Purification of natural products by selective precipitation using Supercritical/Gas Antisolvent techniques (SAS/GAS)

Alicia Gil-Ramírez, Irene Rodriguez-Meizoso*

Lund University, Department of Chemistry, Centre for Analysis and Synthesis, P.O. Box 124, SE-22100, Lund, Sweden

*corresponding author: irene.rodriguez_meizoso@chem.lu.se, irenerome@gmail.com

phone: +46 46 222 81 69, fax +46 46 222 82 09

Abstract

Supercritical fluids offer considerable advantages for the isolation of natural products. Supercritical fluids can be used as antisolvents to selectively precipitate target compounds from a mixture and remove impurities by the Supercritical Antisolvent technique (SAS). The present decade has experienced a considerable increase in the number of publications that apply this technique to natural extracts, especially for the isolation of polyphenols and carotenoids from plants and microalgae. However, the lack of a clear terminology and purpose adds confusion to the topic. The proposed review aims at defining a research field that consists in applying SAS to natural extracts for the purification of target compounds. To do so, we trace back the origin of the field, discuss the different terminology used to refer to such processes, and suggest appropriate terms for the process and for reported results. This work explores the scope of the topic by compiling all works published to date from a scattered literature, using all possible process terminologies for the search. The information given aims to highlight the most promising applications explored so far and possibly inspire further research.

Keywords: Selective precipitation, supercritical antisolvent, fractionation, purification, natural extracts, supercritical fluid.

INTRODUCTION

Natural products are chemical compounds produced by living organisms like plants, algae and animals. In many cases, these substances possess biological activities, technological and nutritional value, making them valuable as nutraceuticals, food and cosmetic ingredients, and as sources of new drugs. Isolation of a particular compound or class of compounds can be the most difficult and time-consuming step when developing new ingredients or drugs. Purification often requires chromatographic or membrane separation, and needs to be followed by drying. Drying improves the stability of active compounds and decrease transportation cost. Furthermore, it is important to produce dry pure substances in particulate form to facilitate processability, bioavailability, absorptivity and dosage.

Supercritical carbon dioxide ($scCO_2$) has been used to make particles of active compounds, for their potential use as drugs and food ingredients (1, 2). In only one step, the compound of interest is collected not only as a particulate but also free of solvent under mild temperature conditions. CO_2 creates an inert environment that minimizes degradation of the compound of interest because of its rather low critical temperature (31 °C). It is non-toxic, non-flammable and accepted by the American Food and Drug Administration and the European Food Safety Authority. By using $scCO_2$, the use of hazardous organic solvents is avoided or decreased. Furthermore, precipitation using $scCO_2$ also enables certain control over particle size and morphology, as well as narrow particle size distribution (3).

Among the different types of techniques that use $scCO_2$ to generate dry particles, the Supercritical Antisolvent technique (SAS) offers the biggest process scope. Unlike other

precipitation agents, CO_2 is easily separated from the product during depressurization. SAS can be used to process any compound as long as the compound is not soluble in $scCO_2$ but it is soluble in an organic solvent. Moreover, the organic solvent must be miscible with CO_2. In a SAS process, the compound of interest is first dissolved in an organic solvent. Afterwards, the organic solution is put in contact with $scCO_2$. As CO_2 penetrates the liquid solution, it induces volumetric expansion of the liquid. The liquid solvent loses its solvation power and the mixture becomes supersaturated. Supersaturation is overcome by precipitation or recrystallization of the solute.

A fundamentally different process occurs when the feed solution, for example a natural extract, contains a mixture of several compounds. In this case, it is likely that not all compounds precipitate at the same process conditions (namely pressure, temperature and proportion between the amount of solvent and antisolvent). The principle of selective precipitation can be exploited as a way to purify or enrich the precipitate in the compound(s) of interest at the same time as the precipitate is dried. In our opinion, this is a powerful idea that has the potential to reduce the number of steps in purification and drying of natural products from an extract. In turn, the process may lead to a reduced use of chemicals and a reduction of waste in cases that otherwise would require chromatographic purification, membrane separation or sequential extraction with multiple solvents.

Seminal works on the application of SAS (or its gaseous version, the Gas Antisolvent technique, GAS) to fractionate mixtures of natural compounds are dated at the beginning of the 90´s, but the number of published studies experimented a noticeable increase in the present decade (Figure 1).

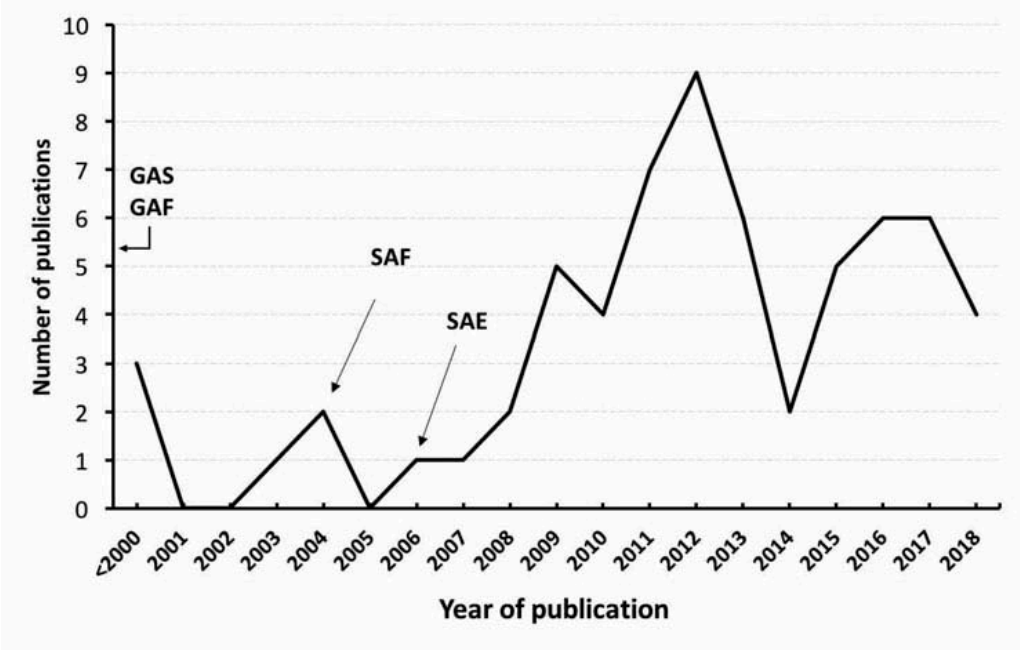

Figure 1. Profile of the number of publications over the years, from 90´s. The search included all the keywords specified in the introduction. Arrows point the specific year in which specific terms (GAS, GAF, SAF, SAE) were coined.

Some applications have been reviewed by Catchpole et al. (2009) (4), Reverchon and De Marco (2006) (5), Torres et al. (2016) (6), Martín et al. (2012) (7) and Baldino et al. (2018) (8). Our initial examination of the literature revealed that the field of research is however not well established, and readers interested in the topic will face several challenges when searching for literature. Scientific articles exploiting the principle of selective precipitation by SAS are published using different terminologies, which generates confusion. Furthermore, there are works in which the main scope was not the fractionation of the extract, even if a purification occurs. Such works are difficult to spot since purification is not explicit in the title of the article nor the abstract. The opposite is also found. Some articles use a generally accepted term for the process, but either purification was not achieved, or no data was provided to evaluate purity. Finally,

purification by phase separation is often expressed under the same process name, regardless if purification is achieved by precipitating the compounds of interest or, on the contrary, by precipitating compounds that are considered to be impurities.

The purpose of this review article is to bring some clarity into the topic. This article aims at defining a research field that consists in applying selective precipitation by SAS/GAS to mixtures of natural compounds, such as plant extracts, for the purification of natural products. We offer a reflection on the fundamentals of the process, as well as a description on instrumental setups and operational modes. We trace back the origin of the process and attempt to formalize the terminology of the field. With the intent to depict the scope of the field, this work tries to be comprehensive in compiling all scientific articles published on the topic, but only significant ones are discussed in detail. Processes in which the precipitate is the compound of interest are highlighted since only such applications are exploiting all the benefits of CO_2 as antisolvent, providing an enriched and dry fraction in one step. The information relayed for each scientific publication focuses on the degree of purification achieved, so as to reflect the potential of the technique and possibly inspire new research in the field.

Reviewing methodology

Combinations of the following keywords have been used to screen the literature: antisolvent, supercritical, ´supercritical antisolvent´, precipitation, gas, fractionation, ´selective precipitation´, extract, carbon dioxide, ´supercritical antisolvent extraction´, ´supercritical antisolvent fractionation´. From the results of the search, we have considered only those works that involve a step in which target compounds are precipitated selectively from a homogeneous mixture in the high-pressure chamber

using a compressed fluid as antisolvent. This step is followed by collection of the two separate phases. Therefore, supercritical extraction from a solid or liquid matrix using $scCO_2$, followed by collection of extract and raffinate, is not considered here since it is a conceptually different process. The same applies to the fractionation of the extract by controlled depressurization, and to column fractionation using CO_2 as aid but that do not produce a precipitate. The reader will however find literature in which these latter cases are misleadingly referred to as Supercritical Antisolvent Fractionation (SAF).

FUNDAMENTALS OF SELECTIVE PRECIPITATION BY SAS/GAS

A mixture of solutes, for example a natural extract, is first dissolved in an organic solvent and then put in contact with a compressed fluid, usually CO_2, that acts as antisolvent. The organic solvent must be able to dissolve all the solutes and be miscible with the antisolvent. Upon contact between antisolvent and the liquid solution, a homogeneous medium is formed in which the liquid solvent loses solvation power due to volumetric expansion. Due to differences in polarity, CO_2-philicity and molecular weight, some of the solutes in the mixture will no longer be soluble in the new medium and precipitate. On the contrary, other compounds interact strongly with the organic solvent and will still be soluble despite the presence of antisolvent. In the latter cases, the organic solvent may be visualized as a cosolvent for CO_2 (9).

The natural extract processed by SAS is a mixture of many components. It is expected that numerous interactions between solutes, solvent and antisolvent will occur in a system containing a multicomponent mixture, an organic solvent and CO_2. Such interactions dictate the phase behavior of the system, but it is impossible to predict the behavior of a multicomponent system of high molecular asymmetry. In practice, to evaluate if selective precipitation by SAS is a feasible process, it is enough to have a

comparative idea on the solubility of the solute(s) of interest(s) and the impurity(ies) in the liquid solvents and in the antisolvent separately. A better alternative is to know the solubility of the solute of interest as well as the impurity in a mixture of antisolvent plus organic solvent. However, this approach is limited since it is still not possible to foresee potential solubility enhancements due to solute-solute interactions. With this information in mind, the process can be further developed by trial-and-error, selecting the concentration of the extract, a combination of solvent/antisolvent ratio, pressure and temperature. These parameters have been shown to affect total yield, recovery and concentration of the compound of interest in the precipitate (10).

As explained in the previous section, it has been observed that the combination of pressure and temperature does not need to be above the supercritical point of the binary mixture (11). Typically, the operating temperature is below the critical temperature of the binary mixture, and it is most likely that the mixture is present in the form of a gas-expanded-liquid (that is, inside the vapor-liquid envelope of a pressure-composition phase diagram) or as a one-phase compressed fluid as the pressure increases away from the vapor-liquid envelope. If the mixture becomes a vapor-liquid equilibrium, that is it separates in two phases, the process may technically be an extraction, in which compounds are transferred from one phase to another, accompanied by precipitation of certain compounds within the liquid phase. For most published works, there is no data to evaluate if the antisolvent has created a homogeneous medium or a vapor-liquid system. Unless there has been a vapor-liquid system clearly created, we have included all works in this review article. We suggest that cases in which the feed contains high amounts of water are better described as extractions, since water and CO_2 are not miscible and therefore it is less likely that a homogeneous mixture was formed.

The amount of CO_2 necessary to force precipitation of solutes depends on the concentration of the feed solution. For instance, concentrated solutions get saturated with a low amount of CO_2, while diluted solutions require higher amounts of CO_2 to induce solid phase formation (12). The type of nozzle used for mixing the feed and the CO_2 may also have an effect on particle size and yield (13).

In the most recent publications (after 2010), particle size, size distribution and morphology of the precipitates is often reported. It has been shown that an increase in pressure may lead to a decrease in particle size and particle size distribution, presumably due to an increase in supersaturation of the liquid feed. As pressure increases so does density of the CO_2, higher amounts of CO_2 are introduced into the liquid solvent forcing precipitation of the solutes (11, 14). Similarly, lower temperature may lead to a smaller particle size (14, 15). If temperature increases solubility of the solutes in the fluid phase, supersaturation is reduced and bigger particles are formed (16). The proportion antisolvent/solvent did also influence particle size. The proportion is adjusted by their respective flow rates during a simultaneous contact mode. Higher proportions of antisolvent may lead to smaller particles (14). In this case, solubility of the solute in the fluid mixture is lower, thus increasing supersaturation.

GENERAL INSTRUMENTAL SETUP AND CONTACT MODES

The instrumental setups described in the literature are the same as those used for a regular SAS process. The basic parts of a setup are an antisolvent cylinder, a cooler, a high-pressure pump for the antisolvent, a high-pressure pump for the liquid solution (optional), a high-pressure chamber for precipitation (normally with a filter to collect the produced particles at the bottom), a chamber heater and a heated micro metering

valve or back pressure regulator in the vent. A separation vessel is needed for cases where the compound of interest is collected in the soluble fraction. No significant developments have been found, although some authors have paid attention to the design of the nozzle used to mix fluids. For a more detailed description of instrumental setups we refer the readers to a recent review by Torres et al. (2016) (6).

The operational mode varies among the literature. In general, we can distinguish between two contact modes, in which the two streams are introduced simultaneously or sequentially into the precipitation chamber. Simultaneous loading is more attractive from an industrial point of view, since the process can be run in continuous and semi-continuous operational mode. In these cases, the high-pressure vessel is initially heated and pressurized with antisolvent or with a mixture of antisolvent plus the same organic solvent as in the liquid feed (Figures 2a, 2b). The latter case requires two high-pressure pumps. The extra high-pressure pump introduces the liquid feed into the vessel. At this point, both antisolvent and liquid feed enter and exit the vessel at a particular flow rate, normally controlled by a back-pressure regulator, while pressure and temperature inside the vessel is kept constant. The liquid feed is normally introduced in the vessel from the top phase and forming an aerosol, which causes a fast contact between solution and antisolvent. A particular nozzle may be used to enhance mixing of the two fluids. Particles are continuously formed. After a desired time, the liquid feed is stopped while neat antisolvent is fluxed through the system to ensure that the particles are dried. In simultaneous contact mode, mixture composition is kept constant throughout processing time, resulting in a homogeneous composition of the particles. Most works in the literature report simultaneous loading.

In the case of sequential loading, the process is often described in the literature as a batch operational mode. Normally, the liquid feed is initially placed in the precipitation chamber. The chamber is heated at the desired temperature. Afterwards, the antisolvent is introduced until the desired pressure is reached. Precipitation of certain solutes occurs as the solution gets in contact with the antisolvent. After a holding time, the mixture of solvent, soluble solutes and antisolvent is removed from the chamber, while the particles are collected on a filter at the bottom of the chamber (Figure 2c). Further drying of the particles may be necessary after collection. An alternative batch mode starts by introducing the antisolvent in the high-pressure chamber, while adding the liquid feed afterwards (Figure 2d). This mode requires two high-pressure pumps, one for the antisolvent and the other one for the feed. In sequential loading, mixture composition changes over time, which may lead to particles of different composition.

ORIGIN OF THE PROCESS AND TERMINOLOGY

The concept of selective precipitation from an organic solution of multiple components using compressed fluids has its origin on the fractionation of polymers. However, the first authors to explore the concept with compressed CO_2 for the purification of natural products were Shishikura et al. in 1992 (17). Their initial work used compressed CO_2 in its gaseous form. The authors called the process Gas Antisolvent Crystallization, which is a term derived from Supercritical Antisolvent Recrystallization, previously coined by Gallagher et al. (18) in 1989. Both terms are currently used by some authors when applied to the purification of natural products.

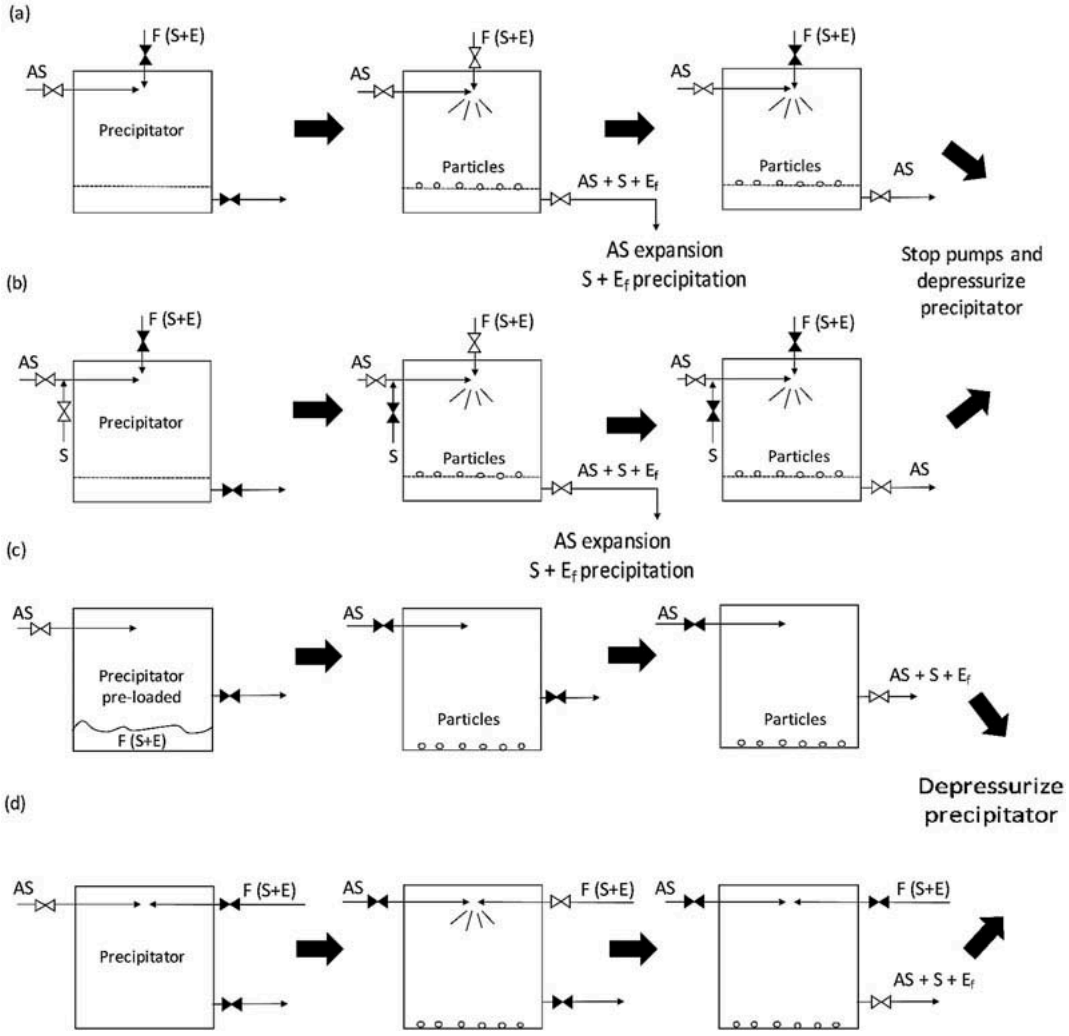

Figure 2. Schematic representation of simultaneous (a, b) and sequential (c, d) contact modes between streams in a selective precipitation by SAS/GAS. AS, antisolvent agent; F, liquid feed; S, liquid solvent; E, extract; E_f, fraction of extract soluble in the solvent mixture.

The term Gas Antisolvent Fractionation was used for the first time to separate natural compounds in a mixture by Catchpole et al. in 1996 (19). Catchpole et al. were also the first ones to coin the term Supercritical Antisolvent Fractionation (SAF) in 2004 (20).

The term ′fractionation′ emphasizes the occurrence of a phase transition leading to separation. It also reflects that the composition of the different fractions varies, which transmits the idea of an enrichment. The newly created phase during a fractionation may be a solid or a vapor (e.g. in a distillation process), and so the term SAF does not necessarily convey the idea of a precipitation.

Reverchon and coworkers referred to the same process as Supercritical Antisolvent Extraction (SAE) in 2006 (see Figure 1). These authors describe SAE as the process in which solid compounds that are initially dissolved in a liquid mixture, precipitate upon contact with the antisolvent. The process is seen as a selective ′extraction′ of the solid compounds that are initially dissolved in the liquid mixture (5). The concept is applied whether the precipitate is the compound of interest or the impurity. Our personal remarks about this definition are that a solid dissolved in a liquid mixture is no longer a solid, unless we are talking of a dispersion. Furthermore, ′extraction′ applies when a certain compound is transferred between two existing phases, while the process at hand starts from one homogeneous mixture created when solvent and antisolvent mix.

The term Supercritical Antisolvent Precipitation was used by Reverchon et al. in 1998, applied to the production of particulate inorganic material (21). Numerous authors currently apply it (or its Gas analogue) when they use compressed CO_2 to selective precipitate specific compounds from a natural extract. Supercritical Antisolvent pulverization has also been used in a few cases. While these terms relay information about the occurrence of a phase separation and formation of a precipitate, they do not transmit the idea of a potential purification or enrichment of the initial liquid mixture.

There is no consensus in the literature on how to name such a process. Among the works that do use a particular acronym, which is very practical, the term SAF prevails over others. The term ´fractionation´ does not distinguish whether the compound of interest precipitates or needs further drying and has been applied in both cases. The majority of authors refer to their process without any specific name, but rather as a purification/selective precipitation/precipitation/micronization by SAS/Supercritical Antisolvent technique or process. Our recommendation aligns with the ISO 704:2009 (22) and it is to use terms that convey all the necessary concepts and are already well described and generally accepted, like for example ´selective precipitation by SAS/GAS´.

It is worth noticing that the use of the word ´supercritical´ or ´gas´ is only reasonable if we assume that it refers to the state in which the neat antisolvent would be at the temperature and pressure used. In fact, most of the works found in the literature operate at pressure and temperature conditions in which the binary mixture organic solvent+CO_2 is not above its supercritical point. This is to say that the terminology would be incorrect should it be applied to the state of the binary mixture organic solvent+CO_2.

Since an important advantage of selective precipitation by SAS is its capacity to enrich extracts, it seems relevant to report values of precipitation yield, purity or concentration of compound(s) in the precipitate and soluble fraction, recovery of target compound or fraction precipitated, and enrichment factor or purity enhancement.

We suggest the following definitions:

- Precipitation yield may be expressed as weight of particles by weight of feed material. When the target compound is the soluble fraction, the same concept may be expressed differently.
- Concentration may be expressed as % by mass of solids or mass of compound/mass dry material.
- Recovery of target compounds may be expressed as weight of compound in the precipitate (or soluble fraction) by weight of compound in the feed material. This value is often given in percentage.
- Enrichment may be expressed as concentration of compound in the precipitate (or soluble fraction) by concentration of compound in the feed. This gives a unitless number that can be considered an enrichment factor. Another option is to report a relative enrichment, by simply subtracting the unit.

The reader however will not find a consistent way of reporting this information in the literature. Some authors report enrichment as a percentage, considering that 100% is the concentration of compound in the feed, and therefore giving values higher than 100%. The word ´enrichment´ is used in some works to indicate the concentration of compound in the final product.

The next section reveals significant works found in the literature where selective precipitation by SAS/GAS was used for the separation of natural extracts.

APPLICATIONS OF SELECTIVE PRECIPITATION BY SAS/GAS

For clarity, this section refers to the separation process as selective precipitation by SAS/GAS, regardless the name used by the respective authors. As it was stated at the beginning of the introduction, there is confusion about what the process entails. The

scope of the technique is unknown due to the difficulty of finding works that exploit its potential for purification and drying.

With the intention to structure an otherwise scattered literature, this section is organized according to target compound or family of compounds, i.e. polyphenols, carotenoids, lipids, terpenoids, carboxylic acids, proteins, polysaccharides, alkaloids and sesquiterpenes. All works are potentially significant and will be mentioned, as they help to define the scope of the technique. However, only those in which SAS/GAS leads to precipitation and purification of the compound of interest will be presented in detail. When possible, we offer a discussion on the progress of the topic within each specific category of compounds.

We report the values of different parameters according to the definitions given in the previous section of this review, which in some cases do not correspond to the definitions given by the authors of a particular application. The aim with this approach is to facilitate comparisons among the literature. The literature has been summarized in Table 1. Table 1 uses raw material as entry and shows only the works that report an enrichment of the original natural extract. Most of the reported works are dedicated to the purification of polyphenols and carotenoids and are operated in the simultaneous contact mode (Figure 3, Table 1).

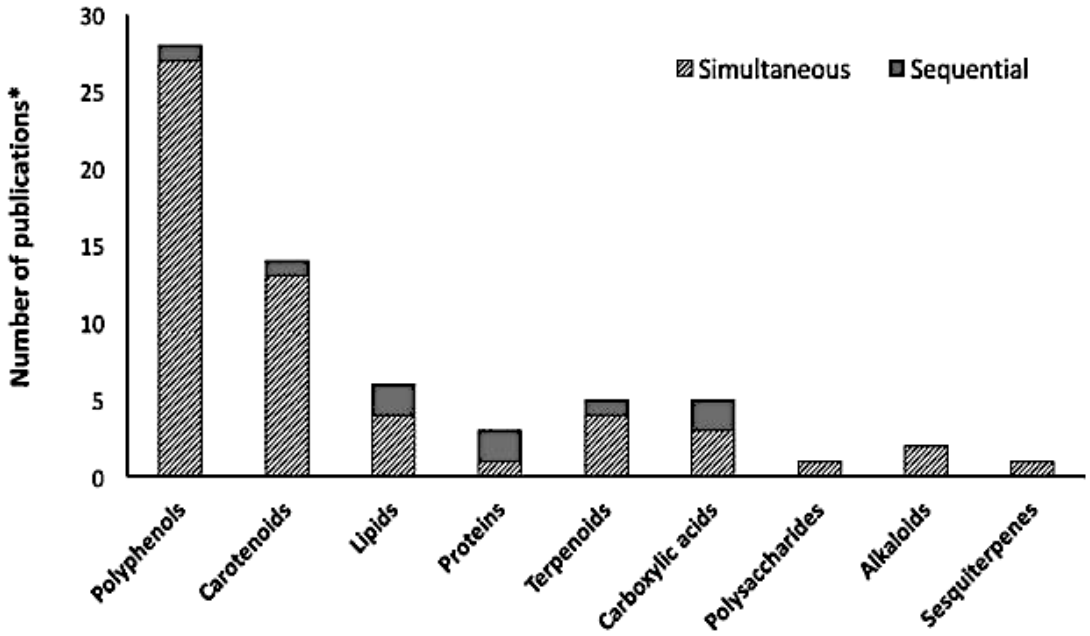

Figure 3. Frequency of appearance of published works classified by the nature of the target compounds. Simultaneous or sequential contact modes are showed in dark or light grey respectively. *The total number of publications is 48.

Polyphenols

Catchpole et al. (2004) (20) used SAS to isolate flavonoids from propolis. Flavonoid pigments are the major constituent of propolis, known to possess antitumoral, anti-inflammatory and antiviral properties among others (23). $ScCO_2$ was used to precipitate high molecular mass components, considered to be impurities, from ethanolic and ethanol/water extracts of propolis (i.e. propolis tincture) to obtain a flavonoids-rich soluble fraction. Although the target compounds were not the precipitated ones, this is a seminal work and therefore it is explained here in further detail. Furthermore, experiments were performed in laboratory and pilot-plant scales, and finally validated in a semi-commercial scale. To this date, this is the only work scaled up to semi-

commercial scale. The concentration of flavonoids in the soluble fraction doubled with respect to the feed solution in the pilot scale. Flavonoids contents of 25-30% by mass were obtained for a tincture concentration of 10% by mass of total propolis solids, at 60 °C, using ethanol as the tincture solvent and a pressure range of 27.5-30 MPa. The precipitates contained 5-10% of flavonoids. These results have not been improved yet, despite recent efforts (24).

Since then, several works have approached the purification of flavonoids. Catechin (flavan-3-ols) are the main type of flavonoids present in green tea. These compounds have shown antioxidant, anticancer, anti-inflammatory, antibiotic and antiviral effects effects (25–29). Sosa et al. (2011) (30) co-precipitated an encapsulating agent and catechins from a green tea extract using acetone as solvent and CO_2 as antisolvent. While the aim of the work was not to enrich the extracts in a particular compound, co-precipitates contained only 13% of the initial caffeine while more than 90% of the catechins, clearly indicating an enrichment in polyphenols compared to the initial extract. A more drastic separation of caffeine from catechins in green tea extracts was achieved by Rodriguez-Meizoso and co. (2015) (31). The feed solution was a catechin-caffeine rich extract obtained by pressurized liquid extraction using ethyl acetate as solvent at 100 °C and 10 MPa. Precipitates with less than 1% by mass of caffeine and 23% by mass of catechins were obtained at 70 °C and 30 MPa, using CO_2/feed flow ratios of 40 mL/mL. 93% by mass of the caffeine in the feed was separated from the precipitate. The total phenolic content of the precipitates was up to 590 mg of gallic acid equivalents/g of precipitate, which represents an increase of up to 25% with respect to the feed solution. Catechin recoveries ranged from 69 to 82%, depending on the specific compound.

Phenolic compounds from yarrow (*Achillea millefolium* L.) have been attributed choleretic, antioxidant, anti-inflammatory, antimicrobial and antimutagenic activity (32–36). Fornari and co. (2017) reported the effect of SAS in the precipitation of phenolic compounds from an ultrasound-assisted ethanolic extract of yarrow (11). The concentration of phenolic compounds in the precipitate was up to 3 times higher than the feed, and particles of 269 μm mean size were obtained at 40 °C, 10 MPa, using a feed concentration of 17.9 mg/mL and flow rates for CO_2 and feed of 50 g/min and 1.6 g/min respectively. The most abundant polyphenol identified was luteolin-7-O-glucoside (a flavonoid) and the highest recovery reported for total phenolic compounds was 40.9%. Adding to this study, the same research constellation confirmed that the higher content of polyphenols in the precipitate translates into a higher antioxidant activity than the feed. The precipitate also presented anti-inflammatory activity, although lower than the soluble fraction (37).

Reporting particle size, size distribution and morphology has become a common practice during the present decade, even if purification was not achieved. Such is the case of flavonoids precipitated from a *Ginkgo biloba* extract using CO_2 as antisolvent (38). Particles of 81.2 nm were obtained, and they showed a great increase of dissolution rate when compared to a non-SAS dried extract. Although no purification was achieved, this work is mentioned here because it shows how particle features translate into a desirable property. This is the only work that not only reports particle sizes but also relates them to a particular benefit.

Finally, rutin, a glycosylated flavonoid present in *Amaranthus paniculatus* leaves, was purified when a water/ethanol extract of the leaves was put in contact with $scCO_2$ (39).

However, the high amounts of water used in the solvent mixture suggest that the process may be better described as an extraction.

Chalcones are precursors in the synthesis of flavonoids. Purification of licochalcone A from licorice root by GAS has been attempted, but there was no data reporting its concentration in the precipitate with respect to the feed (40).

Curcuminoids are a type of polyphenols valuable due to their intense yellow color, anticancer, antibacterial, chemopreventive and chemotherapeutic activities (41, 42). Meireles and co. (2016) (13) purified curcuminoids from turmeric (*Curcuma longa* L.) rhizomes by SAS. An ethanolic extract was obtained from de-flavored turmeric using pressurized liquid extraction, and it was used as feed for the SAS process. The effect of nozzle type, pressure, temperature and CO_2 flow on the curcuminoids precipitation process was evaluated based on an experimental design. This is the only work within the literature that explores the effect of different nozzle design. The results using a T-mixer nozzle showed that a temperature of 40 °C, a pressure of 10 MPa and flow rates of CO_2 and feed of 500 g/h and 0.5 mL/min respectively gave the best conditions considering precipitation yield, curcuminoid content and particle size all together. The concentration of curcuminoids in the precipitates under such conditions was 524 mg/g, with recoveries of 91%. The content of curcumin in the precipitates was 31 times higher than the content in the feed solution. SAS performed with other solvents like dichloromethane and dimethyl sulfoxide was not successful (43).

Lignans are appreciated due to their numerous biological activities (e.g. anti-allergic, anticarcinogenic, liver protective). Their precipitation by SAS has not been achieved so far. Huang et al. (2013) purified lignans from a *Schisandra chinensis* extract by SAS but

they were recovered in the soluble fraction (44). The process required purification of the feed (and ethanolic solution) by column fractionation prior to SAS. The total concentration of lignans increased from 206.3 mg/g in the feed to 581.84 mg/g in the effluent with a recovery of 84%. Perretti et al. (2013) fractionated lignans from flaxseeds (from flax, *Linum usitatissimum* L.) with the aid of $scCO_2$ (45), but the raffinate was not a dry precipitate and we do not consider the process an antisolvent precipiation.

Polyphenols from grapes, and in particular anthocyanins, are valuable for food, pharmaceutical and cosmetics applications due to their antioxidant activity. Their recovery by SAS required in all cases an extra step to purify the liquid feed prior to precipitation. Polyphenols were isolated from grape seeds vinification wastes by Marqués et al. (2013) (46). The feed consisted of an ethanolic extract previously defatted with hexane. Gallic acid, catechin, epicatechin and resveratrol were precipitated with reported relative enrichments of 100, 250, 267 and 78% respectively upon contact with $scCO_2$ at 15 MPa, 40 °C, CO_2 flow rate of 2.38 kg/h and a molar fraction of CO_2 of 0.98. Recovery of polyphenols in the SAS process at those conditions was 70%, although precipitation yield was lower than 30%. Previous attempts had failed to enrich the product, although they managed to produce dry particles containing polyphenols (47, 48). Proanthocyanidins were precipitated by SAS from an ethanolic extract of grape marc (49). The extract had also been previously defatted, in this case by $scCO_2$ extraction. Relative enrichments of 350% of total polyphenols were achieved at operating conditions of 12 MPa, 45 °C and 0.99 CO_2 molar fraction, corresponding to a total amount of polyphenols of 300.9 mg galic acid equivalent/100 g dried grape marc. A relative enrichment of oligomeric and polymeric proanthocyanidin fractions between 300 and 450% was achieved. High CO_2 molar fractions gave the highest recovery of

polyphenols, and proanthocyanidins in particular. Particles had an average particle size of 5 μm. Recoveries ranged from 53 to 94%, depending on the specific compound. The same year, Mezzomo et al. (2016) used SAS to co-precipitate grape pomace from Merlot (*Vitis vinifera*) with poly(-lactic-co- glycolic acid). The goal was to encapsulate and preserve the antioxidant compounds (presumably polyphenols) present in the pomace (50). The work however does not report data on a possible enrichment of the pomace or change in antioxidant activity. Although the antioxidant properties of the compounds of interest was of main interest, none of these works on grapes performed antioxidant assays. In fact, it is mostly in the last 5 years that authors include studies on the biological activity of the precipitate. The following are some examples.

Anthocyanins were precipitated by SAS from an ethanolic extract of blackberry residues (51). The amount of total monomeric anthocyanins and total phenolic compounds in the precipitate doubled with respect to the feed (up to 16.7 mg Cy3GI equivalents/g dry sample, and 101.5 mg gallic acid equivalent/g sample) when CO_2 was used at 30 °C, 12.5 MPa, 2.0 kg/h and for a feed flow rate of 1.0 mL/min. The antioxidant capacity of the precipitates under the same conditions was seven times higher than the feed (3796 μmol Trolox equivalent/g sample). The extract was also co-precipitated with polvinylpyrrolidone (PVP), although the presence of PVP lead to much lower purification values. The co-precipitates obtained at the same conditions as described above, and with a PVP concentration in the extract of 0.5%, lead to particles with the smallest diameters (below 40 μm) and particle size distribution, a recovery of 64%, however the highest hygroscopicity (about 14 g adsorbed water/100 g dry sample).

Polyphenols from rosemary (*Rosmarinus officinalis*) are used by the food industry as antioxidants. Their effect in the treatment of cancer and neurodegenerative diseases has

also been studied (52-54). Therefore, there is an interest in developing processes to efficiently extract and purify the antioxidant fraction from rosemary leaves. The literature shows that rosmarinic acid can be precipitated from water/ethanol extracts (52, 53) when in contact with $scCO_2$, while phenolic diterpenes like carnosic acid can only be recovered as the soluble fraction from ethanolic (55) or water/ethanol (52, 53) extracts. The best conditions to fractionate an ethanol rosemary extract were 50 °C and 30 MPa, giving a fraction with a carnosic acid (CA) concentration 10 times higher than the oleoresin, and with antioxidant properties superior to the synthetic antioxidant butylhydroxytoluene (55). The high amount of water of some of the water/ethanol extracts (52, 53) raises doubts about the process being an extraction rather than a selective precipitation by SAS. Nevertheless, the strength of those works lies in giving a purpose to the fractions by demonstrating that all fractions possessed antioxidant and antiproliferative activity against HT-29 colon cancer cells.

Mango (*Mangifera indica* L.) plant contains phenolic compounds valuable for their antioxidant properties. Mangiferin and benzophenone derivatives such as iriflophenone 3-C-β-D-glucoside and iriflophenone 3-C-(2-O-p-hydroxybenzoyl)-β −D-glucoside were extracted from mango leaves with ethanol, and precipitated by SAS with enrichment factors up to 2.8 (56). As it is common in recent works, the size of the particles and their bioactivity (in this case antioxidant activity) is reported. The smallest particles (0.04 μm) were obtained at 15 MPa and 35 °C with a feed concentration of 20 mg/mL. This sample showed the highest concentration of phenolic compounds and antioxidant activity. The range of flow rates studied (5 and 10 mL feed/min, 30 and 40 g CO_2/min) did not have a significant influence in particle size. Attempts to purify

phenolic compounds from other parts of the plant (i.e. by-products of mango canned fruits and mango juice) by SAS were not successful (57).

A different type of phenolic compounds valued for their antioxidant properties are present in olive (*Olea europea*) leaves. Chinnarasu et al. (2015) obtained an extract by supercritical fluid extraction using CO_2 plus ethanol as solvent mixture. Tyrosol derivatives and oleuropein were precipitated by SAS (15) and separated from flavone glycosides of less polar and less antioxidant character. This work shows how the concentration of the feed could be critical for the process. Only feed concentrations higher than 32 mg/mL allowed precipitation. In addition, lower temperatures (i.e. 50 °C compared to 60 °C), higher CO_2 rates (i.e. 30 g/min compared to 11 g/min) and higher pressures (i.e. 15 MPa compared to 10 MPa) gave smaller particle size and particle size distributions. Spherical particles with mean particles sizes in the range 300–1060 nm were formed. The antioxidant activity of all SAS precipitates was much higher than the feed. Baldino et al. (2018) approached the purification of oleuropein using an ethanolic extract as feed. Their work includes quantitative analysis of the different products and makes it possible to evaluate the degree of purification achieved. The concentration of oleuropein increased from 20 wt.% in the feed to 36 wt.% in the precipitate at operating conditions of 15 MPa, 35 °C and 0.98 CO_2 molar fraction (8).

This section finalizes mentioning the work of Tjandrawinata and co. (2013, 2017). They applied SAS on extracts from curative Indonesian herbs (58, 59). The specific chemical nature of the targeted bioactive compounds is not reported, but it is most likely to be polyphenols. Their first work does not report quantitative measurements, however the latest one is one of the few examples in the literature that aim at separating a compound of interest from a known impurity. A target fraction DLBS3233 was separated from

coumarin during SAS, while the total polyphenol content in the precipitate was the same as the extract, 37%. The bioactive fraction DLBS3233 was obtained by extracting *Lagerstroemia speciosa* and *Cinnamomum burmannii* with water at 70 °C. DLBS3233 was re-dissolved in dimethylformamide and submitted to SAS. The effect of CO_2 pressure (10-20 MPa), temperature (35-55 °C), feed solution flow rate (0.53-2.19 mL/min) and feed concentration (5 -20 mg/mL) on particle size was evaluated. The CO_2 flow rate was 30 g/min. Particles size ranged from 0.107 to 0.298 μm.

Carotenoids

The extraction of carotenoids has been widely explored due to their interest as natural antioxidants. Carotenoids are precursors of retinol and photoprotectors from UV radiation (60). The relation between intake of carotenoids and a decreased risk of cancer and cardiovascular diseases has also been studied (61, 62).

Carotenes and xanthophylls have been purified by selective precipitation using SAS/GAS. From the perspective of product development, the liquid feed would rather be a solution of a natural extract that has not been previously enriched by any other kind of separation technique, so as to develop a process with as few steps as possible. The only example of such kind is the work by Mukhopadhyay and Patel (2009). They recovered of β-carotene recovered from mango (*Mangifera indica* L.) leaves (40). The process was performed in a sequential loading mode with a CO_2 molar fraction of 0.8. The extract was obtained with $scCO_2$ (30 MPa, 40 °C) and dissolved in ethyl lactate to be used as feed solution. Operating parameters for SAS were 25 °C and pressures in the range of 4-7 MPa. The concentration of β-carotene increased from 1.02% in the feed to 5.66% in the crystals obtained.

The rest of the literature describes the purification of carotenes and xanthophylls introducing column fractionation with polystyrene based resins as an enrichment step prior to precipitation by SAS. In a series of articles, Chang and co. purified β-carotene from microalgae *Dunaliella salina* (63, 64), fucoxanthin from microalgae *Hincksia mitchellae* (65), zeaxanthin and zeaxanthin dipalmitates from marine microalgae *Nannochloropsis oculata* (66-71) and boxthorn (*Lycium barbarum*) fruit extracts (72-74). The differences between these works lie on the extraction method (ultrasound-assisted extraction, Soxhlet) and the type of solvent used for extraction and chromatography (tetrahydrofurane, ethyl ether, ethyl acetate, acetone, hexane:acetone). With very few exceptions, these works include an experimental design with purity of the precipitate as response. Some of these works also explore the effect of counter-current vs con-current flow type nozzle configuration. A counter-current flow leads to higher purities and smaller particle size. The best enrichment factors, 2.4, were obtained for zeaxanthin from *Nannochloropsis oculata* (67), with a recovery of 90%.

The same type of procedure was applied by Wu et al. (2017) to purify lycopene from Bitter melon (*Momordica charantia* L.) aril (75). After chromatographic fractionation, the concentration of lycopene increased more than seven times to yield a product almost completely pure. Subsequent SAS treatment could not possible increase purity further, but it produced a particulate dry material when THF was used as feed solvent.

Lipids

The refining of edible oils is an industrial process necessary to improve flavor and color of the product, and to increase stability and processability. Furthermore, certain lipids

like phospholipids are excellent natural emulsifiers, valuable in cosmetic, food and pharmaceutical applications.

In 1996, Catchpole et al. published the first study focused on lipid fractionation using gaseous CO_2 as antisolvent agent (19). Two mixtures were used as feed, lecithin/soya oil/hexane and coriander seed triglycerides/essential oil/hexane. Results showed that the concentration of triglycerides in both mixtures influenced the degree of separation of lecithin from triglycerides in soya oil and triglycerides from coriander essential oil. A soluble fraction with 90% by weight of coriander essential oil was recovered at 35 °C and 7 MPa. At lower pressures and for certain lipid ratios, precipitation of lecithin from the feed solution by GAS was successful, leaving a soluble fraction with virtually no lecithin present.

Based on these findings, Mukhopadhyay and Singh (2004) approached the separation of lecithin from neutral lipids (oil) in a mixture composed by crude soya lecithin and soybean oil in hexane (76). The authors claimed that using low pressures of gaseous CO_2, a high purity lecithin fraction was obtained by GAS. The feed solution was prepared with several lipids to solvent ratios. Lecithin crystals were formed as lecithin solubility decreased in the compressed solvent mixture (hexane + oil + CO_2). Results showed the highest selectivity (defined as the weight % ratios of lecithin and oil in the product divided by the same ratio in the feed solution) of lecithin precipitation at 25 °C and 6.5 MPa for a 100% recovery of lecithin. Similar conditions (25 °C and 5.8 MPa with a 60 wt.% w oil in the feed) resulted in the highest enrichment of the crystals in lecithin (from 60 wt.% to 98 wt.%) with recoveries around 80%.

In later years, the feed went from being model mixtures to natural raw materials. Catchpole et al. (2008) separated a mixture of neutral and polar lipids (mostly phospholipids) from proteins and lactose in milk (77). The feed solution was a liquid beta-serum fraction from milk. Dimethyl ether (DME) was used as antisolvent to precipitate proteins, lactose and most of the water, while it dissolved lipids and some of the water. This is the only example in the literature where a fluid other than CO_2 was used as antisolvent. DME was used in liquid state, at 60 °C and 4 MPa. Feed-to-DME flow rate ratios used varied from 0.1 to 0.35, and feed solids concentrations from 10 to 25 mass%. The lower those values were, the higher the yield of phospholipids. The process was run continuously for 6-12 h with sample collection every 15 or 30 min, which makes it attractive from an industrial point of view. Two liquid phases were obtained, a protein-rich aqueous fraction and a lipid-rich DME fraction. Presumably, the process was technically an extraction. The fractions were collected from the bottom and the upper part of the separator respectively. Since DME is a liquid at normal conditions, the DME fraction was passed through a pressure reduction valve and heat exchanger in order to remove the DME. In general terms, all tested conditions achieved 70% extraction of the total lipids, reaching even higher numbers (~90%) when the protein-rich fraction was re-precipitated in a second antisolvent process. In addition, an enrichment in total phospholipids and total fat content was observed in comparison with the feed solution, i.e. from 14 to 39 wt.% and 34 to 97 wt.% respectively.

Continuing with the purification of phospholipids from natural raw materials, Aro et al. (2009) extracted and isolated phospholipids from egg yolk (78). An ethanolic extract was used as feed solution. CO_2 was used as antisolvent at 27 MPa, 70 °C and a flow rate of 0.25 L/min. The feed was introduced in the precipitation chamber at 6.5 mL/min

through a spray nozzle. Feed concentrations of 10 g/L led to precipitates with a combined phosphatidyl cholines and phosphatidyl ethanolamines amount of up to 990 mg/g, free from cholesterol. This result indicates that up to 50% of the lipids from egg yolk were recovered. The recovery of phospholipids from the feed to the precipitate reached up to 95%. The same research group extracted polar lipids from oats using $scCO_2$ plus ethanol as cosolvent (79). However, applying CO_2 as antisolvent did not lead to further purification.

Other lipid compounds, presumably steroid derivatives, from Brazilian ginseng roots have not been successfully precipitated (80).

Terpenoids

Initial works on the isolation of terpenoids by SAS/GAS were either not successful in purifying the extract (38), did not report data on purification (81) or recovered terpenoids in the soluble fraction (82, 83). The latest examples reported a significant purification and therefore it is described in further detail. Ryanodol is a diterpenoid present in laurel (*Persea indica*) leaves and stems (82). The isolation of ryanodol is especially interesting for its use as biopesticide. A concentrated ethanolic extract (3% of solids content approximately) was used as feed solution and $scCO_2$ as antisolvent. Ryanodol was recovered as the soluble fraction at 15 MPa and 35 °C, with a CO_2 molar fraction of 0.9. The content of ryanodol in the feed solution was 7.5% by mass and, after precipitation of the impurities, it increased up to 37.7% by mass, with a 42.9% recovery. The antifeedant activity of the soluble fraction was also investigated, although it did not correlate with the amount of ryanodol (83).

More recently, Chinnarasu et al. (2015) recovered terpenoids from eucalyptus (*Eucalyptus globulus*) leaves (14). As it is common in recent works, the authors reported the antioxidant activity of the precipitate, as well as particle size. The feed solution was obtained by extraction with $scCO_2$ and ethanol as cosolvent. Precipitates with the smallest particle size (0.27 μm approx.) as well as the highest antioxidant activity were obtained at 35 °C and 15 MPa with flow rates for CO_2 and feed of 20 g/min and 5 mL/min respectively. The applied SAS conditions provoked the precipitation and concentration of some bioactive compounds such as *p*-cymene and DL-limonene initially present in the feed. However, other antioxidants like linalool were soluble in the mixture.

Carboxylic acids

In a seminal work, Shishikura et al. in 1992 (17) used gaseous CO_2 to purify citric acid from an acetone mixture containing mainly citric acid and sugars. The impurities (sugars) were insoluble in acetone in the presence of the antisolvent. The separation was most effective for pressures below 2.7 MPa. At higher pressures, crystallization of citric acid occurred. Most citric acid was recovered in the form of crystals at pressures near 5.3 MPa.

3,5-diprenyl-4-hydroxycinnamic acid (DHCA), known as Artepillin C, has been reported to inhibit the growth of certain cancer cells (84, 85) and to possess antioxidant activity (86). Producing microparticles of DHCA is interesting to increase its bioavailability. DHCA was recovered from de-waxed Brazilian propolis lumps by Soxhlet extraction with ethyl acetate and precipitated by SAS (10, 87). Pressure and temperature used for precipitation were 20 MPa and 55 °C respectively. According to

an experimental design, a CO_2 flow rate of 10 L/min, feed flow rate of 1 mL/min and feeding concentration of 9 mg/mL led to precipitates with DHCA concentration of 29.9% by mass, that is increased by a factor of 1.61 with respect to the feed solution, with DHCA recoveries of 92.6%. Narrow particle size distribution was achieved with higher flow rates of CO_2 and a low feeding concentration. The precipitates displayed anti-carcinogenic properties by inhibiting the growth of human colon and breast cancer cells. Very similar results were reported by the same research constellation previously that year (87). More recently, Monroy et al. (2018) approached the precipitation of DHCA and 4-hydroxycinnamic acid (HCA) from ethanolic and hydroalcoholic extracts by sequentially introducing the feed into four separators (24). The separators were filled with CO_2 at different operating pressures. Recovery and concentration of DHCA in the precipitate did not improve with respect to Wu et al. (2009), and not enough data was presented to evaluate the degree of purification achieved. Based on the descriptions made by the authors, the process was likely to be an extraction when hydroalcoholic extracts were used as feed.

Mukhopadhyay et al. (2009) reported that the phytochemical alpha-hydroxycitric acid (α-HCA) was extracted from kokum (*Garcinia cambogia*) with acetone at 40 °C and precipitated by SAS with CO_2 operating at 25 °C and pressures in the range of 4-7 MPa. The concentration of α-HCA increased significantly from 21% in the feed to 72% in the precipitate and was separated from citric acid (40).

Proteins

Recovery, purification and precipitation of proteins without losing their biological activity is important for drug delivery applications. Successful separation and

purification of proteins was achieved by Winters et al. (1999) using model mixtures (88) of lysozyme + ribonuclease and alkaline phosphatase + insulin. The mixtures were dissolved in DMSO and CO_2 was used as antisolvent at 34 °C and 4.9 MPa. A lysozyme precipitate with no detectable ribonuclease was obtained. Precipitated lysozyme recovered 72 ± 2% of its initial biological activity. Using the same pressure, alkaline phosphatase was precipitated, and the precipitate contained no insulin. Alkaline phosphatase, however, lost its biological activity.

Polysaccharides

An optimized separation of lignin, cellulose and hemicellulose from plant material is essential for effective biorefinery processes. Haimer et al. (2010) used CO_2 at 40 °C and 15 MPa to precipitate hemicellulose from DMSO solutions. Flow rates of CO_2 and feed were 5 kg/h and 2 mL/min. The lignin content of the initial hemicellulose samples decreased from 49% to 85%, depending on the type of hemicellulose studied without affecting the structure of the polymer. Particle sizes were small (around 0.5 μm) and uniform (89).

Alkaloids

Alkaloids have so far been recovered in the soluble fraction. Purification of alkaloids from tobacco has been attempted without success (90). The soluble fraction showed increased concentration of nicotine, but it was also enriched in lipids, and the concentration of proteins did not decrease. In a different study, caffeine was separated from catechins from a green tea extract (31). The aim of the work was however to precipitate catechins. Details can be found under the polyphenols section.

Sesquiterpenes

There is no clear evidence of the purification of sesquiterpenes by SAS. Benelli et al. (2014) obtained particles from the extract of a medicinal Brazilian plant, *Casearia sylvestris* or guacatonga, co-precipitated with the biopolymer Pluronic F127 (91). The extract is expected to contain biologically active sesquiterpenes, but there is no data to evaluate the composition of the precipitates nor the biological activity.

All the works mentioned in this section are research articles or conference proceedings. No patents have been granted on the process. There is one patent on the fractionation of natural extracts using near-critical fluids as antisolvent (92). However, the process has been properly described as an extraction since it uses water/ethanol solutions with high proportions of water.

There is no much data to estimate costs of industrial applications of selective precipitation by SAS. In 2000, Michel Perrut published an interesting dissertation about the possible cost of a supercritical fluid process at an industrial scale (93). His work suggests that the investment cost associated to the technology may be compensated by operating high capacity units. In that sense, it would be economically beneficial to process raw materials that are produced in high amounts, like tea and herbs, or food residues. Minimizing special technological requirements will also reduce the prize. Therefore, processes in which the target compounds are present in the soluble fraction, or those requiring purification of the feed prior to SAS are less favorable. Operational costs may be reduced for processes that can be run in continuous mode or in long-duration batches, since they demand lower manpower operation. Multipurpose plants are also more economically advantageous, although special attention must be paid to cleaning operations. Processes working at low pressures (that is GAS) will minimize costs associated to CO_2 consumption. CO_2 may also be recirculated using a

recompression unit. However, since the amount of solvent used in SAS is higher than for a typical supercritical fluid extraction, a specific unit may be needed to condense and separate the solvent before CO_2 can be recycled.

CONCLUSIONS

Supercritical fluids constitute powerful antisolvents that have the potential to selectively precipitate specific compounds from a mixture, thus enriching the precipitate in the compound of interest at the same time as the precipitate is dried. The antisolvent and the solvent in which solutes are dissolved must be miscible. There is no agreement among the scientific community on what term to use when such process is applied to the fractionation of natural extracts. In this review, we find the expression ´selective precipitation by SAS´ most appropriate.

The concept of selective precipitation by SAS to purify natural compounds from a mixture was first reported in 1992, but scarcely investigated until the year 2010. The present decade has experienced a considerable increase in the number of publications, in particular on the isolation of polyphenols and carotenoids from plant leaves, herbs, fruits, microalgae and rhizomes. Initial works experimented with mixtures of model compounds. The field evolved to fractionate extracts of more complex nature and a few attempts have been made to co-precipitate the extract with an encapsulation agent. The most recent works often report the bioactivity of the particles (mostly antioxidant activity) and particle size, although it is rarely studied how a particular size translates into a beneficial property. Type of organic solvent, concentration of the liquid feed, pressure, temperature, and respective flow rates of feed and antisolvent are parameters that have an effect on total yield, recovery and concentration of the compound of interest

in the precipitate, as well as particle size and size distribution. Experimental designs have been used in some cases to evaluate the extent of these effects and results are case specific.

Despite the potential of selective precipitation by SAS for the fractionation of natural extracts, not all publications report an enrichment in the compound of interest. In our opinion, the most remarkable applications are those that aim at separating a target compound(s) from specific known impurities. Such examples are the separation of proteins from lipids in a beta-serum extract, separation of lysozyme from ribonuclease and separation of alkaline phosphatase from insulin in protein binary mixtures, separation of polyphenols from caffeine in a green tea extract, separation of polyphenols from coumarin in an extract of medicinal herbs, and separation of phospholipids from cholesterol in an extract of egg yolk.

All the research articles published on the topic in the last decade operate in continuous or semi-continuous mode, which could be valuable from an industrial point of view. However, only one process has been scaled up to semi-commercial scale (20), while the rest of the works operate in a laboratory or pilot-plant scale with precipitation chambers of up to 2 L. The lack of economic reports and the difficulty in screening the literature for significantly advantageous applications may explain the lack of industrial-scale processes on selective precipitation by SAS. Nevertheless, several successful examples of potential interest for the industry have already been reported. This review provides a clearer vision of the scope of the topic, which is meant to encourage further research in this promising field.

ACKNOWLEDGEMENTS

The authors acknowledge Lund University as a host institution.

FUNDING DETAILS

This work was supported by the European Union's Horizon 2020 research and innovation programme under the grant Marie Skłodowska-Curie Individual Fellowship Grant Agreement No. H2020-MSCA-IF-2016-746137.

DISCLOSURE STATEMENT

There are no potential conflicts of interest.

REFERENCES

1. Padrela, L., Rodrigues, M.A., Duarte, A., Dias, A.M.A., Braga, M.E.M. and de Sousa, H.C. (2018) Supercritical carbon dioxide-based technologies for the production of drug nanoparticles/nanocrystals – A comprehensive review. *Adv. Drug Deliv. Rev.* 131: 22-78.
2. Temelli, F. (2017) Perspectives on the use of supercritical particle formation technologies for food ingredients. *J. Supercrit. Fluids.* 134: 244-251.
3. De Marco, I., Rossmann, M., Prosapio, V., Reverchon, E. and Braeuer, A. (2015) Control of particle size, at micrometric and nanometric range, using supercritical antisolvent precipitation from solvent mixtures: Application to PVP. *Chem. Eng. J.* 273: 344–352.
4. Catchpole, O.J., Durling, N.E., Grey, J.B., Eltringham,W. and Tallon, S.J. (2009) in A. R. Duarte (ed.) *Current Trends of Supercritical Fluid Technology in Pharmaceutical, Nutraceutical and Food Processing Industries*, Supercritical antisolvent fractionation of plant extracts. Betham Science Publishers: Lower

Hutt, New Zealand.

5. Reverchon, E. and De Marco, I. (2006) Supercritical fluid extraction and fractionation of natural matter. *J. Supercrit. Fluids*. 38: 146-166.
6. Torres, A.E.C., Santana, A.L., Santos, D.T. and Meireles, M.A.A. (2016) Perspectives on the application of supercritical antisolvent process for the purification of plant extracts: effects of operating parameters and patent survey. *Recent Patents Eng.* 10: 88-97.
7. Martín, L., Marqués, J.L., González-Coloma, A., Mainar, A.M., Palavra, A.M.F. and Urieta, J.S. (2012) Supercritical methodologies applied to the production of biopesticides: A review. *Phytochem. Rev.* 11: 413-431.
8. Baldino, L., Della Porta, G., Osseo, L.S., Reverchon, E., and Adami, R. (2018) Concentrated oleuropein powder from olive leaves using alcoholic extraction and supercritical CO_2 assisted extraction. *J. Supercrit. Fluids*. 133: 65-69. doi:https://doi.org/10.1016/j.supflu.2017.09.026.
9. Shimizu, S. and Abbott, S. (2016) How entrainers enhance solubility in supercritical carbon dioxide. *J. Phys. Chem. B*. 120: 3713–3723.
10. Wu, J.J., Shen, C.T., Jong, T.T., Young, C.C., Yang, H.L., Hsu, S.L., Chang, C.J. and Shieh, C.J. (2009) Supercritical carbon dioxide anti-solvent process for purification of micronized propolis particulates and associated anti-cancer activity. *Sep. Purif. Technol.* 70: 190-198.
11. Villanueva-Bermejo, D., Zahran, F., Troconis, D., Villalva, M., Reglero, G. and Fornari, T. (2017) Selective precipitation of phenolic compounds from *Achillea millefolium* L. extracts by supercritical anti-solvent technique. *J. Supercrit. Fluids*. 120: 52-58.

12. Franceschi, E., De Cesaro, A.M., Feiten, M., Ferreira, S.R.S., Dariva, C., Kunita, M.H., Rubira, A.F., Muniz, E.C., Corazza, M.L. and Oliveira, J.V. (2008) Precipitation of beta-carotene and PHBV and co-precipitation from SEDS technique using supercritical CO_2. *J. Supercrit. Fluids*. 47: 259-269.
13. Osorio-Tobón, J.F., Carvalho, P.I.N., Rostagno, M.A., Petenate, A.J. and Meireles, M.A.A. (2016) Precipitation of curcuminoids from an ethanolic turmeric extract using a supercritical antisolvent process. *J. Supercrit. Fluids*. 108: 26-34.
14. Chinnarasu, C., Montes, A., Fernandez-Ponce, M.T., Casas, L., Mantell, C., Pereyra, C., De La Ossa, E.J.M. and Pattabhi, S. (2015) Natural antioxidant fine particles recovery from *Eucalyptus globulus* leaves using supercritical carbon dioxide assisted processes. *J. Supercrit. Fluids*. 101: 161-169.
15. Chinnarasu, C., Montes, A., Ponce, M.T.F., Casas, L., Mantell, C., Pereyra, C. and De La Ossa, E.J.M. (2015) Precipitation of antioxidant fine particles from *Olea europaea* leaves using supercritical antisolvent process. *J. Supercrit. Fluids*. 97: 125-132.
16. Martin, A. and Cocero, M.J. (2004) Numerical modeling of jet hydrodynamics, mass transfer, and crystallization kinetics in the supercritical antisolvent (SAS) process. *J. Supercrit. Fluids*. 32: 203-219.
17. Shishikura, A., Takahashi, H., Hirohama, S. and Arai, K. (1992) Citric acid purification process using compressed carbon dioxides. *J. Supercrit. Fluids*. 5: 303-312.
18. Gallagher, P.M., Coffey, M.P., Krukonis, V.J. and Klasutis, N. (1989) in American Chemical Society (ed.) *Supercritical fluid science and technology* ,

Gas antisolvent recrystallization: new process to recrystallize compounds insoluble in supercritical fluids. ACS publications: Washington, USA.

19. Catchpole, O.J., Hochmann, S. and Anderson, S.R.J. (1996) in Von Rohr (ed) High Pressure Chemical Engineering, proceedings of the 3rd International Symposium on High Presure Chemical Engineering Process Technology Proceedings. 12: 309-314.
20. Catchpole, O.J., Grey, J.B., Mitchell, K.A. and Lan, J.S. (2004) Supercritical antisolvent fractionation of propolis tincture. *J. Supercrit. Fluids*. 29: 97-106.
21. Reverchon, E., Celano, C., Della Porta, G., Di Trolio, A. and Pace, S. (1998) Supercritical antisolvent precipitation : a new technique for preparing submicronic yttrium powders to improve YBCO superconductors. *J Mater Res.* 13(2): 284-289.
22. ISO 704 (2000) Terminology work–Principles and methods. Geneva: International Organization for Standardization.
23. Burdock, G.A. (1998) Review of the biological properties and toxicity of bee propolis (Propolis). *Food Chem. Toxicol.* 36(4): 347-63.
24. Monroy, Y.M., Rodrigues, R.A.F., Rodrigues, M.V.N. and Cabral, F.A. (2018) Fractionation of ethanolic and hydroalcoholic extracts of green propolis using supercritical carbon dioxide as an anti-solvent to obtain artepillin rich-extract. *J. Supercrit. Fluids*. 138: 167-173.
25. Khan, N. and Mukhtar, H. (2007) Tea polyphenols for health promotion. *Life Sci.* 81: 519-533.
26. Stapleton, P.D., Shah, S., Anderson, J.C., Hara, Y., Hamilton-Miller, J.M.T. and Taylor, P.W. (2004) Modulation of β-lactam resistance in *Staphylococcus aureus*

by catechins and gallates. *Int. J. Antimicrob. Agents*. 23: 462-467.

27. Susan, B.M. and Nagi, B.K. (2004) Green tea polyphenols and cancer chemoprevention: multiple mechanisms and endpoints for phase II trials. *Nutr. Rev.*, 62: 204–211.
28. Yanagawa, Y., Yamamoto, Y., Hara, Y. and Shimamura, T. (2003) A combination effect of epigallocatechin gallate, a major compound of green tea catechins, with antibiotics on *Helicobacter pylor*i growth *in vitro*. *Curr. Microbiol.* 47: 244-249.
29. Liu, Z., Ma, L.P., Zhou, B., Yang, L. and Liu, Z.L. (2000) Antioxidative effects of green tea polyphenols on free radical initiated and photosensitized peroxidation of human low density lipoprotein. *Chem. Phys. Lipids*. 106: 53-63.
30. Sosa, M.V., Rodríguez-Rojo, S., Mattea, F., Cismondi, M. and Cocero, M.J. (2011) Green tea encapsulation by means of high pressure antisolvent coprecipitation. *J. Supercrit. Fluids*. 56: 304-311.
31. Villanueva-Bermejo, D., Ibáñez, E., Reglero, G., Turner, C., Fornari, T. and Rodriguez-Meizoso, I. (2015) High catechins/low caffeine powder from green tea leaves by pressurized liquid extraction and supercritical antisolvent precipitation. *Sep. Purif. Technol.* 148: 49-56.
32. Georgieva, L., Gadjalova, A., Mihaylova, D. and Pavlov, A. (2015) *Achillea millefolium* L. - Phytochemical profile and *in vitro* antioxidant activity. *Int. Food Res. J.* 22: 1347-1352.
33. Bobis, O., Dezmirean, D.S., Tomos, L., Chirila, F. and Marghitas, L. (2015) Influence of phytochemical profile on antibacterial activity of different medicinal plants against gram-positive and gram-negative bacteria. *Appl.*

Biochem. Microbiol. 51: 113-118.

34. Huo, C.H., Li, Y., Zhang, M.L., Wang, Y.F., Zhang, Q., Qin, F., Shi, Q.W. and Kiyota, H. (2013) Cytotoxic flavonoids from the flowers of *Achillea millefolium*. *Chem. Nat. Compd.* 48: 958-962.
35. Benedek, B., Kopp, B. and Melzig, M.F. (2007) *Achillea millefolium* L. s.l. - Is the anti-inflammatory activity mediated by protease inhibition? *J. Ethnopharmacol.* 113: 312-317.
36. Benedek, B., Geisz, N., Jäger, W., Thalhammer, T. and Kopp, B. (2006) Choleretic effects of yarrow (*Achillea millefolium* s.l.) in the isolated perfused rat liver. *Phytomedicine*. 13: 702-706.
37. Villalva, M., Jaime, L., Villanueva-Bermejo, D., Lara, B., Fornari, T., Reglero, G. and Santoyo, S. (2019) Supercritical anti-solvent fractionation for improving antioxidant and anti-inflammatory activities of an *Achillea millefolium* L. extract. *Food Res Int*. 115: 128-134.
38. Zhao, C., Wang, L., Zu, Y., Li, C., Liu, S., Yang, L., Zhao, X. and Zu, B. (2011) Micronization of *Ginkgo biloba* extract using supercritical antisolvent process. *Powder Tech.* 209: 73-80.
39. Kraujalis, P., Venskutonis, P.R., Ibáñez, E. and Herrero, M. (2015) Optimization of rutin isolation from *Amaranthus paniculatus* leaves by high pressure extraction and fractionation techniques. *J. Supercrit. Fluids*, 104: 234–242.
40. Mukhopadhyay, M. and Patel, C.K.R. (2009) Purification of phytochemicals by gas antisolvent precipitation with carbon dioxide. *Indian Chem. Eng.* 51: 111-118.
41. Hatcher, H., Planalp, R., Cho, J., Torti, F.M. and Torti, S.V. (2008) Curcumin:

From ancient medicine to current clinical trials. *Cell. Mol. Life Sci.* 65: 1631-1652.

42. Wilson, B., Abraham, G., Manju, V.S., Mathew, M., Vimala, B., Sundaresan, S. and Nambisan, B. (2005) Antimicrobial activity of *Curcuma zedoaria* and *Curcuma malabarica* tubers. *J. Ethnopharmacol.* 99: 147-151.
43. Santana, Á.L. and Meireles, M.A.A. (2017) Coprecipitation of turmeric extracts and polyethylene glycol with compressed carbon dioxide. *J. Supercrit. Fluids*. 125: 31-41.
44. Huang, T.L., Lin, J.C., Chyau, C.C., Lin, K.L. and Chang, C.M.J. (2013) Purification of lignans from *Schisandra chinensis* fruit by using column fractionation and supercritical antisolvent precipitation. *J. Chromatogr. A*. 1282: 27-37.
45. Perretti, G., Virgili, C., Troilo, A., Marconi, O., Regnicoli, G.F. and Fantozzi, P. (2013) Supercritical antisolvent fractionation of lignans from the ethanol extract of flaxseed. *J. Supercrit. Fluids*. 75: 94-100.
46. Marqués, J.L., Della Porta, G., Reverchon, E., Renuncio, J.A.R. and Mainar, A.M. (2013) Supercritical antisolvent extraction of antioxidants from grape seeds after vinification. *J. Supercrit. Fluids*. 82: 238-243.
47. Floris, T., Filippino, G., Scrugli, S., Pinna, M.B., Argiolas, F., Argiolas, A., Murru, M. and Reverchon, E. (2010) Antioxidant compounds recovery from grape residues by a supercritical antisolvent assisted process. *J. Supercrit. Fluids*. 54: 165-170.
48. García-Abarrio, S.M., Marqués, J.L., Scognamiglio, M., Della Porta, G., Reverchon, E., Mainar, A.M. and Urieta, J.S. (2012) Supercritical extraction and

separation of antioxidants from residues of the wine industry. *Procedia Eng.* 42: 1762-1766.

49. Natolino, A., Da Porto, C,. Rodríguez-rojo, S., Moreno, T. and Cocero, M.J. (2016) Supercritical antisolvent precipitation of polyphenols from grape marc extract. *J. Supercrit. Fluids*. 118: 54-63.
50. Mezzomo, N., Oliveira, D.A., Comim, S.R.R. and Ferreira, S.R.S. (2016) Encapsulation of extracts from winery industry residue using the supercritical anti-solvent technique. *Brazilian J. Chem. Eng.* 33: 589-598.
51. Machado, A.P.D.F., Rueda, M., Barbero, G.F., Martín, A., Cocero, M.J. and Martínez, J. (2018). Co-precipitation of anthocyanins of the extract obtained from blackberry residues by pressurized antisolvent process, *J. Supercrit. Fluids.* 137: 81-92.
52. Sánchez-Camargo, A., García-Cañas, V., Herrero, M., Cifuentes, A. and Ibáñez, E. (2016) Comparative study of green sub- and supercritical processes to obtain carnosic acid and carnosol-enriched rosemary extracts with *in vitro* anti-proliferative activity on colon cancer cells. *Int. J. Mol. Sci.* 17: 2046.
53. Sánchez-Camargo, A.P., Mendiola, J.A., Valdés, A., Castro-Puyana, M., García-Cañas, V., Cifuentes, A., Herrero, M. and Ibáñez, E. (2016) Supercritical antisolvent fractionation of rosemary extracts obtained by pressurized liquid extraction to enhance their antiproliferative activity. *J. Supercrit. Fluids*. 107: 581-589.
54. Leal, P.F., Braga, M.E.M., Sato, D.N., Carvalho, J.E., Marques, M.O.M. and Meireles, M.A.A. (2003) Functional properties of spice extracts obtained via supercritical fluid extraction. *J. Agric. Food Chem.* 51: 2520-2525.

55. Visentin, A., Cismondi, M. and Maestri, D. (2011) Supercritical CO_2 fractionation of rosemary ethanolic oleoresins as a method to improve carnosic acid recovery. *Innov. Food Sci. Emerg. Technol.* 12: 142-145.
56. Guamán-Balcázar, M.C., Montes, A., Pereyra, C. and de la Ossa, E.M. (2017) Precipitation of mango leaves antioxidants by supercritical antisolvent process. *J. Supercrit. Fluids*. 128: 218-226
57. Meneses, M.A., Caputo, G., Scognamiglio, M., Reverchon, E. and Adami, R. (2015) Antioxidant phenolic compounds recovery from *Mangifera indica* L. by-products by supercritical antisolvent extraction. *J. Food Eng.* 163: 45-53.
58. Widjojokusumo, E., Veriansyah, B. and Tjandrawinata, R.R. (2013) Supercritical anti-solvent (SAS) micronization of *Manilkara kauki* bioactive fraction (DLBS2347). *J. CO2 Util.* 4: 30-36.
59. Hiendrawan, S,. Veriansyah, B., Widjojokusumo, E. and Tjandrawinata, R.R. (2017) Simultaneous micronization and purification of bioactive fraction by supercritical antisolvent technology. *J Adv Pharm Technol Res.* 8: 52-58.
60. Saini, R.K., Nile, S.H. and Park, S.W. (2015) Carotenoids from fruits and vegetables : Chemistry , analysis , occurrence , bioavailability and biological activities *Food Res. Inter*. 76: 735-750.
61. Costa-rodrigues, J., Pinho, O. and Monteiro, P.R.R. (2018) Can lycopene be considered an effective protection against cardiovascular disease ? *Food Chem.* 245: 1148-1153.
62. Chajès, V. and Romieu, I. (2014) Maturitas nutrition and breast cancer. *Maturitas*. 77: 7-11.
63. Shen, Y.C., Chng, L.M., Wang, Y.C., Shieh, C.J., Lin, K.L., Hsu, S.L., Chou,

H.N. and Chang, C.M.J. (2012) Production of all trans-beta-carotene by using impinging flow of supercritical carbon dioxide anti-solvent pulverization. *J. Chromatogr. A*. 1270: 1-8.

64. Chen, J., Wu, J., Lin, J.C., Wang, Y., Young, C,. Shieh, C,. Hsu, S. and Chang, C.J. (2013) Low density supercritical fluids precipitation of 9-cis and all trans-beta-carotenes enriched particulates from *Dunaliella salina*. *J. Chromatogr. A*. 1299: 1-9.
65. Chen, C.R., Lin, D.M., Chang, C.M.J., Chou, H.N. and Wu, J.J. (2017) Supercritical carbon dioxide anti-solvent crystallization of fucoxanthin chromatographically purified from *Hincksia mitchellae* P.C. Silva. *J. Supercrit. Fluids*. 119: 1-8.
66. Liau, B.C., Shen, C.T., Liang, F.P., Hong, S.E., Hsu, S.L., Jong, T.T. and Chang, C.M.J. (2010) Supercritical fluids extraction and anti-solvent purification of carotenoids from microalgae and associated bioactivity. *J. Supercrit. Fluids*. 55: 169-175.
67. Cho, Y.C., Wang, Y.C., Shieh, C.J., Lin, J.C., Chang, C.M.J. and Han, E. (2012) Countercurrent flow of supercritical anti-solvent in the production of pure xanthophylls from *Nannochloropsis oculata*. *J. Chromatogr. A*. 1250: 85-91.
68. Wu, J.J., Hong, S.E., Wang, Y.C., Hsu, S.L. and Chang, C.M.J. (2012) Microalgae cultivation and purification of carotenoids using supercritical anti-solvent recrystallization of CO_2 + acetone solution. *J. Supercrit. Fluids*. 66: 333-341.
69. Cho, Y.C., Cheng, J.H., Hsu, S.L., Hong, S.E., Lee, T.M. and Chang, C.M.J. (2011) Supercritical carbon dioxide anti-solvent precipitation of anti-oxidative

zeaxanthin highly recovered by elution chromatography from *Nannochloropsis oculata*. *Sep. Purif. Technol.* 78: 274-280.

70. Chen, C.R., Hong, S.E., Wang, Y.C., Hsu, S.L., Hsiang, D. and Chang, C.M.J. (2012) Preparation of highly pure zeaxanthin particles from sea water-cultivated microalgae using supercritical anti-solvent recrystallization. *Bioresour. Technol.* 104: 828-831.
71. Shen, C.T., Chen, P.Y., Wu, J.J., Lee, T.M., Hsu, S.L., Chang, C.M.J., Young, C.C. and Shieh, C.J. (2011) Purification of algal anti-tyrosinase zeaxanthin from *Nannochloropsis oculata* using supercritical anti-solvent precipitation. *J. Supercrit. Fluids*. 55: 955-962.
72. Chang, L.P., Cheng, J.H., Hsu, S.L., Fu, Y.C., Lin, K.L., Shieh, C.J., Zhou, X.Q. and Chang, C.M.J. (2012) Supercritical carbon dioxide anti-solvent purification of anti-oxidative compounds from *Lycium barbarum* fruits by using response surface methodology. *Sep. Purif. Technol.* 100: 66-73.
73. Chang, L.P., Cheng, J.H., Hsu, S.L., Liau, B.C., Wu, T.M. and Chang, C.M.J. (2011) Application of continuous supercritical anti-solvents for rapid recrystallization and purification of zeaxanthin dipalmitates from de-glycosides of *Lycium barbarum* fruits. *J. Supercrit. Fluids*. 57: 155-161.
74. Lin, K.L., Chang, L.M., Lin, J.C., Hsu, S.L., Young, C.C., Shieh, C.J. and Chang, C.M.J. (2014) Effect of anti-solvent conditions on low density supercritical fluids precipitation of zeaxanthin palmitates from *Lycium barbarum* fruits. *J. Supercrit. Fluids*. 87: 104-110.
75. Wu, J., Shen, L., Yin, M. and Cheng, Y. (2017) Supercritical carbon dioxide anti-solvent micronization of lycopene extracted and chromatographic purified from

Momordica charantia L . aril. *J. Taiwan Inst. Chem. Eng.* 80: 64-70.

76. Mukhopadhyay, M. and Singh, S. (2004) Refining of crude lecithin using dense carbon dioxide as anti-solvent. *J. Supercrit. Fluids*. 30: 201-211.

77. Catchpole, O.J., Tallon, S.J., Grey, J.B., Fletcher, K. and Fletcher, A.J. (2008) Extraction of lipids from a specialist dairy stream. *J. Supercrit. Fluids*. 45: 314-321.

78. Aro, H., Järvenpää, E.P., Könkö, K., Sihvonen, M., Hietaniemi,V. and Huopalahti, R. (2009) Isolation and purification of egg yolk phospholipids using liquid extraction and pilot-scale supercritical fluid techniques. *Eur. Food Res. Technol.* 228: 857-863.

79. Aro, H., Järvenpää, E., Könkö, K., Huopalahti, R. and Hietaniemi, V. (2007) The characterisation of oat lipids produced by supercritical fluid technologies. *J. Cereal Sci.* 45: 116-119.

80. Santos, D.T., Barbosa, D.F., Broccolo, K., Gomes, M.T.M.S., Vardanega, R. and Meireles, M.A.A (2012) Pressurized organic solvent extraction with on-line particle formation by supercritical anti solvent processes. *Food and Public Health*. 2: 231-240.

81. Charoenchaitrakool, M., Trisilanun, W. and Srinopakhun, P. (2010) Application of gas anti-solvent process to the recovery of andrographolide from *Andrographis paniculatanees*. *Korean J. Chem. Eng.*, 27: 950-954.

82. Martín, L., González-Coloma, A., Adami, R., Scognamiglio, M., Reverchon, E., Della Porta, G., Urieta, J.S. and Mainar, A.M. (2011) Supercritical antisolvent fractionation of ryanodol from *Persea indica*. *J. Supercrit. Fluids*. 60: 16-20.

83. Gonzalez-Coloma, A., Martín, L., Mainar, A.M., Urieta, J.S., Fraga, B.M., Rodríguez-Vallejo, V. and Díaz, C.E. (2012) Supercritical extraction and supercritical fractionation of natural products from plant material: comparative results on *Persea indica. Phytochem Rev*., 11: 433-466.

84. Messerli, S.M., Ahn, M., Kunimasa, K., Yanagihara, M., Tatefuji, T., Hashimoto, K., Mautner, V., Uto, Y., Hori, H., Kumazawa, S., Kaji, K., Ohta, T. and Maruta H. (2009) Artepillin C (ARC) in Brazilian green propolis selectively blocks oncogenic PAK1 signaling and suppresses the growth of NF tumors in mice. *Phytother. Res.* 427: 423-427.

85. Kimoto, T., Aga, M., Hino, K., Koya-Miyata, S., Yamamoto, Y., Micallef, M.J., Hanaya, T., Arai, S., Ikeda, M. and Kurimoto, M. (2001) Apoptosis of human leukemia cells induced by Artepillin C, an active ingredient of Brazilian propolis. *Anticancer Res.* 21: 221-228.

86. Shimizu, K., Ashida, H., Matsuura, Y. and Kanazawa, K. (2004) Antioxidative bioavailability of artepillin C in Brazilian propolis. *Arch. Biochem. Biophys.* 424: 181-188.

87. Chen, C.R., Shen, C.T., Wu J.J., Yang, H.L., Hsu S.L. and Chang, C.M.J. (2009). Precipitation of sub-micron particles of 3,5-diprenyl-4-hydroxycinnamic acid in Brazilian propolis from supercritical carbon dioxide anti-solvent solutions. *J. Supercrit. Fluids.* 50:176-182.

88. Winters, M.A., Frankel, D.Z,. Debenedetti, P.G., Carey, J., Devaney, M. and Przybycien, T.M. (1999) Protein purification with vapor-phase carbon dioxide. Biotechnol. Bioeng. 62: 247-258.

89. Haimer, E., Wendland, M., Potthast, A., Henniges, U., Rosenau, T. and Liebner,

F. (2010) Controlled precipitation and purification of hemicellulose from DMSO and DMSO/water mixtures by carbon dioxide as anti-solvent. *J. Supercrit. Fluids*. 53: 121-130.

90. Scrugli, S., Frongia, M., Sfacteria, G., Loi, G., Pinna, M.B., Crobu, V. and Carta, D. (2003) in International Symposium on Supercritical Fluids proceedings, Proteic compounds reduction in smoke tobacco by supercritical antisolvent technique (SAS). 3: 1789-1794. *Vandoeuvre France, Institut national polytechnique de Lorraine* publications.
91. Benelli, P., Rosso, S.R., Oliveira, J.V., Pedrosa, R.C. and Ferreira, S.R.S. (2014) Phase equilibrium data of guacatonga (*Casearia sylvestris*) extract + ethanol + CO_2 system and encapsulation using a supercritical anti-solvent process. *J. Supercrit. Fluids*. 93: 103-111.
92. Catchpole, O.J., Bertram Grey, J., Durling, N.E., Dunn, K.G., (2013) Near-critical Fluid Extraction Process, United States Patents (US 8,580,116 B2).
93. Perrut, M. (2000) Supercritical fluid applications: industrial developments and economic issues. *Ind. Eng. Chem. Res*. 39: 4531-4535.

Table 1. Compilation of research articles reporting an actual purification of the natural product by means of Supercritical Antisolvent induced phase Separation (SAS) and its Gas equivalent process. *The antisolvent fluid was dimethyl ether (DME). In all other cases the antisolvent used was carbon dioxide. Q_{CO2}: flow rate of CO_2. Q_{feed}: flow rate of liquid feed. C_{feed}: concentration of liquid feed. UAE: ultrasound-assisted extraction. PLE: Pressurized liquid extraction, MAE: microwave-assisted extraction, DMSO: dimethyl sulfoxide, DMF: dimethylformamide.

Raw material	Pretreatment of SAS feed solution	SAS feed solution	Target compound	Fraction of interest	Fluids contact mode	Process conditions	Results	Reference
***Milk**	Beta-serum from dairy streams	Liquid (aqueous) beta-serum	Proteins	Precipitate	Simultaneous	60 °C and 4 MPa, Q_{feed} /Q_{DME} (mass) = 0.1-	90% of the lipids were separated from the proteins when SAS was	(77)

						0.35, C_{feed}= 10-25 mass%	run twice on the same sample	
Egg yolk powder	Extraction with ethanol	Ethanolic extract	Phospholipids	Precipitate	Simultaneous	27 MPa, 70 °C, Q_{CO2} = 0.25 L/min Q_{feed} = 6.5 mL/min	Phosphatidyl cholines and phosphatidyl ethanolamines amount up to 990 mg/g, free from cholesterol	(78)
Protein mixtures	Lysozyme + ribonuclease mixture	Mixture dissolved in DMSO	Lysozyme	Precipitate	Sequential	34 °C 4.9 MPa	No detectable ribonuclease in precipitate. Lysozyme kept 72 ± 2% of its	(88)

							initial biological activity	
	Alkaline phosphatase + insulin mixture	Mixture dissolved in DMSO	Alkaline phosphatase	Precipitate	Sequential	34 °C 4.9 MPa	No detectable insulin in precipitate	(88)
Mango leaves	Extraction with supercritical CO_2	Extract dissolved in ethyl lactate	β-carotene	Precipitate	Sequential	25 °C, 4-7 MPa	β-carotene concentration increased from 1.02% in the feed to 5.66% in the precipitate	(40)
	Extraction with ethanol	Ethanolic extract	Polyphenols mangiferin and benzophenone derivatives	Precipitate	Simultaneous	15 MPa, 35 °C, Q_{CO2} = 30, 40 g/min,	Enrichment factors up to 2.8. Particles showed stronger	(56)

						Q_{feed} = 5, 10 mL/min C_{feed} = 20 mg/mL	antioxidant activity than feed	
Rosemary leaves	Oleoresins extracted with ethanol	Ethanolic extract	Carnosic acid	Soluble fraction	Simultaneous	50 °C, 30 MPa	CA concentration increased 10 times	(55)
	PLE with water/ethanol mixtures	Water/ethanol (50% v/v) extract	Rosmarinic acid (RA)	Precipitate	Simultaneous	40 °C, 10 MPa, Q_{feed}/Q_{CO2} (mass) = 0.025	Precipitate enriched in RA	(52, 53)
			Carnosic acid (CA) and carnosol (CS)	Soluble fraction			CA+CS concentration of 478.1 mg/g, no RA was detected	

Laurel leaves	Extraction with ethanol	Ethanolic extract	Ryanodol	Soluble fraction	Simultaneous	15 MPa, 35 °C, CO_2 molar fraction of 0.9	Ryanodol concentration from 7.5% by mass in the feed to 37.7% in the fraction, 42.9% recovery	(82)
Olive leaves	Extraction with scCO2+ethanol	Extract in ethanol	Antioxidants	Precipitate	Simultaneous	15 MPa, 50 °C, Q_{CO2} = 30 g/min, Q_{feed} = 2 mL/min, C_{feed} = 32 mg/mL	Less polar compounds in the feed were removed by SAS. The antioxidant activity of all	(15)

						(smallest particles, 300 nm)	precipitates was much higher than the feed	
	Extraction with ethanol	Ethanolic extract	Oleuropein	Precipitate	Simultaneous	15 MPa, 35 °C, Q_{CO2} = 40 g/min Q_{feed} = 1 mL/min, C_{feed} = 20% w/w	Oleuropein concentration from 20% by mass in the feed to 36% in the precipitate	(8)
Eucalyptus leaves	Extraction with scCO2+ethanol	Extract in ethanol	Antioxidants	Precipitate	Simultaneous	15 MPa, 35 °C, Q_{CO2} = 20 g/min, Q_{feed} = 2 mL/min	Smallest particles (0.27 μm) and highest antioxidant activity	(14)

Kokum leaves	Extraction with acetone	Acetone extract	Alpha-hydroxycitric acid (α-HCA)	Precipitate	Sequential	24 °C, 4-7 MPa	α-HCA concentration increased from 21% in the feed to 72% in the precipitate, free from citric acid	(40)
Yarrow leaves	UAE with ethanol	Ethanolic extract	Phenolic compounds (luteolin-7-O-glucoside most abundant)	Precipitate	Simultaneous	10 MPa, 40 °C, Q_{CO2} = 50 g/min, Q_{feed} = 1.6 g/min, C_{feed} = 17.9 mg/mL	3 times higher concentration in the precipitate than in the feed. 269 μm mean size particles. Highest recovery 40.9%	(11)

	UAE with ethanol	Ethanolic extract	Phenolic compounds (luteolin-7-O-glucoside most abundant)	Precipitate	Simultaneous	10 MPa, 40 °C, Q_{CO2} = 50 g/min, Q_{feed} = 1.6 g/min, C_{feed} = 17.9 mg/mL	Doubled antioxidant activity than the feed. Anti-inflammatory activity was detected	(37)
Green tea leaves	PLE with ethyl lactate	Ethyl lactate extract	Catechins (epigallocatechin gallate, EGCG, most abundant)	Precipitate	Simultaneous	30 MPa, 70 °C, Q_{CO2}/Q_{feed} (vol.) = 40	Particles with 23% by mass catechins and <1% caffeine. Enrichment factors for EGCG up to	(31)

							1.40. Recoveries up to 82%	
	MAE with acetone	Acetone extract + polymer	Catechins + polymer	Precipitate	Simultaneous	8-12 MPa, 11-34 °C, Q_{CO2}/Q_{feed} (mass) = 4.22-28.13	>90% catechins from the feed present in the precipitate, while only 13% of the caffeine present.	(30)
***Manilkara kauki* L. Dubard's leaf**	Extraction with ethanol (extract named DLBS2347)	Ethanolic extract redissolved in acetone	Unspecified polar compounds	Precipitate	Simultaneous	15 MPa, 40 °C, 0.95 mol fraction CO_2	Highest active compound concentration (relative quantification) and particle sizes of ≤100 µm	(58)

Lagerstroemia speciosa* and *Cinnamomum burmannii herbs	Hot water extraction (extract named DLBS3233)	Water extract redissolved in DMF	Polyphenols	Precipitate	Simultaneous	10-20 MPa, 35-55 °C, Q_{feed} = 0.53-2.19 mL/min, Q_{CO2} = 30 g/min, C_{feed} = 5 -20 mg/mL	Same total phenolic content as feed (37%) but no coumarin present. Particle sizes 0.107-0.298 µm	(59)
Licorice root	Extraction with ethyl acetate	Ethyl acetate extract	Licochalcone A (Lic-A)	Precipitate	Sequential	25 °C, 4-7 MPa	No data about Lic-A but other components of the precipitate increased in concentration	(40)

							with respect to the feed	
Turmeric rhizomes	PLE with ethanol of previously deflavored turmeric	Ethanolic extract	Curcuminoids	Precipitate	Simultaneous	10 MPa, 40 °C, Q_{CO2} = 500 g/h, Q_{feed} = 0.5 mL/min	Curcuminoids concentration in precipitates (524 mg/g) 31 times higher than in feed. 91% recovery.	(13)
Boxthorn fruit	UAE with THF of previously de-glycoside fruits	Fraction of THF extract, enriched by liquid chromatography, redissolved in THF	Zeaxanthin palmitates	Precipitate	Simultaneous	15 MPa, 55 °C, Q_{CO2} = 15 L/min, Q_{feed} = 0.2 L/min, C_{feed} = 5 mg/mL	82.5% zeaxanthin palmitates in feed to 92.5% in precipitate, 71% recovery	(74)

	UAE with acetone of previously de-glycoside fruits	Fraction of acetone extract, enriched by liquid chromatography, redissolved in acetone	Zeaxanthin palmitates	Precipitate	Simultaneous	20 MPa, 50 °C, Q_{CO2} = 54 g/min, Q_{feed} = 1 mL/min, C_{feed} = 0.6 mg/mL	81.6% zeaxanthin palmitates in feed to 92.9% in precipitate, 59.6% recovery	(73)
	UAE with acetone of previously de-glycoside fruits	Fraction of acetone extract, enriched by liquid chromatography, redissolved in ethyl acetate	Zeaxanthin dipalmitate	Precipitate	Simultaneous	15 MPa, 55 °C, Q_{CO2} = 20 L/min, Q_{feed} = 0.35 mL/min, C_{feed} = 1.2	81.8% zeaxanthin dipalmitate in feed to 93.1% in precipitate, 54.2% recovery	(72)

						mg/mL, for 18 min		
Microalgae	*Nannochloropsis oculata*, UAE with acetone	Fraction of acetone extract, enriched by liquid chromatography, redissolved in acetone	Zeaxanthin	Precipitate	Simultaneous	20 MPa, 40 °C, Q_{CO2} = 54 g/min, Q_{feed} = 2 mL/min, C_{feed} = 0.4 mg/mL	30% zeaxanthin in feed to 50% in precipitate, 70% recovery	(69)
	Nannochloropsis oculata, UAE with acetone	Fraction of acetone extract, enriched by liquid chromatography,	Zeaxanthin	Precipitate	Simultaneous	13.5 MPa, 55 °C, Q_{CO2} = 48.6 g/min, Q_{feed} = 0.5 mL/min,	42.6% zeaxanthin in feed to 84.2% in precipitate, 85.3% recovery	(70)

		redissolved in ethyl acetate				C_{feed} = 1.5 mg/mL		
	Nannochloropsis oculata, Soxhlet dichloromethane (DCM) extraction	Fraction of DCM extract, enriched by liquid chromatography, redissolved in acetone	Zeaxanthin	Precipitate	Simultaneous	Experimental design indicates 20 MPa, 40 °C, C_{feed} = 0.4 mg/mL, Q_{CO2} = 54 g/min gives highest purity	43.6% zeaxanthin in feed to up to 67.4% in particles. Recovery above 70%.	(71)
	Nannochloropsis oculata, UAE with acetone	Fraction of acetone extract, enriched by	Zeaxanthin	Precipitate	Simultaneous	21.5 MPa, 50 °C, Q_{CO2} = 36 g/min,	41.0% zeaxanthin in feed to 58.2% in	(68)

		liquid chromatography, redissolved in acetone				Q_{feed} = 0.5 mL/min, C_{feed} = 0.5 mg/mL, for 24 min.	particles. Recovery above 67%.	
	Hincksia mitchellae, UAE with ethyl acetate	Fraction of ethyl acetate extract, enriched by liquid chromatography, redissolved in ethyl ether	Fucoxanthin	Precipitate	Simultaneous	9 MPa, 40 °C, Q_{CO2} = 15 L/min, Q_{feed} = 0.3 mL/min, C_{feed} = 10 mg/mL, for 10 min.	71.9 % fucoxanthin in feed to 87.0% in particles. Recovery above 97%.	(65)
	Dunaliella salina, UAE	Fraction of THF extract, enriched	Trans-beta-carotene	Precipitate	Simultaneous	18 MPa, 45 °C, Q_{CO2} =	85 % trans-beta-carotene in feed	(63)

	with tetrahydrofuran (THF)	by liquid chromatography, redissolved in THF				15 L/min, Q_{feed} = 0.2 mL/min, C_{feed} = 5 mg/mL, for 40 min.	up to 93% in particles. Recovery above 54%.	
	Dunaliella salina, UAE with ethyl ether	Fraction of ethyl ether extract, enriched by liquid chromatography, redissolved in ethyl ether	Beta-carotenes	Precipitate	Simultaneous	9.9 MPa, 60 °C, Q_{CO2} = 15 L/min, Q_{feed} = 0.6 mL/min, C_{feed} = 5 mg/mL, for 21.5 min.	85 % beta-carotenes in feed up to 95% in particles. Recovery 57.5%.	(64)

Grape seeds (from vinification waste)	Ethanol extraction of previously defatted seeds	Ethanolic extract	Polyphenols	Precipitate		15 MPa, 40 °C, Q_{CO2} = 2.38 kg/h, molar fraction of CO_2 of 0.98	Relative enrichments of 100, 250, 267 and 78% for gallic acid, catechin, epicatechin and resveratrol respectively. Recoveries 70%	(46)
Grape marc	Ethanol extraction of previously defatted marc	Ethanolic extract	Polyphenols	Precipitate	Simultaneous	12 MPa, 45 °C, 0.99 CO_2 molar fraction	Relative enrichments of 350% of total polyphenols, oligomeric and	(49)

							polymeric proanthocyanidin fractions between 300 and 450%. Average particle size 5 μm.	
Blackberry (residues)	UAE with ethanol	Ethanolic extract	Anthocyanins and other polyphenols	Precipitate	Simultaneous	12.5 MPa, 30 °C, Q_{CO2} = 2.0 kg/h, Q_{feed} = 1.0 mL/min, Total $solids_{feed}$ = 1.5%	Total monomeric anthocyanins increased from 6.6 in feed to 16.7 mg Cy3GI equivalents/g dry sample in precipitate.	(51)

							Increased antioxidant activity.	
***Schisandra chinensis* fruit**	UAE with ethanol	Fraction of ethyl ether extract, enriched by liquid chromatography, redissolved in ethanol	Lignans	Soluble fraction	Simultaneous	13 MPa, 40 °C, Q_{CO2} = 0.2 L/min, Q_{feed} = 0.1 mL/min, C_{feed} = 10 g/L	Total concentration of lignans of 206.3 mg/g in the feed increased to 581.84 mg/g in the effluent with a recovery of 84%	(44)
Lecithin/soya oil	Mixed	Mixture dissolved in hexane	Lecithin	Precipitate	Sequential	35 °C, < 7 MPa	Soluble fraction of 90% by weight coriander	(19)

							essential oil was obtained	
Soya crude lecithin	Crude soya lecithin and soybean oil mixed	Mixture dissolved in hexane	Lecithin	Precipitate	Sequential	25 °C, 6.5 MPa	Highest selectivity and 100% recovery of lecithin	(76)
						25 °C and 5.8 MPa with a 60 wt.% w oil in the feed. 25 °C, 5.0 MPa for feed solutions	Highest enrichment of the crystals in lecithin (from 60 wt.% to 98 wt.%) with recoveries around 80%.	

						with <60 wt.% w oil content		
Coriander seed triglycerides/essential oil	Mixed	Mixture dissolved in hexane	Triglycerides	Precipitate	Sequential	35 °C, 7 MPa	Soluble fraction with no lecithin present	(19)
Propolis	Tincture	Ethanol extract	Flavonoids	Soluble fraction	Simultaneous	60 °C, 27.5-30 MPa, C_{feed} = 10% by mass of total propolis solids, mass flow ratio (tincture to CO2) of 1.3	Flavonoids contents of 25-30% by mass. Concentration of flavonoids doubled with respect to feed in pilot-plant scale.	(20)

Propolis Beech xylan, oat xylan, spruce mannan	Powder	Soxhlet extraction with ethyl acetate	3,5-diprenyl-4-hydroxycinnamic acid (DHCA)	Precipitate	Simultaneous	20 MPa, 55 °C, Q_{CO2} = 10 L/min, Q_{feed} = 1 mL/min, C_{feed} =9 mg/mL	DHCA concentration of 29.9% by mass, that is increased by a factor of 1.61 with respect to the feed solution, with DHCA recoveries of 92.6%	(10) (87)
	Hemicellulose used as received	Samples individually dissolved in DMSO	Hemicellulose	Precipitate	Simultaneous	15 MPa, 40 °C,	Hemicellulose spherical particles (0.5 µm), lignin	(89)

						Q_{CO2} = 5 kg/h, Q_{feed} = 2 mL/min	content decreased 49-85%	

8

9