Encapsulation and precipitation of aqueous natural hydroxytyrosol-rich concentrate into a solid lipid matrix through PGSS®

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ABSTRACT

Hydroxytyrosol-rich concentrate is a natural aqueous extract derived from olive-mill residues that present bioactive features such as antioxidant, antimicrobial and anti-inflamatory action. However, its aqueous nature may hinder its incorporation into pharmaceutical, cosmetic or food formulations with large lipophilic characteristics.
In this work, particles of glycerol monostearate (GMS) loaded with hydroxytyrosol were produced by PGSS® (Particles from Gas Saturated Solutions). The solid lipid particles produced were characterized in terms of morphology, size and thermal behaviour. The remained water content, the total concentration of phenolic compounds and their antioxidant capacity were also determined. The optimum mass ratio of GMS:aqueous extract possible to produce handlable particles with high content of hydroxytyrosol was assessed and set at 1:1.

INTRODUCTION

Hydroxytyrosol-rich concentrate is a natural bioactive aqueous extract derived from olive-mill residues that has an important added value as antioxidant, antimicrobial and anti-inflammatory with application in nutraceutical, pharmaceutical and cosmetic formulations[1]. Nevertheless, it is highly desirable to work with solid forms, instead of liquid extracts, since aqueous extracts are chemically unstable being susceptible to rapidly oxidized in presence of sunlight and/or atmospheric oxygen. Moreover, the incorporation of aqueous extracts into products with a large lipophilic component could be hindered, triggering problems of incompatibility with other active agents and product's final forms. The aim of the present work was the precipitation and encapsulation of hydroxytyrosol-rich extract into a lipophilic solid matrix through Supercritical Fluid (SCF) Technology. By using glycerol monostearate as the chosen lipid carrier to encapsulate the extract, it was possible to produce solid lipid particles rich on hydroxytyrosol. Moreover, this lipid carrier presents emulsifying properties suitable for the development of emulsions. One of the advantages of using sc-CO_2-based precipitation processes is the melting point depression of GMS in the presence of compressed CO_2 (reduction of 9.8K at 13.4MPa) [2], thus being an appropriate technique while processing thermolabile compounds [3].

Particles of GMS containing hydroxytyrosol (HT) were successfully produced by PGSS®. The maximum mass ratio of GMS:water possible to produce handlable particles was previously evaluated and fixed at 2:3. The operating conditions used were set at 13MPa and 335 K. Heated compressed air (305K) was used during the atomization step in order to improve the drying capacity of the method. The total concentration of phenolic compounds present in the encapsulated forms was determined according to the Folin–Ciocalteu colorimetric method, being their antioxidant capacity also accessed by Oxygen radical absorbance capacity (ORAC) assay. Additionally, the solid lipid particles produced were analyzed by Differential Scanning Calorimetry (DSC) for the knowledge of their thermal behaviour, by Scanning Electron Microscopy (SEM) for morphology characterization and by Laser Diffraction (LD) for particle size distribution. The remained water content in the particles was also determined.

MATERIALS AND METHODS

Materials

Lumulse® GMS-K (GMS) was kindly provided by Lambent Technologies. Imwitor® 600 (water/oil emulsifier) was kindly supplied by Sasol (Germany). CO_2 (99.95 mol% purity) was delivered by Air Liquide (Portugal). Chlorophorm was purchased from JMGS (Portugal). HT-rich aqueous extract ([Polyphenols]=90mgGAE/mL;ORAC=3325µmolET/mL) was obtained according to the US 8,066,881 B2 patent[1].

Particles from Gas Saturated Solutions (PGSS®)

Hydroxytyrosol-loaded particles were produced through the PGSS® process. The schematic representation of the modified PGSS® equipment (FAME UNIT, Separex, France) used to produce the particles is shown in Figure 1.

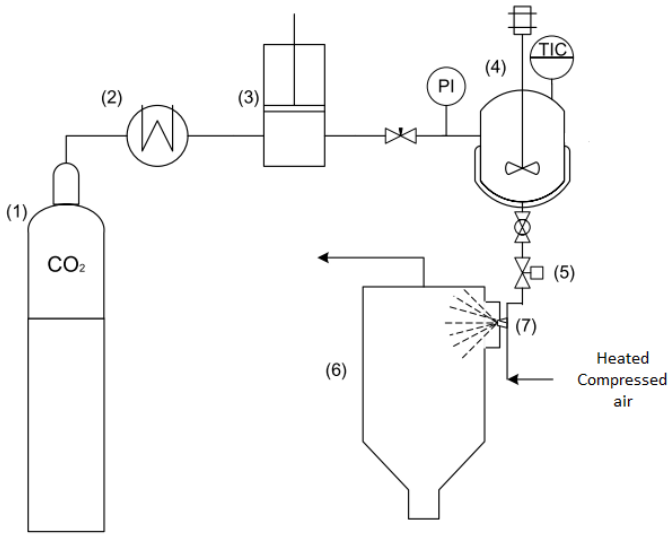

Figure 1. Experimental setup: (1) CO_2 cylinder (2) cryostate (3) pneumatic piston pump (4) stirred vessel (electrically thermostated) (5) automated depressurization valve (6) recovery vessel (7) nozzle

Carbon dioxide was fed by a high-pressure piston pump to a 50 cm^3 electrically thermostated high-pressure stirred vessel, containing the materials to be precipitated, until the desired

working pressure was reached. The operating conditions used were chosen according to previous experiments while processing GMS and water and set at 13MPa and 335.15 K [2]. After 30 minutes of stirring (215rpm), the mixture was depressurized by an automated depressurization valve and atomized through a two fluid nozzle (d=250μm) to a cyclone, where it was mixed with heated compressed air (0.7MPa, 305.15K) for improved drying. Finally the particles were recovered in an 18 L collector vessel.
In this work, GMS acted not only as the carrier agent but also as the emulsifier, since an emulsion was formed while the lipid and aqueous solutions were mixed. This emulsion was further precipitated by PGSS® in order to obtain particles. Table 1 shows a summary of the experiments performed in this work. In experiments A-E, mixtures of GMS:Water were precipitated in order to previously evaluate the maximum % of aqueous solutions capable to be precipitated with the lipid and producing dry particles. The experiments F-H were performed with the natural aqueous HT-extract. In experiments E, G and H an additional emulsifier, Imwitor® 600, was used in the formulation (2% w/w).

Table 1: Summary of the experiments performed.

EXP	Compounds	Mass ratio
A	GMS:Water	3:1
B	GMS:Water	3:2
C	GMS:Water	1:1
D	GMS:Water	2:3
E	GMS:Water	2:3
F	GMS:HT aqueous extract	1:1
G	GMS:HT aqueous extract	1:1
H	GMS:HT aqueous extract	2:3

Product Analysis

Morphology

The morphology of the particles were analyzed and imaged by Field Emission Scanning Electron Microscopy (JEOL 7001F). Before the analysis, particles were covered with approximately 300 Å of a gold-platinum film with a sputter-coater in argon atmosphere (Polaron).

Particle size distribution

Particle size distribution (PSD) was measured with a laser diffractometer (Mastersizer 2000, Malvern). Particles were dispersed in distilled water and measurement was carried out after a gentle rotation of the particles suspension container in order to obtain an ever dispersion of the particles. In this work, particle size measurements are reported as volume distribution and defined as the average diameter ($d_{0.5}$), being the final result the average from 3 measurements. The span value is also reported, that is, the ratio between $d_{0.5}$ and ($d_{0.9}$- $d_{0.1}$); span values near to 1 represent narrow PSD.

Thermal Behavior

Differential Scanning Calorimetry measurements were carried out on a DSC TA instruments Q200 (module MDSC) with the aim of studying the thermal behavior of the particles. The samples were placed in an aluminium pan and sealed; the probes were heated from 253.15 to 473.15K at a rate of 10K/min under nitrogen atmosphere.

Moisture content

Samples of the particles were dried at 55ºC for 48h. The moisture was expressed as g of water per 100g of particles (% w/w).

Total phenolic content by Folin-Ciocalteau method

The total concentration of phenolic compounds present in hydroxytyrosol extract and particles was determined according to the modified Folin Ciocalteau colorimetric method as previously described by Serra et al. [4]. A suspension was prepared by adding 10mg of particles in 1ml of double distilled water and further vortexed. After 10 minutes in an ultrasound bath, the suspension was filtered with a 0.45 µm filter (Whatman) and the aqueous media was used for further analysis.The results were expressed as means of triplicates (mg of gallic acid equivalents (GAE) per liter of extract or per mg of particle).

Evaluation of antioxidant activity by Oxygen radical absorbance capacity (ORAC) method

ORAC assay was carried out by a modified method for the FL800 microplate fluorescence reader (Bio-Tek Instruments, Winooski, VT, USA), as described by Serra et al.[5] .This assay measured the ability of the antioxidant species in the sample to inhibit the oxidation of disodium fluorescein (FL) catalyzed by peroxyl radicals generated from AAPH (2,2'-azobis-2-methyl-propanimidamide, dihydrochloride). A suspension was prepared by adding 10mg of particles in 1ml of double distilled water and further vortexed. After 10 minutes in an ultrasound bath, the suspension was filtered with a 0.45 µm filter and the aqueous media was used for further analysis. The results were presented as Trolox equivalent (TE) per L of extract or mg of particle.

RESULTS

This work started with the study of the maximum percentage of water that can be mixed with GMS suitable to produce handlable particles by PGSS®. As expected, as the initial amount of water in the formulation increased (experiments A-D), so did the moisture in the particles produced (Fig.2). Particles obtained in experiment D were not sufficiently dried and were difficult to handle, being this fact due to the almost 30% of residual water content present in this samples. This experiment was repeated (Exp E) with the addition of a second emulsifier, Imwitor® 600, with the aim of further stabilize the emulsion. The handlable particles obtained in this experiment presented final water content similar to experiment C.

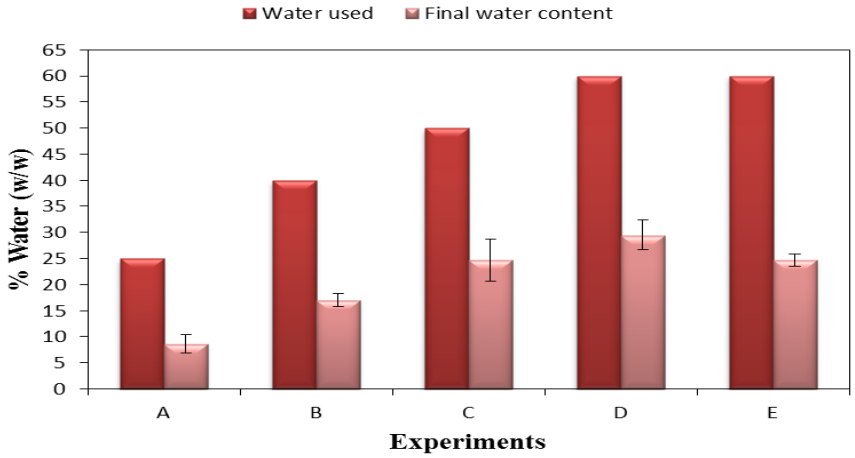

Figure 2. Comparisson between the water present in the initial formulation and the water present in produced particles

Thus, the maximum mass ratio of GMS:water suitable to produce dry particles was fixed at 2:3, in the presence of an additional water/oil emulsifier. This mass ratio was used to further perform the experiments with the HT-rich concentrate extract, being the mass ratio 1:1 also tested, with and without Imwitor® 600. Lipid particles containing HT were successfully produced by mixing GMS with the hydroxytyrosol-rich extract. The results are shown in Table 2 and Figure 3.

Table 2: Product analysis

EXP	Morphology	$d_{0.5}$ (µm)	Span	% water w/w	Melting Enthalpy (J g^{-1})	Phenolic compounds (mgGAE/g particle)	ORAC (µmol TE/g particle)
F	Particles	72.5	1.5	8.7	62.9	38	955
G	Particles with segregated crystalls of HT	76.3	1.3	9.2	56.3	33	878
H	Particles with segregated crystalls of HT	66.9	1.6	13.5	45.2	39	968

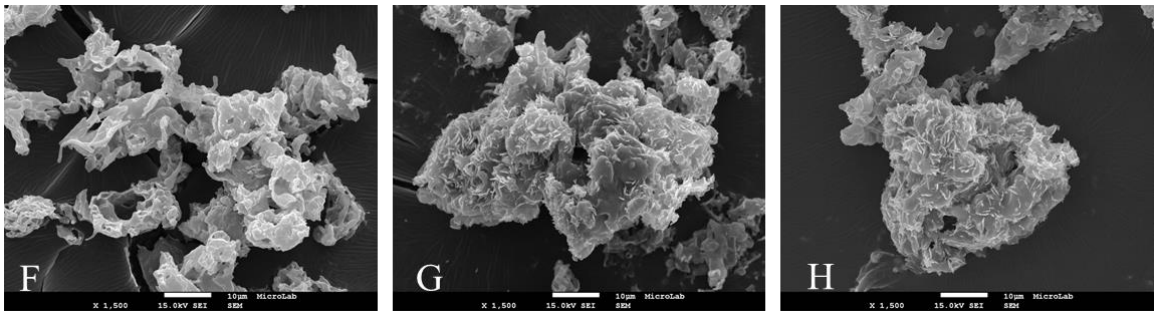

Figure 3: SEM photographs of particles obtained in experiments F-H.

It is possible to verify that particles obtained in experiments G-H present crystals of HT at the surface, leading HT more vulnerable to degradation. Concerning the final water content it is possible to see that it increases from F<G< H. Moreover, by analysing the melting enthalpy of the particles produced, it is possible to verify that it decreased at the same time that the particles' moisture increased. This is explained by the formation of more imperfections in the crystal structure of the lipid due to the presence of water.

Although particles from Exp H present higher concentration of phenolic compounds and consequently higher antioxidant activity, the HT's encapsulation efficiency decreased from F>G>H. Therefore, the presence of Imwitor® 600 does not appear to be beneficial in this case, being the mass ratio of 1:1 (carrier:natural extract) the optimum to produce dry particles with higher concentration of HT.

CONCLUSION

Aqueous natural hydroxytyrosol-rich concentrate was succefully co-precipitated with a solid lipid matrix. PGSS® proved to be a suitable method to dry water, achieving a drying capacity of 65%. The optimum mass ratio to produce dry particles rich in HT was fixed at 1:1 (GMS:aqueous extract). The HT-loaded lipid particles produced presented high antioxidant activity, being appropriate to be further incorporated into cosmetic, pharmaceutical and nutraceutical products.

ACKNOWLEDGEMENTS

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