Drying of Polyphenol Extracts from Bilberry By-products by SAA Process

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In the industrial production of bilberry juice, a solid cake is obtained as by-product. It is a good source of phenolic compounds and other antioxidants, substances that scavenge harmful molecules such as free radicals, preventing or even reversing cellular damage. The bilberry by-product can be treated using ethanol and the obtained solution, antioxidants rich, has to be processed to obtain a stable powder where values compounds are concentrated. The spray dying is an inexpensive method that allows the aqueous ethanolic extracts to be dried using maltodextrin as a carrier. The amount of maltodextrin (MD) should be the minimum that allows the production of dry powder of single particles, not cluster of particles. When the ratio dry residue/MD is 1:5, the typical powder obtained consists of particles smaller than 10 μm in diameter. Alternatively, the processing of ethanol extract using Supercritical Atomization (SAA) technique allows to reduce drying temperatures and a smaller amounts of carrier are needed. The lower temperature needed will be advantageous because protects a part of polyphenols from degradation and thus potentially increases their recovered amount. Moreover, using the SAA process a dry residue/MD ratio 1:2 is enough to obtain regular not connected particles. The particles have a narrow size distribution, the average diameter size being significantly smaller than that of the particles obtained by spray drying. Spherical particles with diameter lower than 3 μm were produced. The PSDs slightly depended on the amount of the carrier. The product with dry residue/MD ratio 1:3 has preserved more than 90% of the polyphenols of the starting extract.

1. INTRODUCTION

An increasing demand of different kind of food supplements as the consumers have become more interested in functional and healthy foods and their health benefits. The increase of nutraceutical market also required available sources of bioactive compounds.

The solid by-products from vegetable and fruit processing have gained industrial interest by the content of bioactive compounds that remains in this matrixes. In the case of berry juice production, large amounts of solid residues are generated and referred to as press cake by-product. This solid matrix, consists of the seeds and skins of the berry and it has not been used extensively as source of bioactive ingredients.

Bilberry (*Vaccinium myrtillus* L.) is in fact an acknowledged source of anthocyanidins (ACNs), an excellent antioxidant [1]. Antioxidant compounds are important by their activity against degenerative illness derived from oxidation and inflammation reactions [2]. Polyphenols and more specifically ACNs are extracted with ethanol or aqueous ethanol solutions and some techniques to recovery ACNs are already available, but the technique used should take into account the light, heat and air sensitivity of ACNs.. However, the antioxidant compounds have to be protected from environment to maintain their beneficial properties. Microencapsulation is the common process to protect antioxidants against destructive agents and also allows obtaining handled powder or ingredients; at the same time, microencapsulation protects the flavours from undesirable interactions with the food matrix. For microencapsulation the most used materials are carbohydrates, such as hydrolyzed starches, emulsifying starches and gums [3]. Maltodextrines of different dextrose equivalents have high solubility in water, low viscosity and are extensively used in food industry. The processing of liquid solutions by spray drying technique is an available and suitable technique to micronize and protect natural antioxidant compounds [4]. Nevertheless the spray drying technique has also some problems, related to the high temperature used in the process and, in some cases, the eventual use of air as drying medium.

Supercritical Assisted Atomization (SAA) is an innovative technique, whose operating conditions respect the fragile nature of antioxidant compounds. SAA allows the processing of water-soluble compounds and uses supercritical carbon dioxide (SC-CO_2) to saturate a liquid solution with the formation of an expanded liquid in a saturator, improve in such a manner the mixing and the residence time. The use of heated N_2 as drying phase generated in the precipitator an inert atmosphere that protects sensitive compounds. SAA process takes advantage from the supercritical CO_2 that generates an expanded liquid in the saturator that has low viscosity and low surface tension. This improves the jet break-up at the nozzle with the production of smaller droplets. SAA has been successfully applied for micronization of sensitive compounds [5] and it has been observed the main effect of pressure and temperature of saturator over the quality of products.

The aim of this work was to micronize an enriched polyphenolic extract from by-products of bilberry juice production, using spray drying technique and SAA technique and evaluate the process effect over antioxidant activity of the product.

2. MATERIALS AND METHODS

2.1 Materials

The berries used in the extraction experiments and for the spray drying experiments had been grown and harvested by MTT Agrifood Research Finland and were kept frozen at – 20°C until experiments. The bilberry used for the SAA experiments were bought frozen from a shop in Salerno, Italy. The used matrix corresponded to the solid part, skin and seeds, from pressing juice production. Maltodextrin 13-17 dextrose equivalent from Sigma Aldrich (Milan, Italy) was used as coating material in micronization

2.2 Methods

Bilberry extracts were prepared using aqueous ethanolic solution (90% v/v); 10 g of sample were mixed with 100 mL of ethanolic solution and stirred continuously at room temperature for 30 min. Then the liquid solution was separated by filtration and the solid residue extracted twice again in the same ratio. Total phenol content and related antioxidant activity were.

For the powder production, the ethanolic solution was mixed with maltodextrin; the final solution consisted of 50 mL of ethanolic solution (containing the dry residue) with 50 mL of water and maltodextrin in a fixed ratio in relation with dry residue. A laboratory apparatus Buchi mini spray drying B 290 (BUCHI Labortechnik, Switzerland) was used for spray drying. The operating parameters were air flow 23 m^3/h, inlet and drying temperature were maintained at 150°C. The powder products were stored at -20°C until its analyses.

The SAA apparatus consists of three feed lines delivering supercritical CO_2, liquid solution and warm N_2, respectively. The plant consists of three main vessel: saturator, precipitator and condenser. Liquid carbon dioxide is delivered by high-pressure pump to a heated bath, and then to the saturator where it solubilizes into the liquid solution. The liquid solution is taken from a graduated glass vessel, pressurized by a high-pressure pump, heated and sent to the saturator. A controlled flow rate of heated N_2 is also delivered into the precipitator to induce the evaporation of the liquid solvent. The saturator is a high-pressure vessel (i.v. 25 cm^3) loaded with stainless steel perforated saddles; it provides a large contacting surface and a residence time long enough (5-6 min) to allow the dissolution of SC-CO_2 in the liquid solution up to the saturation conditions at process pressure and temperature. The obtained solid-liquid-gas mixture, at the exit of the saturator, is sent into a thin wall stainless steel injector (i.d. 80 µm) to produce a spray of liquid droplets in the precipitator. The precipitator is a stainless steel vessel operating at near atmospheric conditions. It is electrically heated using thin band heaters connected to a temperature controller. The powder precipitated into the precipitator is, then, collected at the bottom of the chamber on a stainless steel sintered filter. Further details and the schematic representation of the SAA plant were published elsewhere [5, 6].

2.3 Analytical Methods

Particle size and morphological characteristics of the collected solid from spray drying and SAA were analyzed by scanning electron microscope (SEM, mod. 420, LEO). Diffraction patterns of each sample was obtained using an X-ray diffractometer (mod. D8 Discover, Bruker). The measuring conditions were as follows: Ni-filtered CuKa radiation, l=1.54 Å, 2θ angle ranging between 5° and 70° with a scan rate of 3 seconds/step and a step size of 0.2°. The Folin Ciocalteu method was used for the determination of phenolic compounds, as mentioned by Laaksonen, Sandell and Kallio [7]; a UV/Vis spectrometer was used to measure the variance of absorbance at wavelength 750 nm. Briefly, 1 mL of solution was mixed with 50 mL of water, 5 mL of Folin Ciocalteu reagent, 20 mL of sodium carbonate 20% (w/v) and the final volume was made up to with water until 100 mL. The solution was hardly shaken and allows to react for 30 min before analyzes. The results were expressed as mg equivalent of gallic acid/g of sample using a calibration curve with real standard of gallic acid from 0.1 to 1.2 mg/mL in concentration. Dilutions in water were made if the measurement was out of calibration range. Antioxidant activity was measured by 2,2-diphenyl-1-picrylhydrazyl (DPPH) analysis: DPPH reagent was used as radical to measure the radical scavenging activity of solutions [8]. A stock solution of DPPH was prepared by dissolving 24 mg DPPH with 100 mL of methanol and then stored at -20 °C. The working solution was obtained mixing 10 mL of the stock solution with 45mL of methanol until adjust the absorbance to 1.17 ±0.02 units at wavelength 515 nm. A certain amount of the samples (150 µL) were mixed with 2850 µL of working solution just made. This solution was allowed to react for 24 hours in the dark and then the absorbance was taken at 515 nm. The results were expressed as amount of trolox per dry extract (mg/g).

2. RESULTS AND DISCUSSION

Bilberries were pressed to obtain a solid matrix containing only the peels and the seeds of the berries. Polyphenols were, then, extracted from these matrices (press cake) usually discarded in the fruit juice factories. The extractions were performed using an ethanol/water solution 90% (v/v) and a ratio press cake/ethanol solution 1:10 (g/mL) and were subsequently micronized by spray drying.

The operating condition were:, drying temperature 150°C and air inlet temperature 150°C and flow 23 m^3/hr. The first spray drying experiments were made using dry residue/MD ratio 1:20 then a reduction of this ratio was done trying to improve the concentration of dry extract in the produced powder. The experiments with dry residue/ MD ratio 1:1 and 1:3 produced sticky and not collectable material. The lowest dry residue/MD ratio that gave a collectable powder was 1:5. The SEM photomicrograph in **Figure 2** shows the morphological characteristics of the obtained particles dry residue/MD ratio.

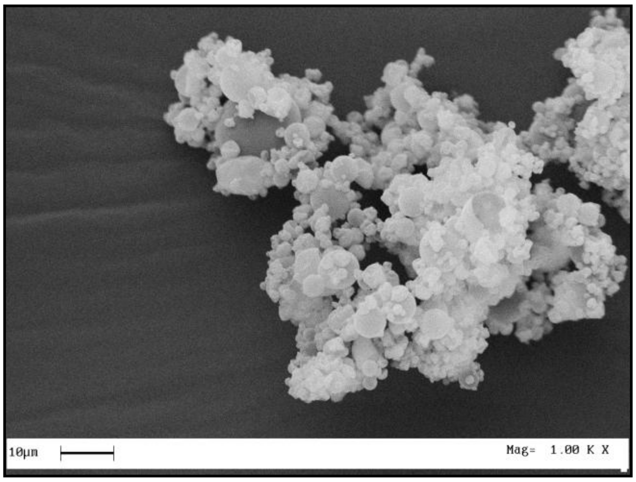

Figure 1. SEM photomicrograph of powder of bilberry press cake obtained by spray drying with a dry residue/MD ratio 1:5.

The PSDs of the powder obtained are reported in the **Figure 2**. Particles have a diameter smaller than 10 µm, independently of the dry residue/MD ratio. The mode increases from of 1.5 µm to 2 µm increasing the dry residue/MD ratio, since in all the cases the concentration of the starting solution is the same, this result might be related to the different characteristics in term of viscosity of the fluid solution due to the presence of higher amounts of MD.

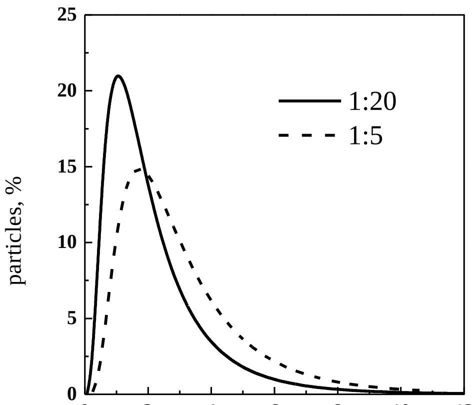

Figure 2. PSDs in terms of number of particles percentage of powder obtained by spray drying at different dry residue/MD ratio

The bilberry extract was dried also by Supercritical Assisted Atomization (SAA) to study the potential use of this process on drying of active food compounds, with the purpose to reduce the degradation during the process using lower temperatures and decrease the percentage of carrier needed with respect to spray drying. Besides, using the SAA process it is possible obtain powder with a lower particle size distribution that the spray drying allowing to extend the application fields. Since polyphenols are sensitive to the temperature, the lowest

temperature with the restriction given by the solvent was used. The operating conditions in each experiment were: solvent EtOH 90%/Water 1:1, concentration of liquid solution 20 mg/mL, CO_2/solution w/w ratio 2.5, temperature at the mixer 70°C, pressure in the mixer 110 bar, precipitation temperature 75°C, N_2 temperature 100°C. The dry residue/MD ratios used were 2:1, 1:2, 1:3.
The collected powder was slightly electrostatic and had a violet colour, whose intensity depends on the amount of maltodextrin present. The powder obtained from a dry residue/MD ratio 2:1 was formed by particles with a branched structure, connected each other; nevertheless, for the other ratios spherical particles well separated were obtained, as can be observed from the SEM photomicrograph reported in **Figure 3**.

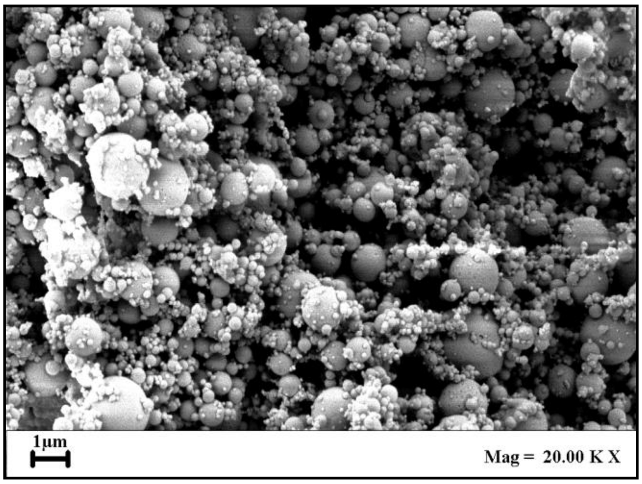

Figure 3. SEM photomicrograph of powder of bilberry press cake obtained by SAA with a dry residue/MD ratio 1:2

The PSDs reported in **Figure 4** show that there is no relevant influence of the dry residue/MD ratio: in both experiments the size of the particle is smaller than 3 μm and the mean diameter is about 250 nm.

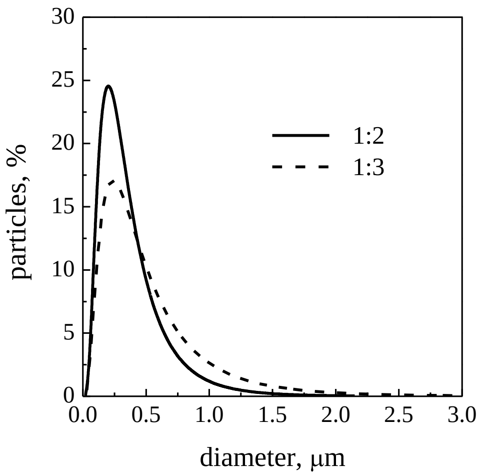

Figure 4. PSDs in terms of number of particles percentage of powder obtained by SAA at different dry residue/MD ratio

The X-ray diffraction analyses showed that the powder obtained using spray drying and by SAA technique had an amorphous structure.

The total amount of phenolic compounds in the extract used for the experiments and in the powder obtained by spray drying and by SAA was analysed by spectrophotometric *Folin-Ciocalteu* method. The results as amount of phenolic compounds per dry extract (mg/g) are reported in the **Table 1**. The amount of polyphenols recovered in the powder obtained by SAA is higher with respect to the one obtained by spray drying, except for the case of dry residue/Md ratio of 2:1. In all the other cases, the recovery of total phenolic compounds increased when higher amount of maltodextrin was used. When a dry extract/MD ratio 1:3 in the SAA experiment was used, a recovery higher than 90 % was obtained.

Table 1. Total phenolic compounds in the ethanolic extract and in the dried powder by spray drying and SAA

sample	Dry residue/MD	Cr [mg/g]	Recovery %
Extract		214	
Spray drying	1:5	114	56
SAA	2:1	92	43
SAA	1:2	188	88
SAA	1:3	197	92

The antioxidant power of the powder produced by SAA technique did not change if the samples were stored at room temperature in amber glass and it slightly changed when the samples were exposed at the effect of the UV light and of the temperature. The analysis was performed also on the ethanolic extract after exposure at 40°C and UV light: it had a colour change and degradation of the antioxidant activity of 100%.

3. CONCLUSIONS

The SAA process is an excellent alternative to the traditional dry methods for the collection and storage of polyphenols. Using this technique lower amount of carrier to obtain a dry and stable product is used and as a consequence powder containing an higher amount of active principle compare to the traditional spray drying is obtained. Furthermore, it is possible to obtain a powder with better control of particle size distribution compared to the other technique.

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