PARTICLE FORMATION BY SUPERCRITICAL FLUID TECHNOLOGY AS DRYING PROCESSES FOR ANTIOXIDANT EXTRACTS

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ABSTRACT

In this work, different devices were designed to carry out two particle formation processes based on supercritical fluids (SAS, supercritical antisolvent, and RESS, rapid expansion of supercritical solution) as a novel way to dry complex extracts with antioxidant activity from natural sources. Particles of quercetin, a strong antioxidant from onion, were obtained by a SAS process from an ethanolic solution. The influence of different precipitation conditions on the size and morphology of the particles was studied by SEM (scanning electron microscopy). On the other hand, a SFE of rosemary leaves was carried out and directly coupled to a RESS process to obtain dried particles enriched in carnosic acid (a potent antioxidant from rosemary). Several devices were tested in order to achieve the complete precipitation of carnosic acid, which was confirmed by HPLC-DAD and the particles obtained studied by SEM.

INTRODUCTION

Increasing the amount of antioxidants in the diet strengthens the natural protection of the human body against oxidative stress [1, 2] that is related to severe diseases such as cancer, diabetes[3], atherosclerosis or cataracts [4].

Supercritical Fluid Extraction (SFE) and Pressurized Liquid Extraction (PLE) satisfy the demand of environmentally clean extraction processes to produce new functional compounds. Natural sources are also preferred; thus, some vegetables and herbs are suitable to provide extracts with antioxidant activity [5-8]. Even though the amount of solvents used in both SFE and PLE are small compared to traditional extraction processes, usually a second drying step is needed to be able to use them as functional ingredients or nutraceuticals.

At present, the most promising techniques for particle formation are based on supercritical fluid technology and have been used to obtain pure compounds with different morphology [9, 10]. For the food industry, the particle size and morphology could be interesting since it can be linked to a different activity, stability or even bioavailability of the functional food ingredients.

In this work, two different particle formation techniques have been tested as a novel way to dry complex extracts with antioxidant activities. To do so, a new device (see Figures 1 and 2) has been designed that can be easily modified and adapted to different applications. Different instruments were assembled to carry out SAS (see Figure 3) and RESS (see Figure 4) processes, depending on the characteristics of the extracts.

Different antioxidants, with different chemical characteristics, have been selected to carry out the present study. Quercetin is a major dietary flavonoid found in onions and other vegetables, extractable with PLE, and it is known to possess strong antioxidant activity [5]. In this work, a SAS process was developed to obtain quercetin particles, as might be done to dry the PLE extract. Rosemary leaves are a natural source of antioxidants. They contain carnosic acid, the main responsible of the antioxidant activity in supercritical extracts of rosemary [11]. In this work, we have obtained a supercritical extract of rosemary, enriched in carnosic acid, and formed particles of the complex extract by a RESS process.

The effect of temperature, pressure, solvent composition and flow rate on the complex particle size and morphology were investigated by SEM. Furthermore, the chemical composition of complex particles obtained was studied by HPLC-DAD.

MATERIALS AND METHODS

1.-Samples and Reactants:
Quercetin dihydrate, >98% from Fluka. Ethanol from LabScan (Dublin, Ireland).
The rosemary sample (*Rosmarinus officinalis* L.) consisted of dried rosemary leaves obtained from an herbalist's shop (dried using the traditional method, as follows: once collected, the plant is ventilated to remove humidity, covered with a blanket to avoid sunlight, and allowed to dry in a ventilated place for 20-30 days, depending on the season, Murcia, Spain) [12]. Samples were ground under cryogenic carbon dioxide and stored in amber flasks at -20 °C until use.

2.-Home-made device for the particle formation.

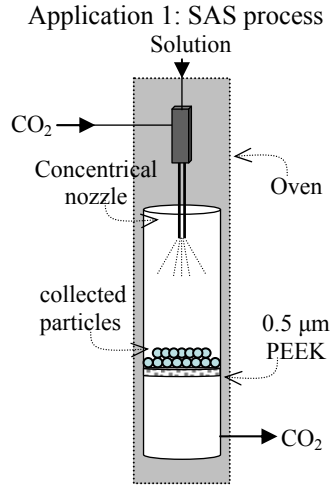

Figure 1. The solution is sprayed into the $scCO_2$

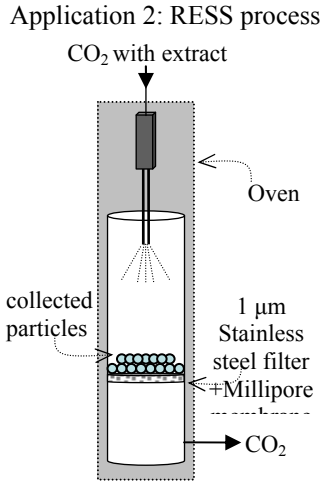

Figure 2. The $scCO_2$ carrier is expanded in the chamber

3.-Supercritical antisolvent process (SAS).
Supercritical CO_2 was pumped into the chamber by one ISCO pump (see Figure 3). Commercial quercetin was dissolved in ethanol (3 mg/mL) and pumped from another ISCO pump into the device. The pressure was controlled automatically, but the flow rate of both $scCO_2$ and quercetin solution were controlled manually by the needle valves. After 30 min running, quercetin solution flow was stopped and $scCO_2$ continued purging the line 10 min more. Particles were collected over the PEEK filter inside the chamber. The chamber was placed inside the oven.

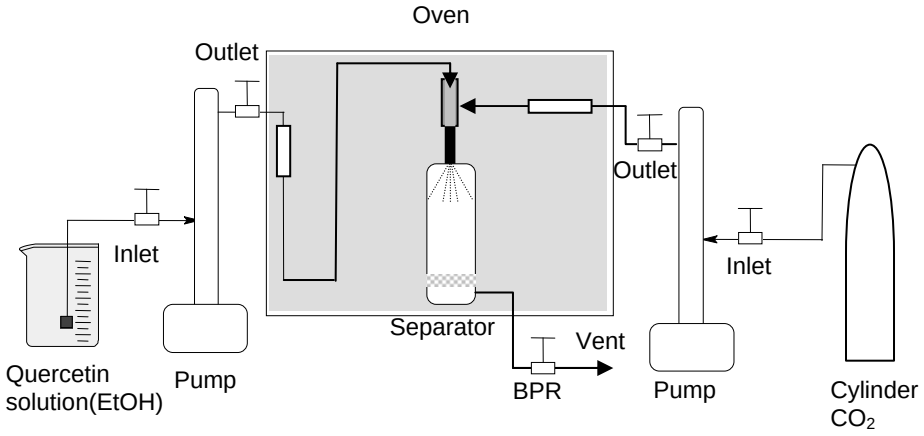

Figure 3. Scheme of the instrument assembled for SAS process.

Table 1 shows de different operational conditions (temperature, pressure, flow rate of the quercetin solution) that have been studied in the present work since they may affect the complex particle size and morphology of the particles formed.

Table 1: Conditions studied for the SAS process

Experiment	P/bar	T/ºC	Real FCO_2/mL·min^{-1}	Real Fsolution/mL·min^{-1}	Observations
A	100	40	3.5	0.3	
B	100	150	3.5	0.2	No particles
C	150	40	2.5	0.2	
D	100	80	3.5	0.2	
E	100	40	3.5	1.0	No particles
F	100	40	3.5	0.2	

3.-**Rapid expansion of supercritical solutions (RESS).**

Carnosic acid is known to be soluble in $scCO_2$ in the presence of a small amount of ethanol as cosolvent [13]. A supercritical rosemary extract enriched in carnosic acid [14] was obtained in a THAR system equipped with a CO_2 pump, a cooling bath, and a 100 mL extraction cell. The exit of the extraction cell was directly connected to our expansion chamber (Figure 2) where the RESS process took place (see Figure 4). In this case, the filter inside the chamber was a stainless steal 1 μm porous diameter, with a Millipore 0.45 μm porous diameter membrane on top (see Figure 2).

The extraction cell was filled with 6 g of ground rosemary, 9 g of glass beads and 6 mL of ethanol. Static extraction (V1 and BPR_1 closed) was performed at 150 bar and 40 °C for 30 min, followed by dynamic extraction (V1, BPR_1, V2 and BPR_2 open) during 15 min. A back pressure regulator (BPR_1) was placed between the extraction cell and the expansion chamber to control the dynamic extraction. The expansion chamber was placed inside an oven, at 50 ºC during the particle formation.

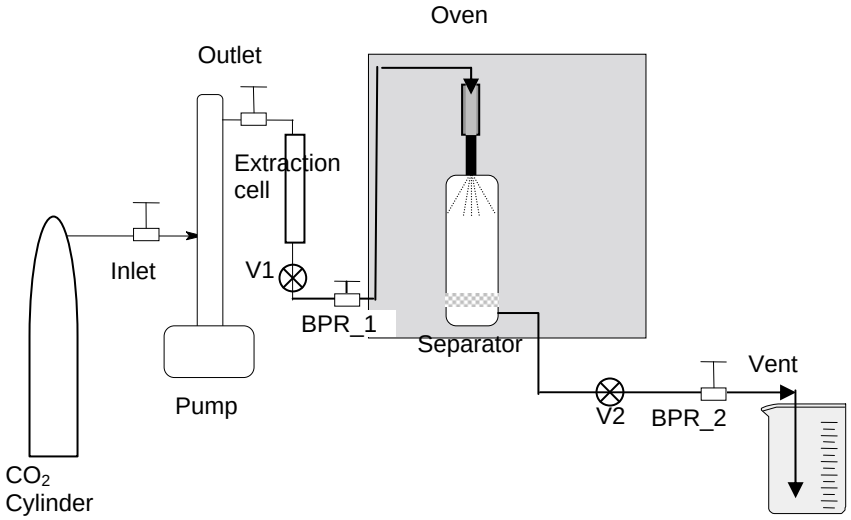

Figure 4. THAR extractor equipment coupled to a RESS chamber.

4.-**HPLC-DAD analysis of the particles**

Analysis were performed with an Ultimate 3000 Dionex, equipped with a Symmetry® C18 column 4.6x150 mm, 3.5 μm particle size, placed in a column compartment, and an autosampler. The mobile phase was a mixture of solvent A (1% acetic acid in water) and solvent B (1% acetic acid in acetonitrile) according to a step gradient lasting for 35 min, starting from 50% B at 5 min to 70% B at 15 min and 100% B at 30 min. Flow rate was kept to 0.7 ml/min. Detection was accomplished by using a photodiode array system at a wavelength of 230 nm. Data were analysed with a Chromaleon software.

RESULTS AND DISCUSSION

1.- **Supercritical antisolvent process (SAS).**

This technique is used to recrystallize solid compounds that are not soluble in supercritical fluids. It is suitable for quercetin as this antioxidant is soluble in ethanol but insoluble in $scCO_2$.

In this technique, a supercritical fluid acts as an antisolvent for the compound solution. This compound is dissolved in a liquid solvent and the solution is sprayed into a chamber where a supercritical fluid (antisolvent) already exists, causing rapid contact between the two media. This generates higher supersaturation ratio of the solution, resulting in fast nucleation and growth, and consequently creates smaller particles. The governing principle for this system is the solvent-induced phase separation, or compositional quench.

Figure 5 shows the SEM pictures for the different experiments in which particles were obtained (A, C, D, F). According to the results, excessive flow rates of the solution (1.0 mL/min, experiment E) do not lead to particle formation, probably because the high flow rates of the solution must probably need $scCO_2$ flow rates of more than 100 ml/min to dry out. With excessive high temperatures (150 ºC, experiment B) the quercetin does not precipitate, probably due to some technical problems in this experiment . In the experiment A, a kind of needles can be observed. The bigger ones reach around 1400 nm length and 300 nm wide, they are conglomerated, and this could be due to an excess of solution flow rate (0.3 mL/min). In experiment C the picture is similar, the needles are bigger (around2300 nm length and 400 nm), but are still agglomerated, maybe because the CO_2 flow rate (< 2,5 ml/min and unstable) was not enough to dry the quercetin flow, and also high pressures (150 bar) may solubilize quercetin. In the experiment D we achieved the longer needles (around 9000 nm length and 400 nm wide). In experiment F a thinner cloud of cross linked needles, not agglomerated, can be observed (around 5000 nm length and 130 nm wide).

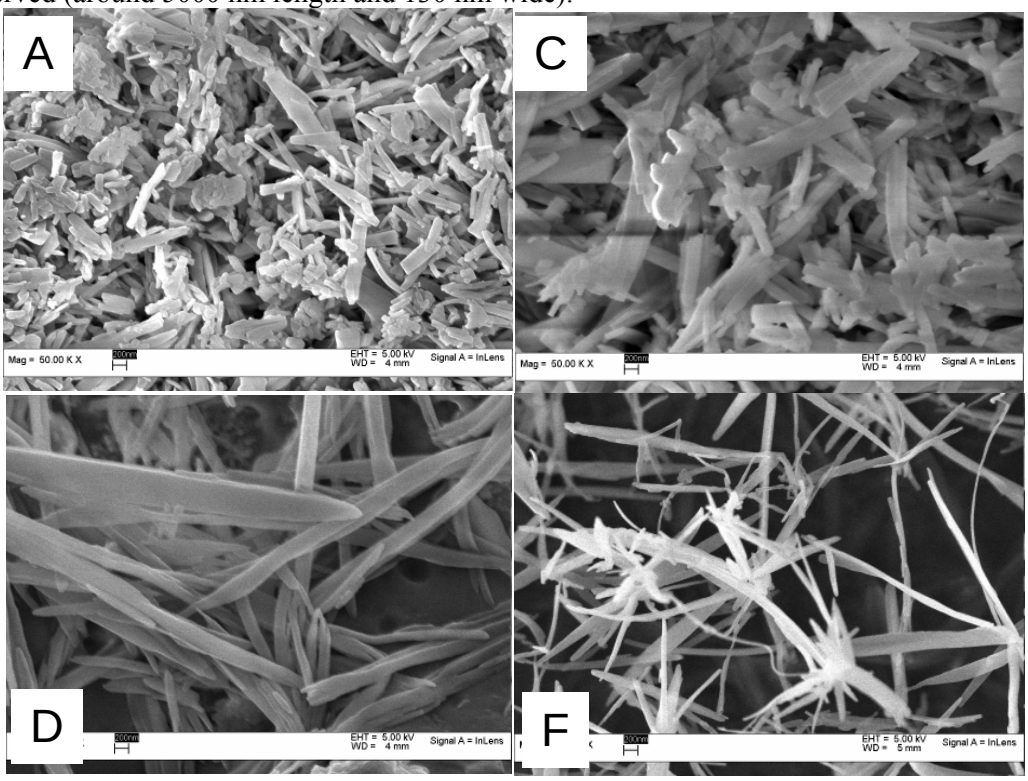

Figure 5. SEM pictures of quercetin particles.

From these results it could be concluded that low solution flow rates (0.2 mL/min or less), high CO_2 flow rates (around 3.5 mL/min), high temperatures (lower than 150 ºC) and moderated pressures (100 bar) are the best to obtain the longer and not conglomerated quercetin needles.

2.- **Rapid expansion of supercritical solutions (RESS)**

In a RESS process, the compound is dissolved in a supercritical fluid and this high-pressure solution is rapidly depressurized through an orifice to lead to polymer precipitation at a low pressure. The process is based on the solubility difference of the compound in supercritical fluids at high and low pressures, respectively. The governing principle is pressure-induced

phase separation. Along with the pressure quench, the solution experiences a temperature quench as well.
Carnosic acid is soluble in $scCO_2$ in the presence of high amount of ethanol. For this reason, a SAS process for a ethanolic extract of rosemary enriched in carnosic acid would not succeeded, since only the more polar compounds may precipitate. A REES process, using a small amount of ethanol as co-solvent to extract carnosic acid, might be a more promising process to particle formation in this case.

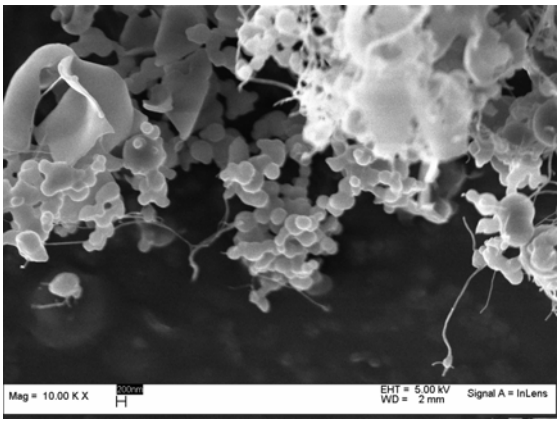

Figure 6. Rosemary extract particles by SEM

Round particles about 300 nm diameter seem to be the dried extract, whereas the fibers around should be associated with pieces of the Millipore membrane filter used to collect them.

The HPLC-DAD analysis of the particles revealed a peak for carnosic acid at 20.39 min. According to the calibration curve for the pure carnosic acid, the particles present 0.04 mg carnosic acid/mg sample.

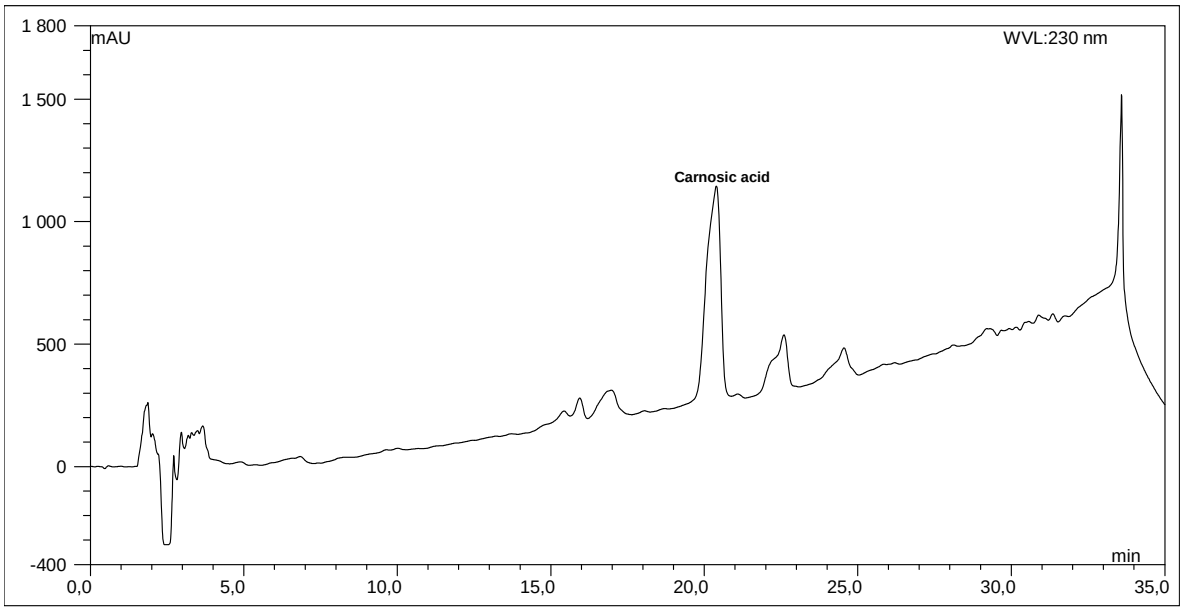

Figure 3. Chromatograma of RESS particles Peak 19 is carnosic acid.

CONCLUSIONS

In this work we have demonstrated the possibility of drying complex extracts from plants with antioxidant compounds, using different particle formation process based on supercritical fluids. Furthermore, the home-made device developed can be easily modified and adaptable to the process depending on the chemical characteristics of the extract.

LITERATURE

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