimicrobials, dyes, and flavorings [11].

2.1. Bioactive Compounds and Their Properties

In the food industry, important bioactive compounds, such as curcumin (from turmeric, Brazilian saffron), chlorophyll (usually extracted from green leaves), anthocyanins (usually extracted from grape hulls), betanin (from beets), carotenoids (alpha, beta and gamma carotene and lycopene) and xanthophylls, have been found [12].

Among these natural pigments, anthocyanins have been well studied for their coloring properties that are responsible for the bright orange, pink, red, violet and blue colors in the flowers and fruits of some plants. They are also known for their antioxidant properties, which play an important role in the prevention of neuronal and cardiovascular diseases, cancer and diabetes [13, 14]. Anthocyanins can be found in the bran and powder of purple wheat, such as cyanidin-3-glucoside, cyanidin-3- (6-malonyl glucoside), cyanidin-3-rutinoside, peonidin-3-glucoside and peonidin-3-(6-malonylglucoside) [15]; in black rice bran (*Oryza sativa* L.), such as cyanidin-3-O-glucoside, cyanidin-3-O-rutinoside, delphinidin, cyanidin, pelargonidin and malvidin, which have been identified as a potential source of dark purple pigment [16]; and in black raspberry (R*ubus occidentalis* L.), such as cyanidin-3-glucoside, cyanidin-3-sambubioside, cyanidin-3-xylosylrutinoside and cyanidin-rutinoside [17]. Additionally, two anthocyanin groups have been isolated in black currants, such as cyanidins and delphinidins (delphinidin-3-glucoside, delphinidin-3-rutinoside, cyanidin-3-glucoside, cyanidin-3-rutinoside) [18].

Carotenoids are responsible for the yellow-orange and red color of food. According to Eggersdorfer and Wyss [19], the main carotenoids found in foods are lutein, β-carotene, zeaxanthin and lycopene. These compounds have properties beneficial to human health due to their antioxidant effects and ability to act individually through other mechanisms; for example, β-carotene has a pro-vitamin A function, while lutein/zeaxanthin is a macular pigment in the eye that benefits ocular health. In addition, they improve cognitive function and cardiovascular health and can help prevent some types of cancer. It is possible to find carotenoids in the fruit of Goji berries (L*ycium barbarum* L.), mainly the zeaxanthin dipalmitate [20], the peels and the melon seed contain mainly lutein, β-carotene and phenolic compounds [21].

On the other hand, aromatic compounds are organic species belonging to different chemical classes, such as alcohols, esters, acids, ketones, aldehydes, terpenes and sulfur compounds [22,23]. These compounds can be found in sources such as rosemary (R*osmarinus officinalis* L.) α-pinene, α-fenchone, eucalyptol, trans-sabinene hydrate, camphor, isoborneol and myrtenal [24]; the clove (*Syzygium aromaticum*) contains eugenol, α-caryophyllene, and 2-methoxy-4-(2-propenyl acetate) phenol [25]. In basil and oregano, α-pinene, limonene, camphor and citronellol

(terpenes) can be found [26]. In fennel, two main aromatic compounds, anethole and fenchone, have been identified [27].

Food color and aroma are important attributes of quality in the food industries, as they influence the choice and preference of the consumer [28]. The quantity of these compounds is not limited since their functional properties improve the quality and nutritional value of foods. Notably, the use of natural compounds becomes a challenge for industries because of the sensitivity of these substances to pH changes, to heating, to the presence of oxygen and oxidizing agents that can cause their decomposition [29]. Currently, the demand and requirements of consumers for natural foods are constantly growing, and competition among food producers encourages researchers to seek new technological alternatives in terms of the application of these compounds.

3. Supercritical Fluids and Their Application in the Food Industry

Supercritical fluids are those in which the temperature and pressure are simultaneously a value higher than the critical point, as shown by the shaded region in Figure 1, which is characterized by a density like that of liquids, a diffusivity greater than that of liquids and a very high compressibility higher than that of the ideal gas [30].
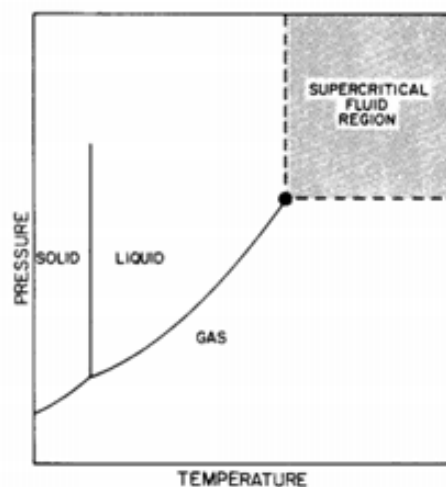

Figure 1. Phase diagram, adapted from Jean and Debenedetti [30]

These fluids, whose properties can be adjusted by changing the fluid density between liquids and gases, achieve the versatility of their use as follows [31]:

(a) Alternative solvents for classical separation processes such as extraction, fractionation, adsorption, chromatography, and crystallization;

(b) Reaction media as in polymerization or depolymerization;

(c) Reprocessing fluids as in the production of particles, fibers, or foams.

Carbon dioxide is the most used supercritical fluid in food applications because of its availability in high purity in addition to the fact that it is economical and safe to handle [32] and has low critical parameters (Tc = 304.1 K and Pc = 7.38 MPa), no toxicity, inertia, nonflammability, solvation and easy separation of solutes [33,34]. The use of CO_2 has been important in the food and pharmaceutical industries, minimizing the use of organic solvents due to the low critical pressure and temperature parameters that allow working at temperatures close to the room temperature, avoiding the degradation or alteration of natural compounds and contributing to the improvement of conventional processes such as extraction, micronization, and encapsulation of compounds of high commercial value [35].

In the last thirty-year period, supercritical fluids have been often proposed as an alternative to organic solvents in different industrial fields [36]. In particular, for the production of bioactive compounds and their encapsulation through the formation of micro-and nano-particles [37]. The processes in which supercritical fluids are used are classified according to the role they play in the processes, as follows: solvent [Rapid Expansion of Supercritical Solutions (RESS)]; solute [Particles from Gas-Saturated Solutions (PGSS)]; or antisolvent [Supercritical Antisolvent (SAS)] [38]. Currently, the application of the SAS technique in natural compounds is being widely studied to form microencapsulated particles and/or nanoparticles from individual food ingredients [39].

4. Supercritical Precipitation Technique Antisolvent (SAS)

The supercritical antisolvent process (SAS) is based on the precipitation of the solutes dissolved in conventional liquid solvent (dichloromethane, ethanol, methanol, dimethylsulfoxide, etc.) using a supercritical fluid. The supercritical fluid saturates the liquid solvent, resulting in precipitation of the solute due to the antisolvent effect [34].

It is important to keep in mind that this technique is based on two prerequisites: first, the liquid solvent and the anti-solvent (CO_2) must be completely miscible under the process conditions, and finally, the solute must be insoluble in the solvent-antisolvent mixture [40].

In the experimental part, the procedure normally begins with the delivery of supercritical CO_2 at a constant flow rate to the precipitation chamber until the desired pressure is reached. Then, a pure solvent is sent through an injector to the pressurized chamber to obtain a stable composition condition during the precipitation of the solute. At that point, the flow of the organic solvent stops, and the liquid solution formed by the organic solvent and the solute to be micronized are delivered through the nozzle. Once the fixed amount of organic solution is injected, the pump for liquids is stopped. However, the supercritical CO_2 continues to flow to wash the chamber of the residual content of liquid solubilized in the supercritical anti-solvent. If the final purge with pure CO_2 is not performed, the solvent condenses

during depressurization and can solubilize or modify the dust [40,41]. In Figure 2, general schematization of the procedure is shown.
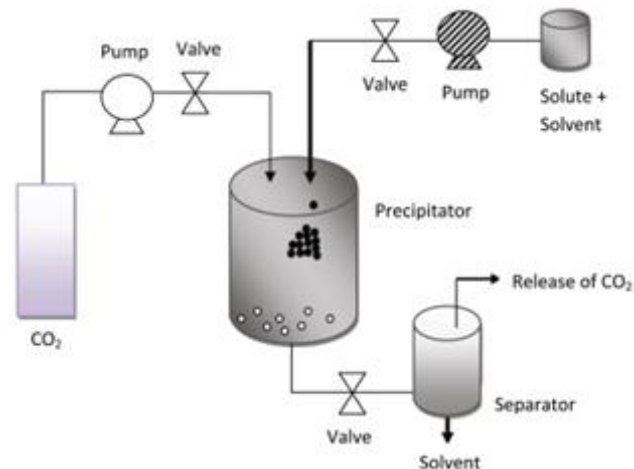

Figure 2. Schematic process the supercritical antisolvent process, adapted from Fahim, t. k. et al [34]

The choice of the SAS technique for the formation of particles is advantageous because the heat-sensitive compounds can be processed at low temperatures to obtain powders with little or no residual solvent [42]; the powder is produced by rapid supersaturation of a solute dissolved in the liquid and the precipitation of the solute as micronized particles due to the high diffusivity of supercritical fluids that can be up to two orders of magnitude higher than that of liquids [43]; the use of a single container during the process allows elimination of the initial solvent and drying of the particles almost at the same time of the particle formation [44]; the availability and solubility are improved by reducing the particle size in micron and nanometer scales to maximize the area of the surface, taking into account the principle of the Noyes-Whitney equation: the dissolution rate is proportional to the surface area exposed to the dissolution medium [45,46]; The equipment allows adaptations to perform extraction and micronization processes online, minimizing the handling of the extracts (exposure to light or oxygen) and the degradation of sensitive compounds [47]. The application of these online processes in the industry could provide benefits, such as simplification and intensification of processes that require fewer operations and space per unit and probably improve profitability [47]. Application studies for the SAS technique in obtaining dried precipitates from plant extracts have recently increased, as shown in Table 1.

It is convenient to mention the use of SAS for the coprecipitation of labile molecules using a polymer that becomes an effective transport for this procedure. The considerations for the use of this process [4]: are the choice of solvent, polymer, and supercritical fluid. The use of CO_2 as a supercritical fluid is again highlighted because most polymers have a very limited or almost zero solubility in carbon dioxide. Moreover, the relatively high solubility of polymers in ordinary liquid organic solvents may provide

ideal conditions to employ both the organic solvents and CO_2 as solvents and as antisolvents, respectively [48]. The Reduction of melting temperature of polymer in presence of carbon dioxide plus a solvent, it can occur at moderate pressures will be desirable to form a polymer film that leads to segregated particles of the polymer and the active compound [49]. Finally, it should be considered that the process efficiency is mainly dependent on the initial concentration of the drug and polymer and the affinity between them.

Table 1. Application of the SAS process in plant extracts

ARTICLE TITLE	FRACTIONED COMPOUND	CONDITIONS OF PROCESS	RESULTS
On-line process for pressurized ethanol extraction of onion peels extract and particle formation using supercritical antisolvent [47]	Quercetin	The flow rates were CO_2 17 g/min and ethanol 0.4 g/min. Temperature variation: 313.15 and 333.15 K; pressure: 6 and 12 MPa.	The best condition for the precipitation process was 313.15 K and 10 MPa, obtaining a quercetin content of 26 $\pm$2% and a particle size of 119 $\pm$ 1 (μm).
Precipitation of Curcuminoids from an ethanolic turmeric extract using a supercritical antisolvent process [61]	Curcuminoids	The flow of the ethanol extract was 0.5 cm^3/min; temperature variation, 313 and 333 K; pressure, 10 and 12 MPa; and CO_2 flow, of 500 and 800 g/h. There were two types of mixing nozzles: T-mixer nozzle and coaxial nozzle.	The best process conditions were the use of the T-mix nozzle combined with a temperature of 313 K, a pressure of 10 MPa and a flow rate of 500 g/h, obtaining a 53.9% global yield of solids, a 547 mg/g content of curcuminoids and a particle size of 111 (μm).
Precipitation and encapsulation of rosemary antioxidants by supercritical antisolvent process [65]	Antioxidant compounds of rosemary (rosmarinic and carnosic acid)	**Precipitation:** Variation in temperature: 298.15, 308.15, 313.15 and 323.15 K; pressure, 8 to 12 MPa. The mass flow rate of CO_2 and the flow rate of the solution were fixed at 0.7 kg/h and 1 ml/min, respectively. **Coprecipitation:** 50 ℃ and 10 MPa; 14 MPa and 50 ℃.	**Precipitation:** The overall yield of solids was 90% with the parameters of 313.15 K and 10 MPa, with particle sizes < 1 μm and with agglomerates approximately 50 μm. **Coprecipitation:** 100% efficiency at 14 MPa and 323.15 K; particle size < 1 μm agglomerated between 5 and 20 μm, due to the increase in pressure (14 MPa) and the presence of the polymer.
Green tea encapsulation by means of high pressure antisolvent coprecipitation [66]	Polyphenolic compounds (caffeine, epicatechin and epicatechin gallate).	Variation of pressure (8 to 12 MPa), temperature (284 to 307 K), concentration of polymer ($C_{polymer}$) in the solvent (0.25, 1.5, 2.2 and 3.5% W/W) and mass flow rate of CO2 (1.11E-3 and 5.56E-4 kg/s). Polymer: Poly-ε-caprolactone, MW: 25,000.	For temperatures 284, 285, 288 K, average particle sizes of 2.3, 3.6, and 4 μm respectively were obtained and accompanied by 9 MPa, F_{CO2} = 1.11E-3 kg/s, and $C_{polymer}$ = 1.5% W / W. The influencing factor for the successful formation of the encapsulation was the temperature, which correspond to values below the T_C of the mixture of solvent and anti-solvent.
Precipitation of mango leaves antioxidants by supercritical antisolvent process [67]	Antioxidant compounds of mango leaves (mangiferin and benzophenone derivatives such as iriflophenone 3-C-β-d-glucoside and iriflophenone 3-C-(2-O-p-hydroxybenzoyl)-β−d-glucoside).	Variation of pressure (10 and 15 MPa), temperature (308.15 and 323.15 K), flow rates of the solution (5 and 10 ml/min), initial concentration of the extract (8, 20, 30 and 40 mg/ml) and supercritical CO_2 flows (10, 30 and 40 g CO_2/min).	The best condition was 15 MPa, 308 K, an initial concentration of 20 mg/ml extract, CO_2 flow rate of 30 g/min, and solution flow rate of 10 ml/min for a mean particle size of 0.06 $\pm$ 0.02 μm. The influencing factor for the particle size was the initial concentration of the extract: the higher the concentration, the smaller was the particle size.
Selective precipitation of phenolic compounds from *Achillea millefolium L.* extracts by supercritical anti-solvent technique [68]	Polyphenolic compounds (3,5-dicaffeoylquinic acid, glycoside of apigenin and luteolin, aglycones)	The temperature was 313.15 K, and the CO_2 and extract fluxes were 50 g/min and 1.6 g/min, respectively. The concentration of the solution was 17.9 mg/ml. The only variation involved pressure: 10, 15, and 20 MPa.	The best result was at 15 MPa with a mean size of 136 μm and a precipitation yield of 30.9% total phenolic compounds.

Several studies have evaluated the influence of the ratio and concentration between the active compound and the polymer. First, Garcia et al. [50] evaluated different solution ratios (1:1 to 1:10) of mangiferin/cellulose acetate phthalate (MNG/CAP) through the injection of one nozzle (SAS1) and two nozzles (SAS2) into the tank precipitation using fixed variables such as temperature (323.15 K), and pressure (18 MPa). Evidence for SAS1, an average particle size between 0.25 and 0.41 μm and the MNG / CAP ratio did not affect the size of the particles or the morphology of the resulting precipitates. Additionally, higher loads of mangiferin were found for lower relations between the mangiferin and polymer. For the SAS2 process, researchers prepared solutions with the same mixture of solvents separately from MNG and CAP, injected by different nozzles for the evaluation of other factors such as the variation of the internal diameters of the nozzles and the flow rates of the liquid solutions. The authors observed from this technique (SAS2) precipitates formed by tiny threads of mangiferin on spherical microparticles of the polymer where a certain degree of MNG crystallinity such as the raw material was perceived, it was also evident that the different nozzle diameters and the solution flow rates had no significant effect on particle size and the morphology of the precipitates.

Prosapio et al. [51] evaluated the effect of the relations among mass of polymer/mass of drug, pressure, temperature and total concentration of fat-soluble vitamins such as α-tocopherol (TOC) and menadione (MEN). Starting the procedure with 9 MPa, 313 K and 20 mg/ml and with the determination of the effect of the relationship between the polymer and the vitamin, varying the weight ratio between polymer polyvinylpyrrolidone (PVP) and the vitamin in the liquid solution, in this case, microparticles were observed in the ratios of 1:1 and 2:1, and when the ratio was increased to 3:1 and 5:1, the average particle size increased; however, the microparticles were maintained. An increase in temperature to 323 K allowed the formation of a liquid phase in the lower part of the precipitator, which shows that under these process conditions (363 MPa and 323 K), the precipitation was not successful, and the operation point was performed in the CO_2/dimethylsulfoxide system miscibility orifice. The opposite occurred when the temperature decreased to 308 K, which favored the formation of micro particles. Evaluation of a concentration increase to 60 mg/ml revealed that microparticles with an average diameter of 4.06 μm (PVP/TOC) and 5.09 μm (PVP/MEN) were also produced, demonstrating that the temperature increase did not have a significant effect on the average size and particle size distribution, Also in this work [51] it was evidenced that increasing the pressure to 15 MPa does not significantly alter the particle size.

These results demonstrate the influential factor of the ratio of the active compound and the polymer for the realization of good co-precipitation. Moreover, successful results were obtained in the PVP/TOC systems, composite microparticles with average diameters of 1.69 to 4.08 μm, and for the PVP/MEN system from 2.64 to 5.09 μm in the range of 9-15

MPa and 308-313 K. Coprecipitates of vitamin B (folic acid) have also been produced [52].

Some limitations were also found in the literature when applying the SAS technique, related to the difficulty of processing some categories of materials [53]: (a) molecules soluble in CO_2 and/or in the solvent/anti-solvent mixture, (b) water-soluble compounds, due to the wide miscibility gap between water and CO_2 in normal SAS conditions (313.15-333.15 K, 10-25 MPa). This last limitation has been overcome by using an SAS modification called Enlarged Liquid Anti-solvent (ELAS), which employs a mixture of CO_2 and a polar organic solvent under expanded liquid conditions to improve the water solubility in the gas mixture. Considering the limitations of the technique, it is important to know the properties of the compound that will be micronized or encapsulated with respect to the interaction with solvent and supercritical CO_2.

4.1. Influencing Factors in Particle Formation and Morphology

Many factors of importance for production or control in the formation and size of the particles were found; several studies have been realized to describe this phenomenon:

First is the influencing factor of the solubility of the solute in the mixture of solvent and antisolvent for the formation of amorphous particles or the production of crystals. If the substance to be processed is slightly soluble in the mixture of solvent and antisolvent, the level of supersaturation is moderate, and crystallization is produced. If the solute is slightly soluble in the mixture, the supersaturation level is much higher and the precipitation of amorphous particles occurs; the pressure plays an important role in this behavior, since when increasing the pressure in a system where the solute is slightly soluble in the mixture, the solubility of the solute increases, decreasing the level of supersaturation, and when the solute is poorly soluble in the mixture, then the level of supersaturation increases [54].

The gas-liquid phase balance between the solvent and the supercritical CO_2 is another important consideration in the SAS process. Campardelli et al. [55] stated that it is important to know the equilibrium of the solvent-CO_2 phase and to consider that the solubility behavior of a binary system can change with the addition of a third component. Therefore, it was realized three experiments. First, it was evaluated the dependence of the morphology in the ternary phase of dimethylsulfoxide (DMSO) plus cefonicid (CEF) and CO_2, obtaining at 313K the values of the critical points of the mixture of 8.81 MPa; $XCO_2 = 0.923$ and 10.71 MPa; and $XCO_2 = 0.927$ for the binary and ternary systems, respectively. In the second place, the authors performed tests with to values lower than the critical point of the mixture (10.5 MPa) observing expanded microparticles or the formation of two phases in the precipitator. Finally, it was used values higher than the critical point of the mixture (18 Mpa-333K) to the precipitation of the CEF, observing nanoparticles corresponding to conditions visualized by the ternary equilibrium system. Similarly, Cardoso et al. [56]

mentioned the importance of the phase behavior of the ternary system where the results of particle size and morphology could be anticipated; however, the absence of information leads to the need to study each particular system (antisolvent, organic solvent and compounds for micronizing) or to preside the behavior through the binary phase diagram that should be performed first to clarify the behavior of the solvent-CO_2 and thereby analyze the considerations of fluid dynamics, nucleation or mass transfer to a given ternary system.

In the literature, measurements of the phase behavior of binary systems, such as CO_2-ethanol, CO_2-water, CO_2-methanol, and CO_2-dichloromethane [57-59] have been reported.

The temperature, pressure, and concentration of the liquid solution (solute-solvent) are also important. In a previous study [60], researchers evaluated these three parameters using gadolinium acetate (GDAC) as a model compound and DMSO as a solvent. First, the pressure variation was evaluated from 9 to 20 MPa, keeping the temperature (313 K) and the concentration of the liquid solution of 60 mg/ml constant, observing that with the increase in pressure, a smaller particle size is produced, achieving a transition from microparticles to nanoparticles (0.21 μm at 20 MPa) in the proximity of the critical point of the DMSO-CO_2 system mix and in fully developed supercritical conditions. Then, the behavior of the concentration of the liquid solution of 20 to 200 mg/ml was evaluated, keeping the temperature (313 K) and the pressure (15 MPa) constant. For this case, it was observed that an increase in the concentration of GDAC in the liquid solution was accompanied by an increase in the average particle size and the size distribution, where only the nanoparticles were present at concentrations of 20 and 25 mg/ml (0.09 μm) for both cases. Finally, a temperature variation of 308 to 333 K was carried out, maintaining constant values of pressure (15 MPa) and concentration (60 mg/ml) for this evaluation, which revealed only microparticles and extended micro particles, considering that the experiments carried out with higher temperatures show a sequence of reverse precipitation mechanism observed for the increase in pressure.

The type of mixer for CO_2 fluids and extract (solute-solvent) in the precipitation vessel is influential; the use of a T-mixer causes turbulence between the fluids, generating increases in the mass transfer between the CO_2 and the organic solvent because of the rapid diffusion of CO_2 into the drop. This phenomenon accelerates supersaturation and nucleation, which results in the formation of smaller particles, and when using a T-connector, the opposite effect occurs [61].

A study by De Marco et al. [62] evaluated the effect of soluble solvent mixtures such as ethanol, dimethylsulfoxide and N-methylpyrrolidone with a slightly soluble solvent (acetone) on a polyvinylpyrrolidone biopolymer (PVP). These experiments demonstrated that the use of suitable solvent mixtures is effective for the decrease in the average particle size of PVP and the contraction of the particle size distribution generating the production of nanoparticles with an average diameter of 0.22 μm, 0.16 μm to 0.11 μm. The acetone acts as a modifier of the particle morphology, interpreting the behavior with the relation of "a bad solvent" - "a good solvent", where the polymer tends to reject the acetone molecules, precipitating in a conformation that is more compact [63].

Petit-Gas et al. [64] studied the role of hydrodynamics in describing the jet dispersion of pure liquid organic solvents and the influence of the presence of solute, temperature, pressure, feed configuration and variation of the liquid flow rate of 0.1 at 9 ml/min. As a result, for ethanol, the formation of an asymmetric jet on the shaft at 313 K and 11 MPa (jet velocity of 0.09 m / s) was shown. For acetone, there was an asymmetric jet at 308 K and 12 MPa (speed of 0.13 m/s) and an atomized jet for acetone at 308 K and 12 MPa (jet velocity of 0.89 m/s), highlighting that even under conditions of complete miscibility between the binary systems, a boundary between the dispersed and continuous phase was observed because it was not above the value of the transition pressure between the biphasic and monophasic regions. The rates of critical atomization for the systems were between 0.33 and 0.42 m/s for the pure solvents and 0.39-0.41 m/s for the systems in the presence of solute (sulfathiazole). The authors affirm that obtaining closed and low values did not influence the chemical nature of the solvent, the variations in the physicochemical properties of the dispersed phase, the internal diameter of the capillary used (only generating differences in the Reynolds number) or the presence of the solute in the critical atomization rate. After studying the crystallization under conditions like those for analyzing the dispersion of the jet, it was evident that the key factor for the formation of finer particles seems to be the mixture's state more than the state of atomization, even if the characteristics of the powder are more homogeneous when atomized by the jet.

5. Conclusions and Future Expectations

The SAS processing of natural extracts is an effective way to obtain micro- and nanoparticles, thereby achieving availability, protection, and concentration as well as consequently increasing the quality of natural extracts.

Due to the feasibility of using the SAS technique and its excellent results for the micronization and encapsulation of natural compounds, it is necessary to increase research on other bioactive compounds in the food industry. Furthermore, it would also be interesting to achieve relevant applications of this technique in industry.

A prerequisite for the micronization or formation of precipitates is the gas-liquid phase diagram of the supercritical fluid and the solvent that will be used as a starting point for the choice of operating parameters. Although good results have been obtained with this criterion, thermodynamic studies involving the presence of two or more solutes in the equilibrium of the phases are needed since this approach could improve the prediction of particle

formation and the coprecipitations would be completely successful.

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