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Encapsulated olive leaf extract in maltodextrin-trehalose amorphous matrices by freeze-drying

Rodrigo Gonzalez Ortega, Carla di Mattia, Paola Pittia

Faculty of Bioscience and Technology, University of Teramo, Italy, email: rgonzalesortega@unite,it

INTRODUCTION

Micro-and nano-encapsulation are nowadays representing an interesting strategy to enhance the stability and functionality of bioactives, biomolecules and/or extracts they are present. From a technological perspective, encapsulation techniques need to be optimized in order to keep the compositional quality and bioactivity of such compounds during storage, upon processing and diverse formulation conditions. The development of dried and amorphous encapsulated products is largely applied for the higher easiness to be used and increased stability during storage and transportation (Fang and Bhandari, 2012). Quality changes and functionality (eg. release) of amorphous encapsulated systems are governed by the glass transition temperature (Maydannyk *et al*., 2017) that, in turn depends on the matrix composition and presence and concentration of plasticiser. Carbohydrates (high molecular weight are largely used for dry encapsulation, where the stabilization of the bioactive ingredient is achieved by vitrification of the matrix and the core ingredient becomes entrapped in an environment with reduced molecular mobility and reactivity. Spray-drying and freeze-drying are technologies used to this aim, in some cases also combined with other dispersing and encapsulation techniques. Process conditions to enhance retention efficiency of bioactives and functionality of the dried encapsulates need, however, to be optimized.

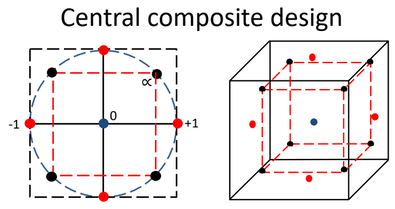
MATERIALS AND METHODS

A commercial olive leaf extract (OLE) with high oleuropein content (48 wt.%) was encapsulated in a carbohydrate matrix by freeze-drying according to preliminarily optimized process parameters The effect of wall material composition using maltodextrin (MD, DE 8-10)-trehalose system, ratio between core and wall material and total solids were the independent variables studied and a response surface optimization approach was used for systems preparation. The following analysis of the freeze-dried systems differently formulated were carried out: OLE retention (encapsulation efficiency) and in-vitro antioxidant activity, thermal analysis (glass transition temperature, Tg), microstructural properties.

Experimental design (response surface methodology)

	Independent variables		
	V1	**V2**	**V3**
	% solids(w/v)	MD:TR(%MD)	Core:Wall
Low(-1)	10	0	0.05
Medium(0)	20	50	0.15
High(+1)	30	100	0.25
-∝	14.23	21.13	0.09
+∝	25.77	78.87	0.21

Central composite design



Experimental formulation	Coded level of variables			Experimental levels of variables		
	V1	**V2**	**V3**	**%solids**	**(MD:TR) %MD**	**core:wall**
1	-1	-1	-1	10	0	0.05
2	+1	-1	-1	30	0	0.05
3	-1	+1	-1	10	100	0.05
4	+1	+1	-1	30	100	0.05
5	-1	-1	+1	10	0	0.25
6	+1	-1	+1	30	0	0.25
7	-1	+1	+1	10	100	0.25
8	+1	+1	+1	30	100	0.25
9	-∝	0	0	14.23	50	0.15
10	+∝	0	0	25.77	50	0.15
11	0	-∝	0	20	21.13	0.15
12	0	+∝	0	20	78.87	0.15
13	0	0	-∝	20	50	0.09
14	0	0	+∝	20	50	0.21
15	0	0	0	20	50	0.15
16	0	0	0	20	50	0.15
17	0	0	0	20	50	0.15

Encapsulated OLE powders analysis

Encapsulation efficiency

Surface and encapsulated OLE bioactives

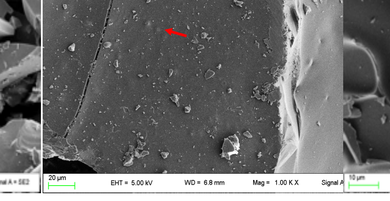
- Total phenolic content (Folin assay)
- Oleuropein (HPLC)

Antioxidant capacity of encapsulated fraction (ABTS-TEAC)

Glass transition temperature (Tg)

Onset of Tg of encapsulated OLE and control (without OLE) freeze-dried powders

Differential scanning calorimetry



Microstructure and OLE distribution

OLE distribution

Natural fluorescence of OLE phenolics using **Confocal Laser Scanning Microscope**

Powder microstructure

Morphology and microstructure of powders using **Scanning Electron Microscope**

RESULTS

Response surface optimisation of OLE bioactives encapsulation

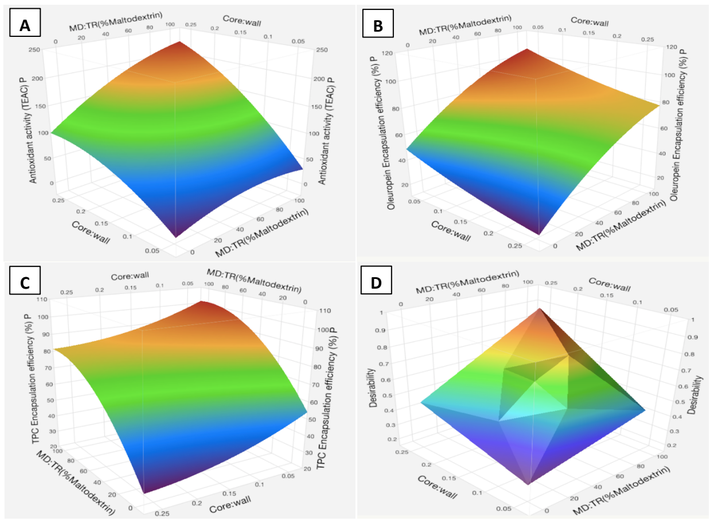


Figure 1. Response surface plots showing variation of AA of encapsulated fraction (A), oleuropein EE% (B), total phenolic content EE% (C) and desirability (D), as a function of changes in factors V2 (ratio MD:TR) and V3 (ratio core:wall).

Experimental response values for formulations prepared according to CCD showed **high correlation with model responses** (R^2 >0.98) at at p<0.01 implying adequacy of the applied regression model

Summary of effects of independent variables on response variables showed that **V2** and **V3 had a significant effect (p<0.0001)** on response variables, while all other effects were not.

Desirability value for each formulation was obtained as a result of simultaneous optimization of the three responses.

Presence of **trehalose led to lower bioactives encapsulation efficiency**, indicating a detrimental effect of its presence in the carrier matrix. This suggest that trehalose, a disaccharide, misstructured the continuous tighter complexes formed by maltodextrin (high molecular weight

The **higher bioactive core:wall ratio**, **the lower the encapsulation efficiency** of the system was found. This logically results as a consequence of reduced wall surface in relation to bioactive entrapped core.

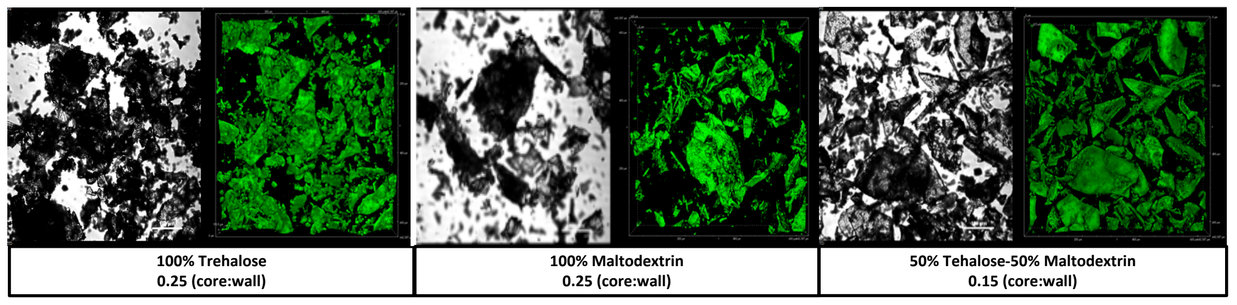
Glass transition temperature

Experimental values of variables				Controls		Encapsulates	
%solids	MD%	TR%	core:wall	Tg onset (ºC)	ΔCp (Jg^{-1} ºC $^{-1}$)	Tg onset (ºC)	ΔCp (Jg^{-1} ºC $^{-1}$)
30	0	100	0.05	98.5 ± 0.2	0.48 ± 0.00	102.9 ± 2.1*	0.48 ± 0.02
10	0	100	0.25	98.4 ± 1.5	0.51 ± 0.02	97.0 ±1.6	0.47 ± 0.03
14	50	50	0.15	129.9 ± 2.3	0.43 ± 0.06	123.0 ± 0.6*	0.34 ± 0.01
26	50	50	0.15	131.5 ± 0.3	0.39 ± 0.01	122.7 ± 1.4***	0.34 ± 0.04
20	21	79	0.15	111.5 ± 0.2	0.41 ± 0.1	107 ± 0.5**	0.44 ± 0.02
20	79	21	0.15	174.5 ± 1.9	0.27 ± 0.12	154.7 ± 1.8***	0.19 ± 0.05

- All samples showed a single Tg, meaning that two components were miscible.
- Tg of the encapsulates increases with increasing MD concentration (↑ Mw) and mixtures of both components showed an intermediate Tg between single components Tg

- Samples with only **MD as carrier**, there **was no observed glass transition** in anhydrous samples, probably due to extremely low mobility of large molecules in anhydrous conditions
- Presence of **OLE had a plasticizing effect**, depressing the Tg, except in those samples were only trehalose is present as carrier. Majority of components present in OLE are of low molecular weight and this might explain this effect.

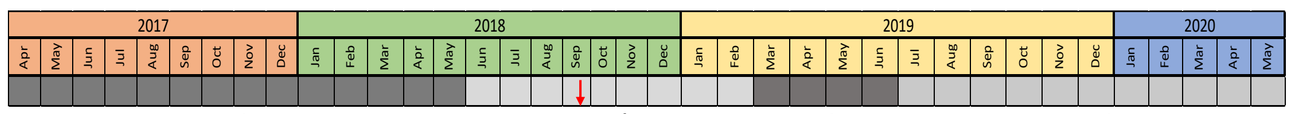
OLE distribution and microstructure



CLSM micrographs show OLE was distributed all across the carrier matrix with a homogeneous distribution although localized microregions showed higher intensity, more remarkably in samples in which maltodextrin was a matrix component. OLE is mostly composed of low molecular weight molecules that may partially partition into this locally concentrated microregions.

- **SEM micrographs** of samples made of trehalose as carrier showed a continuous amorphous structure, while those containing maltodextrin as main carrier component showed a flaky structure with regularly distributed cavities (ice crystals)
- Encapsulated OLE microcapsules gave rise to **rougher surface** with characteristic spots as compared to control powders, and more remarkably when maltodextrin is present as carrier matrix, as observed in CLSM micrographs.

FUTURE WORK



University of Teramo:

- Freeze-drying encapsulation (70%)
- Co-milling encapsulation (70%)

University of Ljubljana:

- Spray-drying encapsulation (0%)
- Proliposomes/liposomes (30%)

University of Bolzano:

- Extrusion encapsulation (0%)

University of Teramo:

- Stability and health properties of produced encapsulated extracts/oil (20%)
- Development of model and formulated real food systems (0%)

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XXIII WORKSHOP ON THE DEVELOPMENTS IN THE ITALIAN PhD RESEARCH ON FOOD SCIENCE, TECHNOLOGY AND BIOTECHNOLOGY

ORISTANO 19th · 20th · 21st SEPT 2018