The Effect of the Matrix System in the Delivery and In-Vitro Bioactivity of Microencapsulated Oregano Essential Oil.

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Introduction

Microencapsulation allows bioactive compounds protection from external factors. Innovation in food industry often requires adding functional ingredients, to tailor flavour and texture, to improve preservation, to control bioactive compounds stability and controlled release during processing/storage. Oregano, besides richness in aroma compounds, is also known by potential antioxidant and antimicrobial activities. These sensitive compounds need protection in order to allow their use in a wider range of processes.

Spray drying and freeze-drying are most of the times very effective to provide high yields of entrapment of flavours and excellent product characteristics. Mechanisms explaining entrapment of volatiles in freeze and spray drying are similar. The mechanism of entrapment of dispersed molecules in spray drying is explained (Thijssen and Rulkens 1968) by a decrease of diffusion coefficient of volatile components at a higher rate than the diffusion coefficient of water during drying and also related to size of molecules. Since spray drying is an economical and effective method for protecting materials it is most widely employed, in large-scale production of encapsulated flavours and volatiles (Deis, 1997). This technique provides a high retention of aroma compounds during drying (Teixeira et al. 2004) due to the low temperatures that the core material reaches (Dziezak, 1988)

In this study, oregano essential oil (EO) was microencapsulated by spray/freeze drying in: rice starch (with/without bonding agents), gelatine/sucrose and inulin. Microencapsulates were analysed for morphology and structure, releasing ability of entrapped EO, as evaluated by diffusion coefficient (*D*) and bioactivity.

Materials and Methods

Materials

Rice starch, Gelatine, and sucrose (MW 342.30 g/mol)(Panreac Quimica S.A) and Raftiline ST (Beneo-Orafti, Tienen, Belgium) (>90% inulin; DP≥10, obtained from chicory roots) were used as shell materials .

Oregano (*Oregano virens L.*) EO, was extracted by steam distillation for 1 h at atmospheric pressure. Essential oil was transferred into a dark glass flask and kept at a temperature of 4°C until used.

Preparation of microencapsulated oregano essential oil

Central composite rotatable experimental designs with two central points were used in developing all the proposed matrices. The tested independent factors were for rice matrix solids content and bonding agent (gelatine or CMC at different concentrations) for inulin solids concentration (5-15%) and drying temperature(120-190ºC) and for gelatine/sucrose system gelatine total solids content (5-10%), gelatine(20-100%) and sucrose (80-0 %)(w/w) and inlet spray dryer temperatures (120 -190 º C). The essential oil was added at the solutions (15% solid basis) and the strongly homogenised emulsions subjected to spray drying in a LabPlant SD-04 (Leeds, UK) co-current spray dryer equipped with a 0.5 mm diameter nozzle. The pressure compressed air for the flow of spray was adjusted to 1.9 bar.

To prepare freeze-dried matrices the mixtures were frozen in Petri dishes at – 45 º C for 3 h. Frozen mixtures were then freeze-dried using a TELABE- J.D.F. LF.10/BFC freeze-dryer.

Analysis

Physical structures of micro particles were observed in a Jeol JSM-5410 scanning electron microscope. Particle size and particle size distribution were calculated from SEM observations. Confocal Laser Scanning Microscopy (CLSM) (Olympus Fluoview, FV 1000) was used to visualize the encapsulated oil phase in the microcapsules. Visualization of the oil in the microcapsules was achieved by dissolving fluorescent β-carotene in the oil phase prior spray-drying.

Fourier transform infrared spectroscopy (FTIR) was used to confirm the presence of the EO.

Oregano essential oil release profiles were obtained by a dialysis method. About 100 mg of microcapsules were added into a dialysis bag and the bag subsequently placed into 100 ml of appropriate phosphate buffer solution (PBS) with magnetic stirring. At regular time intervals,

1 ml samples were taken from the PBS and EO concentration followed as a function of time by measuring the absorbance (Elisa Biotech Synergy HT) at λ = 277 nm. The diffusion of entrapped oregano EO from micro particles can be described by Fick's law diffusion for spherical particles (Prata et al., 2008; Zhang et al., 2006; Romero-Cano & Vincent, 2002). In diffusion-controlled release of EO, the relationship between the size of the particles and the released fraction of the oregano oil at a time *t* can be described by the following equation (Zhang *et.* al., 2006):

$$\frac{M_t}{M_\infty} = 6\left(\frac{Dt}{\pi r^2}\right)^{0.5} - \frac{3Dt}{r^2} \quad \text{para } \frac{M_t}{M_\infty} \leq 0.7 \qquad \text{Eq.1}$$

where *Mt* is the solute mass released at time *t*; M_∞ the solute mass released at infinite time when equilibrium is achieved; *r* is the radius and *D* can be calculated from the Eq.1 by non-linear parameter estimation.

Antioxidant activity (AA) was evaluated by ORAC assay (Wada and Ou. 2002) and the antimicrobial effect was determined by plate count assay.

Statistical analyses were carried out using the Statistica® 7 (Statsoft, Tulsa, OK, USA).

Results and Discussion

Rice starch spherules, presenting interconnecting cavities, were formed. Spray-dried inulin and gelatine/sucrose systems formed continuous walled and smooth surface spherical capsules (3-4.5 and 0.9-10 μm, respectively). EO was uniformly distributed inside the structures (CLSM) and its presence confirmed by FTIR. Depending on the system, *D* varied among 10^{-13} (starch), 10^{-13}-10^{-15}, (gelatine/sucrose) and 10^{-16} m^2/s (inulin). In starch system, *D* was mainly influenced by the gelatin concentration, increasing with it. X- ray diffraction and FTIR suggest some kind of linkage between gelatine and one fraction of starch. On the other hand FTIR results seem to show a linkage between EO and other fraction of starch.

Spray-dried gelatine/sucrose system, revealed to be unsuitable for EO encapsulation due to capsules disintegration but freeze-drying was effective in EO retention.

The D of EO from inulin capsules decreases when these are produced above 140 ºC.

The impact of encapsulation method on EO bioactivity and product stability was verified through the determination of microcapsules AA, using free EO value as reference. Among all

samples, gelatine/sucrose microencapsulates exhibited the highest ORAC values (100-350 µmol TEAC/100g). In this system, capsules formulated with 100% gelatine showed the best results and freeze-drying was the most effective method to obtain antioxidant rich particles. For rice starch system, the presence of gelatine slightly increased the ORAC value of microencapsulates. In the case of inulin system, the amount of matrix used in formulation compromised the antioxidant activity of the final capsule. In all systems, the AA of microencapsulates decreased with high processing temperature.

It is important to highlight that the AA of all microencapsulates was preserved during, at least, 3 months.

The antimicrobial activity of microencapsulates was also evaluated against five bacteria strains: *Escherichia coli*, *Staphylococcus aureus, Bacillus cereus*, *Listeria monocytogenes* and *Enterococcus faecalis.* Results obtained showed that freeze-dried gelatine 100% was the most efficient system in inhibiting the growth of these foodborne pathogens- (inibition > 87% using 5mg/mL).

Conclusions

The results obtained provide information on the release/stability of oregano EO from different matrices, relevant for functional ingredients microencapsulation, since the releasing efficiency and bioactivity is not the same and should be kept in mind in food formulations

References

Thijssen, H.A.C.; Rulkens, W.H. (1968). De Ingenieur, *47*, 45–56.

Dziezak, J.D. (1988). Food Technology, 42, 136–151

Deis, R.C. (1997). Food Product Design, 7, 97–113.

Teixeira, M.I., Andrade, L.R., Farina, M. & Rocha-Leão, M.H.M. (2004). Materials Science and Engineering C, *24*, 653–658

Romero-Cano, M. S. & Vincent, B. (2002). Journal of Controlled Release, *82*, 127–135

Prata, A.S., Zanin, M. H.A., Ré, M.I. & Grosso, C. R. F. (2008). Colloids and Surfaces B: Bioninterfaces, *67*, 171-178

Zhang, Z., Grijpma, D. W. & Feijen, J. (2006). Journal of Controlled Release, *111*, 263-270

Wada L. & B. Ou. (2002). Journal of Agricultural and Food Chemistry, *50*, 3495-3500.