NOVEL PROCESS FOR WATER EXTRACTION AND PARTICLE FORMATION ON-LINE (WEPO)®

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Abstract

In this work, a new on-line process for Subcritical Water Extraction of plants and drying of the extract in one step by particle formation based on the use of supercritical CO_2 has been developed. With this process, complex dried extracts that preserve their intact antioxidant activity have been obtained as a fine powder with particle sizes lower than 140 μm length. An auxiliary inert gas (hot N_2) was used to enhance the drying process. The influence of water flow rate in the size and morphology of the particles has been studied. Other parameters such as temperature (200 ºC), scCO2 pressure (80 bar) and flow rate (2.5 ml/min) have been settled in order to optimize the spray formation.

The efficiency of the WEPO® process has been tested by means of the antioxidant activity and morphology of the particles (by Scanning Electron Microscopy).

Keywords

Water extraction; particle formation; plant; antioxidant

Introduction

In the search of green processes to obtain valuable compounds from natural sources, the extraction with subcritical water has been shown as one of the most selective and environmentally friendly technique. However, the extracts require later freeze or hot drying, and this is the major limitation, as it is energy and time consuming.

On the other hand, particle formation based on supercritical fluids have raised different solvent-antisolvent processes that can be interpreted as a way to dry pure compounds from organic solutions. In the case of aqueous solution, these processes are not suitable for drying due to the low solubility of $scCO_2$ in water. At the moment, only CAN-BD (Carbon dioxide assisted nebulisation- bubble dryer) process have been applied to obtain powders from pure proteins [1, 2], vaccines [3], antibiotics, antiviral [4] and other water soluble drugs [5]. However, regarding to complex extracts, there is only one publication by PGSS process [6].

In this work, we have developed a new process combining subcritical water extraction plus particle formation on-line, as a novel way to obtain dried complex extracts from natural sources in one step. This process has been patented as WEPO® meaning for (Water Extraction and Particle formation On-line).

As an example of this promising technique, we have carried WEPO with several aromatic herbs such as rosemary and oregano, leading to dried particles with intact antioxidant properties. These particles could be used as functional ingredients to develop functional foods.

Materials and methods

Samples and reagents

Both the oregano (*Origanum vulgare* L.) and the rosemary (*Rosmarinus officinalis* L.) samples consisted of dried oregano or rosemary leaves obtained from Murciana de Herboristeria (Murcia, Spain). The leaves were collected during September and then dried by using a traditional method previously described [7]. Cryogenic grinding of the sample was performed under carbon dioxide. The size of the particle (between 250 and 500 µm for oregano, and between 500 and 1000 µm for rosemary) was determined by passing the ground plant material through sieves of appropriate size. The whole sample was stored in amber flasks at – 20ºC until use.

1,1-diphenyl-2-picrylhydrazyl (DPPH, 95% purity) was from Sigma-Aldrich (Madrid, Spain). Methanol, acetonitrile (ACN) and acetone were HPLC grade from Lab Scan (Dublin, Ireland). Ethanol (99.5%) was obtained from Panreac (Spain) and acetic acid from Merck (Schuchardt, Germany). Milli-Q water was obtained using a purification system (Millipore Corporation, Billerica, MA, USA) and deoxygenated in an ultrasounds bath for 15 min before its use. CO_2 (UN1013, Supercritical Fluid Chromatography Grade quality) and N_2 (extra pure 50) were obtained from Praxair (Madrid, Spain). Anti-return and micrometric regulation valves were obtained from Swagelok (OH, USA), meanwhile on/off valves were obtained from Scientific Systems Inc. (PA, USA).

Equipment Description

Figure 1 shows a scheme of the home-made equipment designed to carry out the water extraction with particle formation on-line (WEPO)®.

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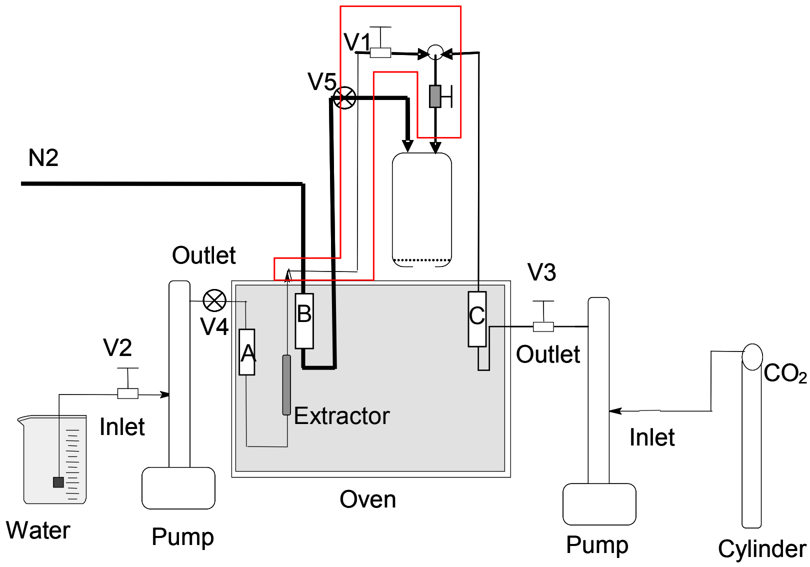

Figure 1. Scheme of the WEPO® equipment. V1, V2 y V3: on/off valves; V4 y V5: anti-return valves, A,B y C: coils to heat the different fluids (water, N_2, CO_2).

The equipment designed in this work combines two processes: the dynamic SWE of rosemary and oregano leaves and the continuous production of an aerosol from the extract, which is instantaneously dried by a hot N_2 current by a supercritical CO_2 assisted nebulization system. Thus, the extraction and precipitation take place in the same system with a small time delay between both.

A modified Suprex PrepMaster (Suprex, Pittsburgh, PA, USA) extractor was used to pump the CO_2. The water was pumped with a modified Suprex Modifier pump. All the tubings were stainless steel 1/16 inch, except for the N_2 (1/8 inch). The length of the tubings ensures time enough for the fluids to reach the desired temperature inside the oven. The extraction cell was also placed inside the oven.

Experimental procedure of WEPO®.

The extraction cell was filled with a mixture of the plant leaves (1 g of rosemary or 850 mg in the case of oregano) plus washed sea sand (2 g).

The process starts by filling the cell with water at room temperature and high flow rate (0.5 ml/min). Then the water flow is stopped and the CO_2 flow and the heating systems (oven and heating tape) are started. When the conditions are reached (80 bar and 2-3 mL/min for the CO_2, 200 ºC for the oven), N_2 flow is started and water is pumped dynamically through the extraction cell, at the different flow rates described in Table 1.

The water extract was mixed under pressure with the CO_2 in a low dead volume T type connection, forming an emulsion that goes through a restrictor and reaches the expansion-drying chamber. In this chamber, the pressure and temperature are below the critical point of the CO_2, so the emulsion is rapidly expanded. After 30 min, valve V1 is closed and the water flow is stopped while CO_2 and N_2 flows continue for 10 more minutes. Thus, the entrance of extract droplets in the expansion-drying chamber due to a possible residual pressure in the extract line is avoided. Particles were collected from the chamber wall.

Characterization of particles

Antioxidant activity analysis

Antioxidant activity of the different extracts was determined by the DPPH radicals capture method, which description can be found elsewhere [7-9].

Scanning electron microscopy (SEM).

Particles were observed by a Scanning Electron Microscopy (SEM) (Phillips, mod. XL30). Particles were previously coated with a 4 nm gold layer by a sputter coater (Polaron, mod. SC7640).

Results and Discussion

Experimental procedure of WEPO®.

The experiments were carried out at three different water flow rates (0.1, 0.2, 0.3 mL/min). The optimal flow rate for the formation of the spray depends on the sample. The oven temperature is also critical. While carnosic acid from rosemary is extracted mainly at 200 ºC, the best antioxidant extract from oregano is obtained at 150 ºC [10]. However, 150 ºC in the oven is not enough to ensure a right spray formation and drying of the extract, so for all the experiments the oven temperature was settled at 200 ºC.

The fast depressurization of the supercritical fluid, together with the explosive release of the supercritical fluid mixed with the aqueous solution, acts as an atomizer of the

solution and generates a continuous aerosol. This aerosol, formed by small droplets of the aqueous solution, is lead to a hot N_2 current. This inert gas evaporates the solvent that forms the droplets, leading to particle formation.
In Figure 2, a picture of rosemary (A) and oregano(B) particles obtained by WEPO® are shown.

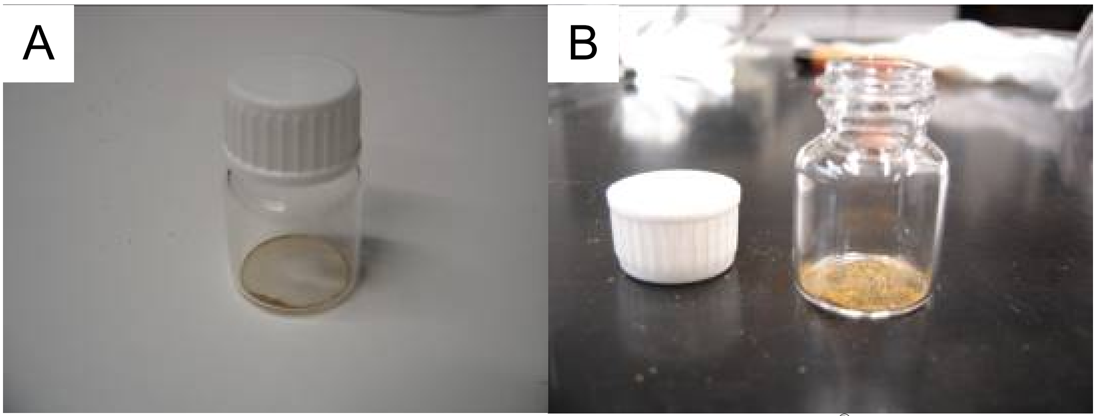

Figure 2. Dried particles of A) rosemary and B) oregano obtained by a WEPO® process.

Table 1 shows the experimental conditions used, together with the particle sizes obtained and their antioxidante activity (expressed in EC50 values).

Table 1. Values of the different parameters used, maximum sizes (μm) and EC_{50} (μg/mL) values of the particles.

Sample number	Plant	Water flow rate (mL/min)	CO_2 Flow rate (mL/min)	N_2 Flow rate (mL/min)	Extraction T ºC	Pressure CO_2 bar	Minimum and maximum particle sizes /μm	EC_{50}/μg·mL^{-1} (6.25 μg/mL sample concentration)
1	Rosermary	0.1	2.5	0.6	200	80	14-93	10.5
2	Rosermary	0.2	2.5	0.6	200	80	8-75	
3	Rosermary	0.3	2.5	0.6	200	80	4-62	
4	Oregano	0.1	2.5	0.6	200	80	16-140	9.9
5	Oregano	0.2	2.5	0.6	200	80	7-104	
6	Oregano	0.3	2.5	0.6	200	80	9-73	

Antioxidant activity analysis of the particles

The water flow rate does not influence the EC50 value of the particles. Thus, Table 1 shows only one antioxidant activity for each plant tested. It is important to point out that antioxidant activity values for both plants are comparable to those obtained with a commercial SWE extractor plus a freeze-drying process.

SEM analysis of the particles

As can be observed in Figures 3 and 4, particles precipitate in agglomerates formed by smaller particles with no defined shape.

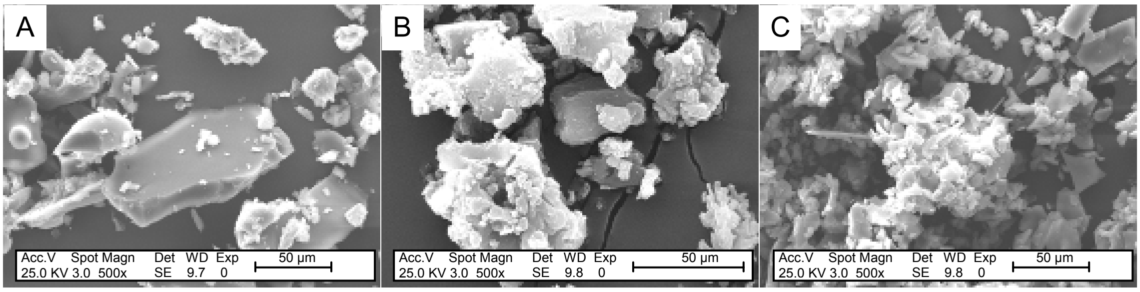

Figure 3. Rosemary particles observed by SEM, obtained at: A) 0.1mL/min, B) 0.2 mL/min C) 0.3 mL/min water flow rates.

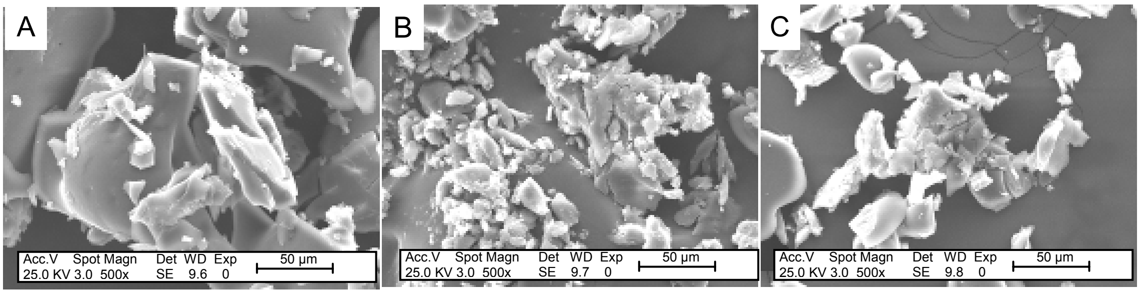

Figure 4. Oregano particles observed by SEM, obtained at: A) 0.1mL/min, B) 0.2 mL/min C) 0.3 mL/min water flow rates.

The size of the particles and agglomerates can influence the solubility of the sample in different food matrixes and its bioavailability. The maximum and minimum size values for each sample are shown in Table 1. As can be observed, the water flow seems to influence the particle size, leading to smaller particles when increasing the flow rate, being the highest flow rates responsible of the smaller particles.
According to literature [11, 12], conditions that enable fast drying processes lead to bigger particles than those that enable slower drying. A fast drying lead to a higher volume structure in the initial moments and does not allow swelling of the particles during the drying process.
The extract concentration also influences the particle size, but no data is available to correlate the water flow rate with the extract concentration. If higher water flow rates would lead to lower extract concentrations, the extract would be less viscous, which is related to smaller particles during the spray formation.
As can be observed in Table 1, particle sizes are around 102 μm fold, so no nanoparticles but microparticles are collected. However, their small size could favour their solubility in different food matrixes and/or its bioavailability in the intestine [13].

Conclusions

A new on-line process for Subcritical Water Extraction of plants and drying of the extract in one step by particle formation based on the use of supercritical CO_2 has been developed. With this process, complex dried extracts that preserve their intact antioxidant activity have been obtained as a fine powder with particle sizes lower than 140 μm length that could enhance the solubility and/or bioavailability of the compounds in the intestine. The WEPO® process developed in this work can be considered as a suitable and promising process to obtain, in only one step, fine and dried particles with intact antioxidant activity, directly from the plant leaves.

Acknowledgements

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