Encapsulation of polyphenol rich extract from *Syzygium aromaticum* in solid lipid carriers

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INTRODUCTION AND OBJETIVE

Clove (*Syzygium aromaticum*) is one of the richest source of phenolic antioxidants (Pérez-Jiménez 2010). Eugenol, a poorly water soluble molecule, is the main compound of clove linked to their pharmacological activities, such as antioxidant, antimicrobial, analgesic and antitumor (Kamatou 2012).

The development of formulations containing instable, volatile and poorly water soluble compounds as eugenol is a challenging task. Recently lipid based formulations have gained special attention due to the high encapsulation efficiency, product stability and the possibility of modification of solubility. Different methods can be used to produce lipid nanoparticles, however, high pressure homogeneization and ultrasound are commonly used techniques due to the simplicity and scaling up easiness (Guimarães 2011).

Powdered forms of lipid formulations represents a new tendency (specially for oral applications), since they are more stable than liquid encapsulated formulations and could be easily redispersible when needed. Spray- and freeze drying are commonly used technologies for this purpose, although the addition of drying aids is generally needed.

The aim of this work was to produce powdered dried lipid particles containing polyphenol-rich clove extract, evaluating the influence of the solid lipid, surfactant, drying carrier, homogeneization methods and drying technology on the physicochemical product properties.

MATERIAL AND METHODS

Clove extract preparation

Milled clove buds (Valença, BA, Brazil) were maintained under agitation with ethanol 70% in glass containers coupled to a thermostatic bath set at 50°C for 30 min in a plant to solvent ratio of 1/10(v/w). The extract was filtered and concentrated in a rotary evaporator (55°C and 600mmHg) until a solids content of 8% (w/w).

Preparation of lipid formulations

Lipid formulations were prepared according to the compositions described in Table 1, using the ultrasonication method. The solid lipid was heated in a water bath 10°C above its melting point and mixed with the liquid lipid (Buriti oil). The surfactant was dissolved in hot water, mixed with the concentrated clove extract and then heated to the same temperature of the lipid phase. The aqueous phase was homogeneously dispersed into the lipid phase by using a high-speed stirrer (UltraTurrax T18, IKA) at 18.000 rpm/min. The suspension was ultrasonicated at 20 kHz, 70% intensity for 3 min (SONICS-Vibracell). Finally the drying aids were added and the resulting compositions submitted to spray drying. Solids content of compositions was standardized at 33%.

Table 1. Encapsulating compositions

Component	Formulation				
	F1	F2	F3	F4	F5
Clove extract	63.0	63.0	63.0	63.0	63.0
Compritol®	8.1	8.1	8.1	-	8.1
Stearic acid	-	-	-	8.1	-
Buriti oil	0.9	0.9	0.9	0.9	0.9
Polysorbate ^{80}	0.9	0.9	0.9	0.9	-
Poloxamer ^{188}	-	-	-	-	0.9
Lactose	-	18.0	-	-	-
Maltodextrin^{DE10}	18.0	-	9.0	18.0	18.0
Arabic gum	-	-	9.0	-	-
Water	9.0	9.0	9.0	9.0	9.0

Chromatographic analysis

The concentration of eugenol in the dried samples was determined by high performance liquid chromatography (Shimatzu LC-20A), using a C18 column employing an isocratic mobile phase of methanol:water 60:40, monitoring at 280nm.

Comparison of homogenization and drying technologies

The formulation 5, was selected to compare the homogenisation process (high shear mixing, ultrasound and high pressure homogeneization) and drying methods (spray drying and freeze drying). High pressure homogenisation (HAP) was performed in a Panda Plus (GEA Niro Soavi, Parma, Italy) applying three homogenization cycles at 500 bar, and compared with the one homogenized just by UltraTurrax (T18, IKA-Wilmington, NC, USA) at 18.000 rpm/min and with the one sonicated at 20 kHz, 70% intensity for 3 min (SONICS, Vibracell).

Spray drying (SD)

The compositions were spray dried (Lab-Plant, United Kingdom SD-05), with a two-fluid atomizer of 1.0 mm operating in a co-current flow regime using a gas flow rate of 60 m^3/h. Dried product was collected in a Lapple cyclone. The formulations were fed at 4 g/min,

the atomization pressure and air flow rate were set at 2 bar and 15 L/min respectively and inlet gas temperature of 90°C.

Freeze drying (FD)

Samples were frozen at -20 C for 12h in plastic tubes and then frozen at -80C for 4h. The freeze drying process was performed in a Thermo Fisher Scientific model SNL108B for 24 h.

Particle Morphology

The morphology of the particles was analyzed using scanning electronic microscopy with field emission gun (SEM-FEG) in an Inspect F-50 (FEI, Nederland) at 5 kV.

RESULTS AND DISCUSSION

Figure 1 shows the retention of eugenol after drying process of each formulation. It was observed that formulation F5 presented the highest concentration of eugenol and formulation F2 and F4 presented the lowest concentrations.

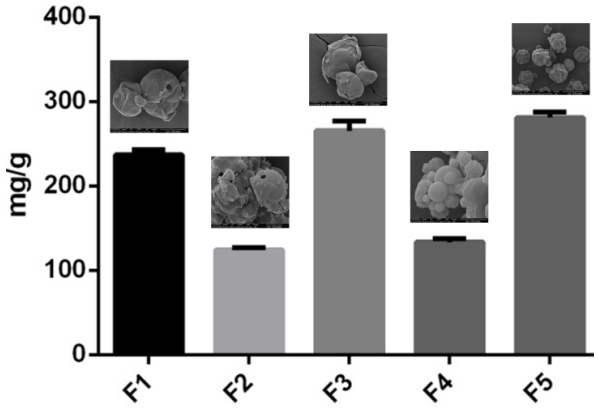

Figure 1. Concentration of eugenol in the powdered formulations (mg eugenol per g of extract, dry base)

The affinity of the solid lipid with the eugenol has direct influence on the final concentration in the powder product. Therefore, proper selection of lipid carrier, which in turn will affect emulsion stability is important to promote the encapsulation of the active substance. Results showed that the retention of eugenol in the formulation containing Compritol® was higher. Other factors as the film forming properties emulsifying capacity and glass transition temperature of the drying carriers has a strong influence with the degree of protection conferred to clove polyphenols. In this work Maltodextrin DE10 presented better product recovery and encapsulation efficiency. Surfactant agent and concentration also have significant impact on encapsulating process. In this work, poloxamer 188 showed better results. The formulations composition have direct impact on the particles morphology as can be observed in Figure 1.

The influence of the three homogenisation techniques and two drying process on eugenol retention is presented in Figure 2. Freeze dried samples showed remarkable higher eugenol concentration, although this method is more time consuming and expensive. The samples homogenized by the different methods did not show marked differences especially when samples were spray dried.

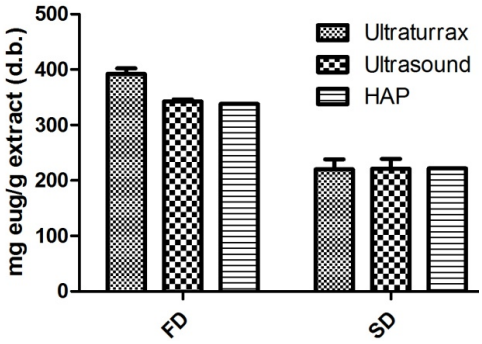

Figure 2. Eugenol retention as affected by the homogenization and drying process

CONCLUSIONS

The spray dried formulation composed by Compritol® as solid lipid, Poloxamer 188 as surfactant and Maltodextrin DE10 as drying carrier presented higher eugenol retention. The concentration of eugenol was higher in the freeze drying powders than in the spray dried ones. Powdered lipid carriers could be an interesting alternative to increase stability and facilitate dosage of pharmaceutical or nutraceutical preparations since could be easily redispersed when needed.

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