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Encapsulation technologies for stabilization and functionality of olive leaves bioactive compounds

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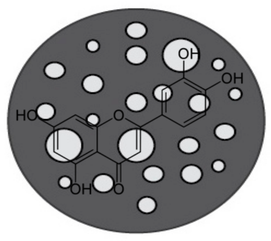
Introduction

Micro-encapsulation or nano-encapsulation is nowadays representing an interesting strategy to enhance the functionality of bioactives and other biomolecules. From a food technological perspective, it is essential to maintain physicochemical quality and bioactivity of such compounds during prolonged storage at diverse conditions. It is also more convenient and applicable to benefit from bioactive phenolic extracts in powdered form that can be easily handled during storage and transportation (Fang and Bhandari, 2012).

Carbohydrate matrices are commonly used, where the stabilization of the bioactive ingredient is achieved by vitrification of the matrix where the core ingredient is trapped in an environment with reduced molecular mobility and reactivity. Therefore quality changes are strongly governed by a physical stability parameter like glass transition temperature (Maydannyk *et al*., 2017)

In this work, an olive leaf extract (OLE) was encapsulated in a carbohydrate matrix by freeze-drying. The effect of wall material component using maltodextrin-trehalose system, ratio between core and wall material and total solids were studied on encapsulation efficiency of the process using a response surface optimization approach. Glass transition of different systems was studied and also the microstructure and OLE distribution in the matrix.



Method

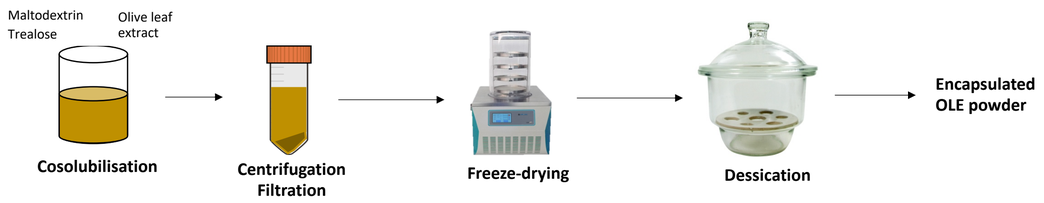
- **Experimental design for optimization using CCD by response surface methodology**

	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

Data optimization,model fitting and response surface contours were done using JMP 14 software

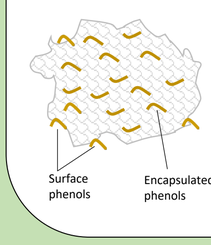
Experimental formulation	Coded level of variables			Experimental levels of variables		
	V1	**V2**	**V3**	**%solids**	**(MD:TR) %MD**	**core:wall**
1	-1	-1	-1	10	0	0.05
2	+1	-1	-1	30	0	0.05
3	-1	+1	-1	10	100	0.05
4	+1	+1	-1	30	100	0.05
5	-1	-1	+1	10	0	0.25
6	+1	-1	+1	30	0	0.25
7	-1	+1	+1	10	100	0.25
8	+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

- **Freeze-drying encapsulation procedure**



- **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

Differential scanning calorimetry

Analysis of Tg on anhydrous encapsulated OLE powders and control powders (without OLE)

Microstructure and OLE distribution

Confocal laser scanning microscopy

Thanks to autofluorescence properties of OLE (oleuropein), distribution in matrix could be observed

Results

- **Response surface optimisation**

Table 1. Response values for experimental formulations of central composite design

Experiment formulation	Experimental values of variables			Responses			
	%solids	(MD:TR) %MD	core:wall	%EE(TP)	%EE(Ole)	TEAC (µmol TE/g)	Desirability
1	10	0	0.05	57.73 ± 1.13	55.35 ± 1.25	43.67 ± 4.08	0.25
2	30	0	0.05	64.83 ± 0.33	55.10 ± 1.56	45.69 ± 2.06	0.26
3	10	100	0.05	98.51± 0.14	99.23± 0.16	65.41 ± 1.85	0.42
4	30	100	0.05	99.33 ± 0.18	100.00 ± 0.00	69.78 ± 6.45	0.44
5	10	0	0.25	38.61 ± 1.52	29.32 ± 1.03	113.70 ± 9.27	0.41
6	30	0	0.25	50.22 ± 1.05	39.61 ± 1.36	136.87 ± 6.81	0.51
7	10	100	0.25	81.07 ± 0.75	74.80 ± 0.67	214.04 ± 13.56	0.86
8	30	100	0.25	86.61 ± 0.56	82.12 ± 0.91	220.48 ± 6.61	0.91
9	14	50	0.15	74.96 ± 0.26	65.76 ± 2.11	132.26 ± 9.16	0.59
10	26	50	0.15	78.85 ± 0.58	70.59 ± 0.32	132.57 ± 5.49	0.61
11	20	21	0.15	54.09 ± 0.58	43.38 ± 1.64	100.06 ± 3.04	0.43
12	20	79	0.15	91.60 ± 0.30	87.78 ± 0.68	150.09 ± 1.12	0.71
13	20	50	0.09	85.90 ± 1.11	77.12 ± 1.86	98.67 ± 1.85	0.51
14	20	50	0.21	68.06 ± 0.68	58.82 ± 0.82	149.59 ± 12.57	0.62
15	20	50	0.15	75.20 ± 0.26	68.69 ± 1.12	134.25 ± 5.17	0.6
16	20	50	0.15	77.64 ± 0.84	71.72 ± 0.40	137.56 ± 4.85	0.62
17	20	50	0.15	76.94 ± 1.03	69.42 ± 1.50	136.35 ± 2.61	0.61

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. Higher (in red), medium (yellow) and low (green) desirability values for each formulations are shown in table 1.

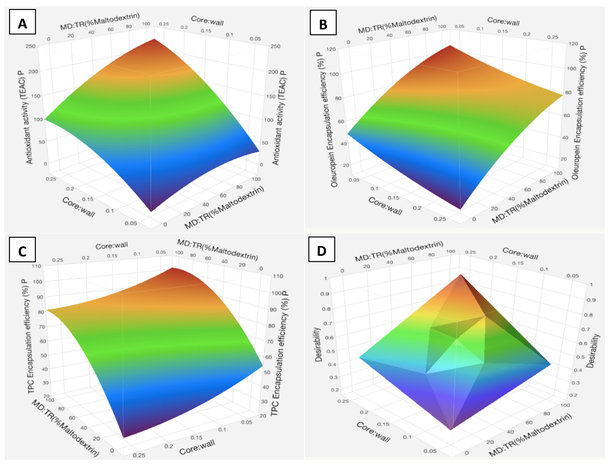


Figure 1. Response surface plots showing variation of AA of encapsulated fraction (A), oleuropein EE% (B), total phenolic content EE% (C) and desirability (D), as a function of changes in factors V2 (ratio MD:TR) and V3 (ratio core:wall).

Presence of trehalose results in lower encapsulation efficiency for both total phenolics and oleuropein, indicating a detrimental effect of its presence in the carrier matrix where olive leaves bioactives are present. This suggest that trehalose, a disaccharide, misstructured the continuous tighter complexes formed by maltodextrin (high molecular weight

The higher the amount of bioactive core in relation to wall material,(core:wall ratio) the lower the encapsulation efficiency of the system. This logically results as a consequence of reduced wall surface in relation to bioactive entrapped core, being this further exposed (a smaller fraction is encapsulated)

- **Glass transition temperature (T_g)**

Table 2. Onset, midpoint and associated specific heat increase of glass transition (T_g) of some encapsulated OLE and control powders.

Experimental values of variables				**Controls**			**Encapsulates**		
%solids	MD%	TR%	core:wall	Tg onset (ºC)	Tg midpoint (ºC)	ΔCp (Jg^{-1} ºC $^{-1}$)	Tg onset (ºC)	Tg midpoint (ºC)	ΔCp (Jg^{-1} ºC $^{-1}$)
30	0	100	0.05	98.5 ± 0.2	101.8 ± 0.8	0.48 ± 0.00	102.9 ± 2.1	105.5 ± 1.5	0.48 ± 0.02
10	0	100	0.25	98.4 ± 1.5	101.4 ± 1.7	0.51 ± 0.02	97.0 ±1.6	101.1 ± 2.0	0.47 ± 0.03
14	50	50	0.15	129.9 ± 2.3	136.7 ± 2.8	0.43 ± 0.06	123.0 ± 0.6	131.6 ± 0.2	0.34 ± 0.01
26	50	50	0.15	131.5 ± 0.3	138.8 ± 0.8	0.39 ± 0.01	122.7 ± 1.4	130.8 ± 2.4	0.34 ± 0.04
20	21	79	0.15	111.5 ± 0.2	115.1 ± 0.9	0.41 ± 0.1	107 ± 0.5	111.5 ± 1.6	0.44 ± 0.02
20	79	21	0.15	174.5 ± 1.9	177.8 ± 0.9	0.27 ± 0.12	154.7 ± 1.8	160.1 ± 1.8	0.19 ± 0.05

Tg increases with increasing maltodextrin concentration. This is explained by its higher molecular weight. Trehalose is a disaccharide with a lower Tg, and as a result mixtures of both components have an intermediate Tg between single components Tg. All samples showed a single Tg , meaning that two components were miscible.

In samples were only maltodextrin was present as carrier, there was no observed glass transition in anhydrous samples, probably due to extremely low mobility of molecules in anhydrous conditions
Presence of OLE components also had a plasticizing effect, depressing the Tg, except in those samples were only trehalose is present as carrier. Most components present in OLE are of low molecular weight and this might explain the mild effect over the Tg. On the other hand, LMW molecules in OLE had a depressing effect of Tg on systems were maltodextrin was present as carrier (**Table 2**).

- **Microstructure and OLE distribution**

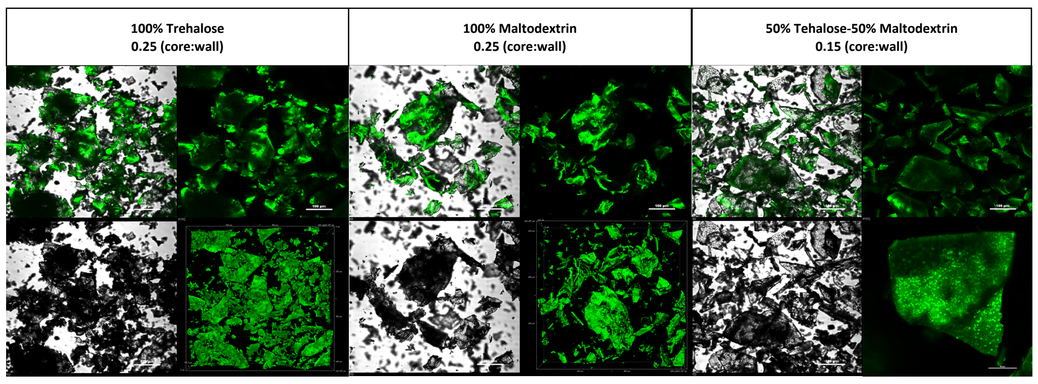


Figure 2. CLSM micrographs of OLE encapsulates

In samples with single matrix component, a more homogeneous distribution of signal was observed. Interestingly, in mixed systems a characteristic distribution with localized spots with stronger signal (higher concentration of bioactives) was observed. It is possible that during maximal freezing concentration, trehalose partitions in locally concentrated regions embedded in a continuous maltodextrin matrix, carrying along other low molecular weight components from OLE.

References

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Conclusion

- A design of experiment optimization approach using RSM was applied to study freeze-drying encapsulation of olive leaf extract in maltodextrin-trehalose carriers systems and effect of process variables on encapsulation efficiency.
- Formulations with MD-TR systems showed a single intermediate Tg. The higher the trehalose content as wall material the lower the glass transition temperature
- OLE appeared to be homogeneously distributed although some local spots with higher intensity were observed, more remarkably in formulations of MD-TR systems