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Analytical Methods

# Supercritical fluid extraction and microencapsulation of bioactive compounds from red pepper (*Capsicum annum* L.) by-products

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ABSTRACT

The objective of this work was to obtain and stabilize natural vitamins from red pepper by-products. The method of obtainment was supercritical carbon dioxide extraction, studying different parameters that affect the yield. The highest extraction yield was found at 60 °C, 24 MPa extraction, with no modifier added and 0.2–0.5 mm particle size. The recovered extract was a red-coloured oil. The extract was subsequently microencapsulated by spray-drying using gum arabic as wall material to avoid the degradation of vitamin over the storage time. The thermal stability of microcapsules was analysed by thermal gravimetric analysis (TGA), while size, shape and morphology of microcapsules were studied by scanning electron microscopy (SEM). The microcapsules containing pepper extract were particles of spherical shape with dents on the surface, the average size of these particles was 5.46 μm.



## 1. Introduction

Worldwide agrifood industry generates several million tonnes of waste every year. The percentage of waste generated in the development of processed vegetables is highly variable, which is determined by several factors including the type of raw material to be processed. In general, solid wastes from organic raw materials processed may represent more than 50% in weight of the original raw material source (MAPA, 2006). This fact constitutes a serious problem for the environment. In some cases, organic solid wastes produced in the processing of vegetables and fruits can be considered by-products. Almost all of them contain compounds of interest for their potential use as nutritional or functional ingredients (proteins, vitamins, pigments, antioxidants, antimicrobials, fragrances, etc.). Therefore, obtaining these ingredients from by-products is one of the most interesting alternatives for the reuse of these by-products (Louli, Ragoussis, & Magoulas, 2004).

The Piquillo red pepper variety (*Capsicum annum* L.) is highly demanded by the global food industry, on account of its aromatic, colouring, and flavouring properties. The Spanish region of Navarre is certified by the Protected Geographical Indication (PGI) "Pimiento del Piquillo de Lodosa". After processing of Piquillo red pepper, a by-product is produced, which amounts to approximately 65% in weight of raw material. This by-product is currently disposed of as municipal solid waste.

The intense red colour of ripe peppers is due to the presence of carotenoid pigments (Deepa, Kaur, Singh, & Kapoor, 2006). β-Carotene is also known as provitamin A since it is the major source of vitamin A for people worldwide. Vitamin A is required for normal development, growth, and eyesight. Thus, dietary β-carotene is essential for humans (Burri, 1997). Furthermore, red pepper is a good source of tocopherols in general, and more particularly of α-tocopherol (vitamin E) (Koch, Arango, Mock, & Heise, 2002). Vitamin E has a protective effect against a number of disorders, for example, atherosclerosis, ischaemic heart disease and different tumours (Azzi, Ricciarelli, & Zingg, 2002).

The acquisition of these vitamins requires their extraction from red pepper by-products without altering their natural original properties. With increasing concerns over the use of organic solvents and their disposal, supercritical fluid extraction is becoming a promising alternative. Among several gases and liquids studied, carbon dioxide remains the most commonly used fluid because of its low critical constants ($T_c$ = 31.1 °C, $P_c$ = 7.38 MPa), its non-toxic and non-flammable properties and its availability in high purity at low cost. Moreover, the extracts obtained by supercritical carbon dioxide extraction are regarded as Generally Recognised As Safe (GRAS) for the American Food and Drug Administration, being possible to add them to any food without undesirable effects for health. Numerous works have been done on the application of supercritical carbon dioxide extraction from vegetable by-products (De Lucas, Martinez de la Ossa, Rincón, Blanco, & Gracia, 2002; Herrero, Cifuentes, & Ibáñez, 2006), citing many advantages such as higher diffusion coefficient and lower viscosity than liquid solvents and the possibility of manipulating selectivity by varying

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extraction temperature, pressure and other parameters. None of these works considered the supercritical fluid extraction of natural vitamins from Piquillo red pepper by-products.

On the other hand, natural vitamins are easily degradable by the action of light, oxygen and temperature. The microencapsulation method seems to be useful to solve this problem. Several techniques are employed to form microcapsules, including spray drying, spray chilling, extrusion coating, fluidized bed-coating, liposome entrapment, coacervation, etc. The most common and economical way to carry out microencapsulation is spray drying. The selection of wall materials for spray drying is vital for efficient microencapsulation. Gum arabic is a cheap and easy to obtain wall material that has been used to microencapsulate different extracts (Krishnan, Bhosale, & Singhal, 2005; Kanakdande, Bhosale, & Singhal, 2007).

The aim of this study was to obtain and stabilize natural vitamins from Piquillo red pepper by-products. Supercritical fluid extraction was used for the obtainment of the vitamins and microencapsulation was used for their stabilization. The effects of supercritical fluid extraction conditions such as temperature, pressure, extraction time, particle size and addition of ethanol as modifier, on the recovery of vitamin E and provitamin A were investigated. Additionally, the microencapsulation of the extract obtained was carried out to avoid its degradation by the action of the light. The microencapsulation process was performed using gum arabic as wall material. Thermal analysis was used to study the stability of the microcapsules. Finally, the stability of vitamins within the extract and the microcapsules was studied.

## 2. Materials and methods

### 2.1. Materials

Pepper (*Capsicum annum* L. variety Piquillo) by-products were constituted by seeds, skin leftovers and stems 15.9%, 34.7% and 49.4% (percentage in weight), respectively. The by-products were obtained from a producer of canned foods located in Navarra (Spain).

Standards of vitamin E (α-tocopherol) with a purity of 99%, provitamin A (β-carotene) (95% purity) and trans-β-Apo-8′carotenal (96% purity) were supplied by Sigma–Aldrich (Sigma–Aldrich Chemie GmbH, Germany). Acetonitrile, *n*-hexane, methanol and 2,6-di-tert-butyl-4-methylphenol (BHT) were acquired from Merck (Merck KGaA, Germany). Absolute ethanol, trichloromethane and acetone were purchased from Panreac (Panreac Quimica S.A.U., Spain). Gum arabic was supplied by Scharlau (Scharlau Chemie S.A., Spain) and Tween 80® was purchased from Acros Organics (Acros Organics, Belgium).

### 2.2. Methods

#### 2.2.1. Supercritical fluid extraction (SFE) from red pepper by-products

Extraction experiments were carried out using a SFE system (Iberfluid, Spain). The unit consists of two pumps ($CO_2$ and modifier), a 350 mL extraction vessel and two 40 mL separation vessels. The extractor is pressurised with the fluid ($CO_2$ or $CO_2$ + ethanol as modifier) and is introduced bottom-up. The system is equipped with temperature controllers and pressure valves. The fluid and the compounds extracted are transported to separation vessels. The extraction system is fully automated and controlled by computer (see Fig. 1).

The extraction process can be described as follows: first, the pepper by-products were frozen (−40 °C) and freeze-dried (Lyoalfa 6-80, Telstar, Spain) to a humidity content below 1%, then they were crushed and sieved through three different pore size sieves (0.2, 0.5 and 1.25 mm). For SFE, 30 g of pepper by-products were loaded into an extraction vessel. The $CO_2$ flow rate used for all experiments was fixed at 2000 mL/h. Different conditions of extraction were assayed: temperature 45 and 60 °C; pressure 20, 24 and 30 MPa, time 90 and 120 min, without or with modifier (300 mL/h).

The extraction yield of vitamin was defined as the percentage rate between the concentration of vitamin present in the pepper extract and the amount analysed in the by-products. The extraction yield for vitamin E and provitamin A were calculated separately.

#### 2.2.2. Microencapsulation of red pepper extract

Microencapsulation of the synthetic vitamins and red pepper extract was performed by emulsion followed by a drying process. The emulsion was spray dried using a Büchi B-290 (BÜCHI Labortechnik AG, Switzerland) equipped with an inert loop developed to specifically allow the use of organic solvents.

In a first step, synthetic vitamin E and provitamin A were used to optimise the microencapsulation process and compared to microencapsulated natural red pepper extract. A wall mixture emulsion was prepared by dissolving 40 g of gum arabic and adding 2 drops of Tween 80®. Different amounts of synthetic vitamin E (0.377 g), synthetic provitamin A (0.076 g) and red pepper extract (0.752 g) were weighted in a flask, the wall mixture was subsequently added to the vitamin and continuously stirred for an hour at room temperature. Afterwards, distiled water was added up to a final volume of 200 mL and the resulting mixture was spray-dried using the following parameters: inlet temperature of 185 °C, outlet temperature of 103 °C, aspiration rate 90% and pump flow rate 10%.

The actual vitamin entrapment was calculated as the difference between total and surface vitamin. To quantify surface vitamin, 10 mg of microcapsules were extracted three times with 3 mL of absolute ethanol, the solvents from the extractions were put together and brought to a volume of 10 mL. For total vitamin, 10 mg of microcapsules were dissolved by sonication in distiled water (25 mL) and, absolute ethanol was added up to 50 mL.

Therefore, the efficiency of the microencapsulation process is defined as the percentage rate between the amount of microencapsulated vitamin and the total amount of vitamin in the microcapsule (surface and entrapped).

#### 2.2.3. Analytical methods

For the determination of provitamin A, 5 g of sample were weighted in a 50 mL conical tube, and 20 mL of extraction mixture (hexane:acetone:ethanol 50:25:25 v/v/v) were added. The conical tube was shaken on an orbital shaker for 30 min at 480 r/min, and it was finally centrifuged at 5000 r/min for 10 min. The supernatant was collected and the pellet was extracted twice more. The supernatants were mixed and 5 mL of the organic phase were pipetted into a test tube, dried in a vacuum evaporator (Turbovap LV, Caliper LifeSciences, MA, USA), and dissolved in a mixture of methanol and acetonitrile (25:75). All extraction processes were carried out in the absence of light.

High Performance Liquid Chromatography (HPLC) was used for the determination of provitamin A (Olives Barba, Cámara Hurtado, Sánchez Mata, Fernández Ruiz, & López Sáenz de Tejada, 2006). The chromatograph was equipped with a Waters 2795 autosampler and a Waters 2996 UV–visible detector (Waters, MA, USA). A Waters Spherisorb ODS II C18 column (250 × 4.6 mm, 5 μm) was used as stationary phase. The mobile phase (methanol:acetonitrile 40:60 volume) flow-rate was fixed at 0.8 mL/min. Peak identifications were based on retention time, and quantification was based on peak area using trans-β-Apo-8′carotenal as internal standard.

For the determination of vitamin E, 2 g of sample were placed in a 15 mL conical tube and extracted with 3 mL of ethanol 96% (stabilized with 0.1% BHT) by vortex mixing and subsequent