Subject:

Optimization the release kinetics of polyphenols of encapsulated *Olea europaea* leaves' extracts

Student name

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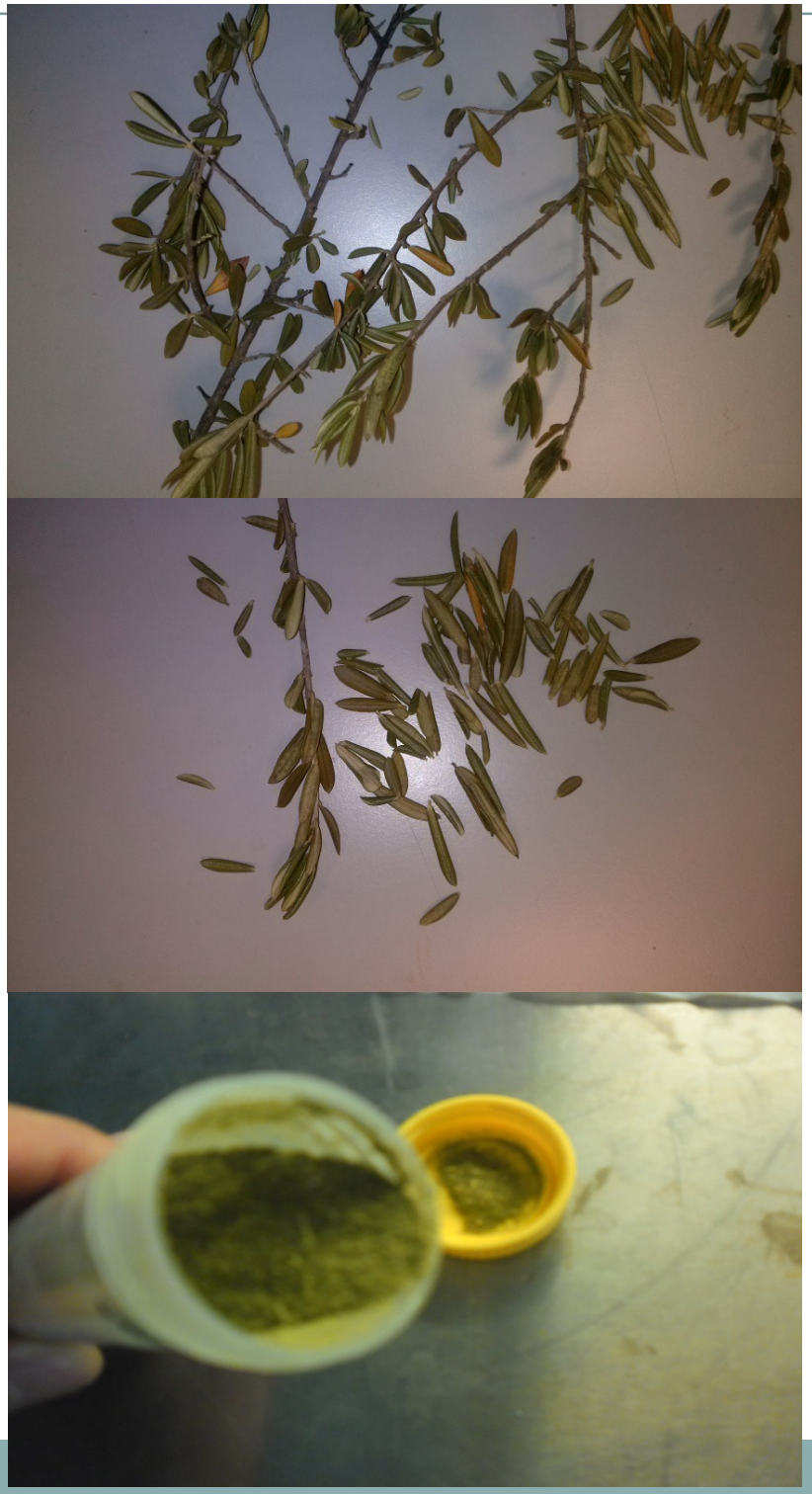
Olea europaeae leaves

- Historically, olive leaf is used for the therapy of malaria and associated fever (Benavente-García, Castillo, Lorente, Ortuño, & Del Rio, 2000).

- Olive leaves' infusions have offered a capability to reduce blood pressure and raise blood circulation in the coronary arteries (Khayyal et al., 2002).

- Hydroxytyrosol, tyrosol, p-coumaric and verbascoside are the main discovered bioactive compounds in olive leaf.

- The infusions of olive leaves have posed antioxidative capabilities (Somova, Shode, Ramnanan, & Nadar, 2003; Škerget et al., 2005) as well as germicidal potentials versus *Campylobacter jejuni, Helicobacter pylori*, and *Staphylococcus aureus* (Sudjana et al., 2009).



Encapsulation

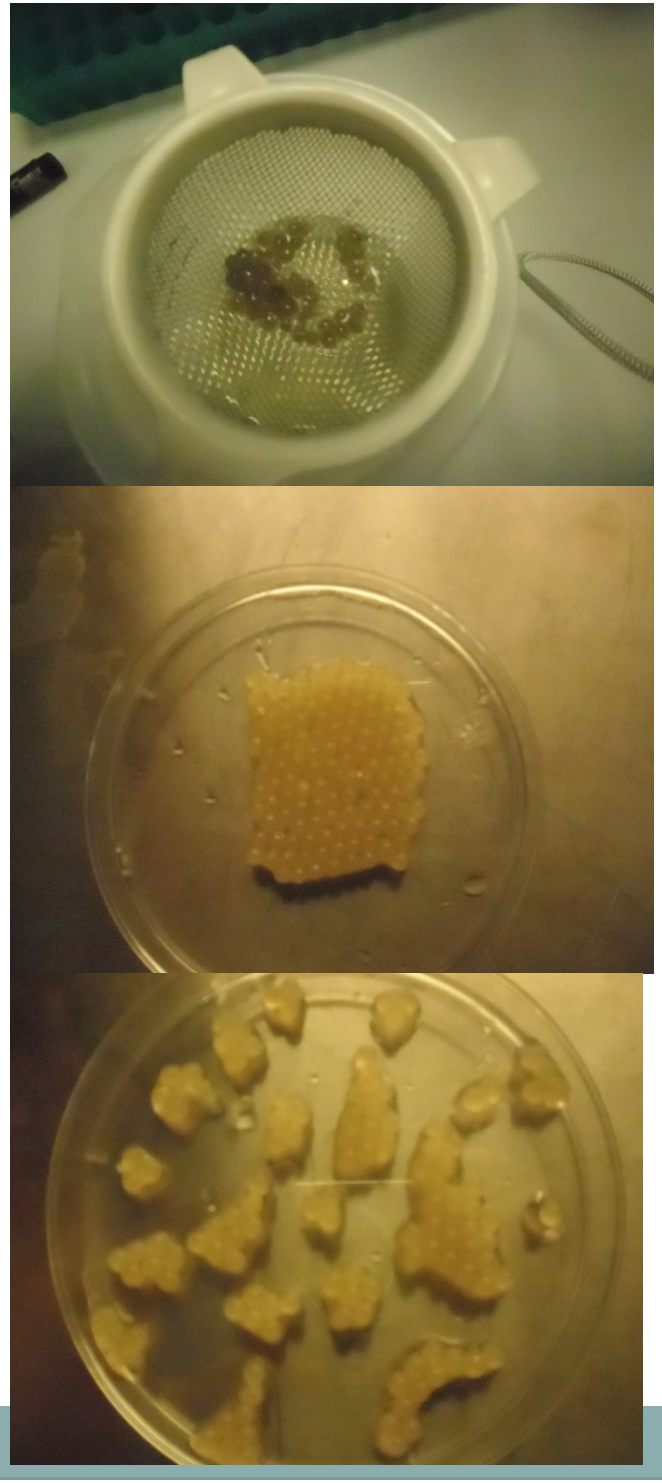
- Due to the high demand to bioactive natural compounds, application of hydrogels acquired from polysaccharides in nutraceutical and pharmaceutical industries is continuously getting increased (Farris, Schaich, Liu, Piergiovanni, & Yam, 2009).

- Sodium alginate was selected for preparing hydrogels.

- Although this is a plain and quick procedure of affording encapsulating systems, the method indicates a substantial limitation comprising in dissipation within bead providing.

- We obviated this undesirability by mixing the alginate with starch of potato.

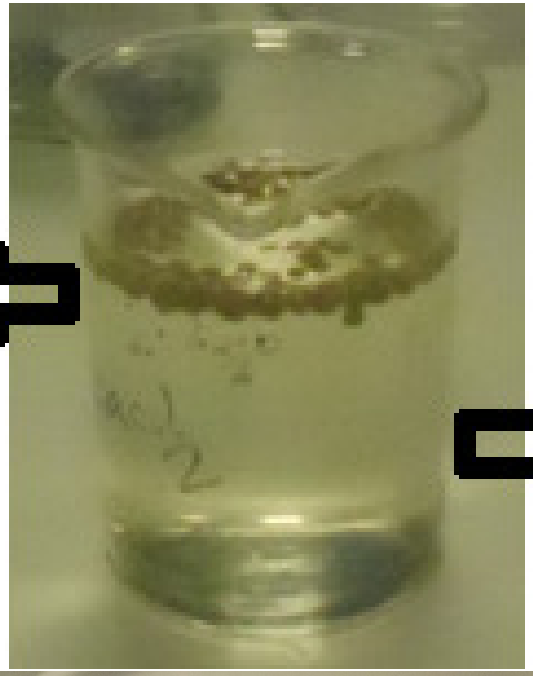
- The incorporation of a filler substance into alginate matrix is a strategy for reducing the disadvantages.



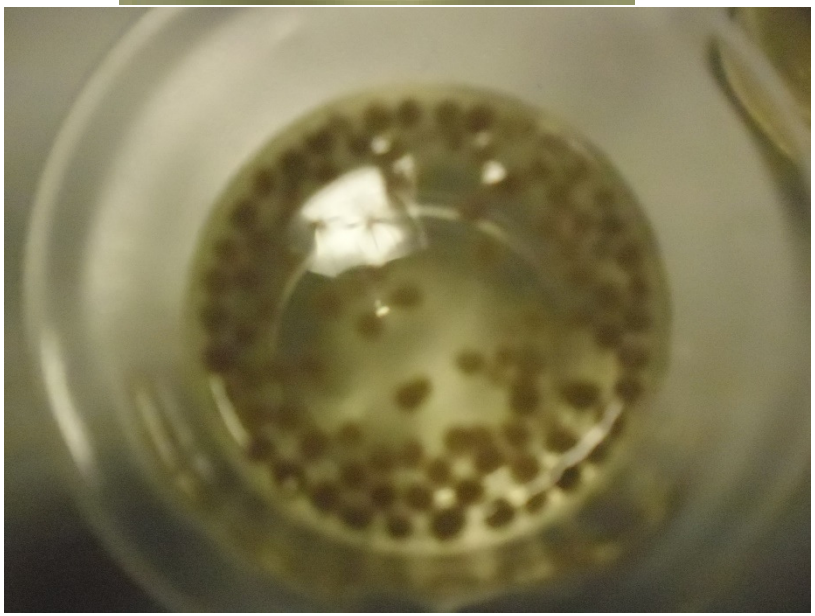
Objectives

- The objective of this study is, optimization of the release kinetics of phenolic compounds of encapsulated *Olea europaea* infusions

Prepared Capsules



$CaCl_2$ Solution



Why encapsulation?

- The use of phenolic extracts as value-added ingriedients have been restricted due to their bitterness and astringency.

- The promising way to mask those unpleasent tastes, is the encapsulation of phenols with carriers like proteins and /or polysaccharides before adding to the food products (Fang & Bhandari, 2010; Lesschaeve & Noble, 2005).

- Protecting the sensitive core materials against undesirable effects such as light, moisture, and oxygen (Shahidi & Han, 1993).

- Encapsulation, developed about sixty years back, is a technology of packaging solids, liquids, or gaseous materials in miniature, sealed capsules that can release their contents at controlled rates under specific conditions (Desai & Jin Park, 2005).

- Food ingredients of acidulants, flavoring agents, sweeteners, colorants, lipids, vitamins, minerals, enzymes, bioactive compounds as well as microorgaisms have been encapsulated in various investigations.

The objectives of encapsulation in food industry

- Protection of the core material from degradation (by reduction of their reactivities with outside environment)
- Reduction of the evaporation or transfer rate of the core material to the outside environment
- Modification of the physical characteristics of the original material to allow easier handling
- Tailoring the release of the core material slowly over time, or at a particular time
- To mask an unwanted flavor or taste of the core material
- Dilution of the core material that only small amounts are required, while achieving uniform dispersion in the host material
- To help separate the components of the mixture that would otherwise react with one another

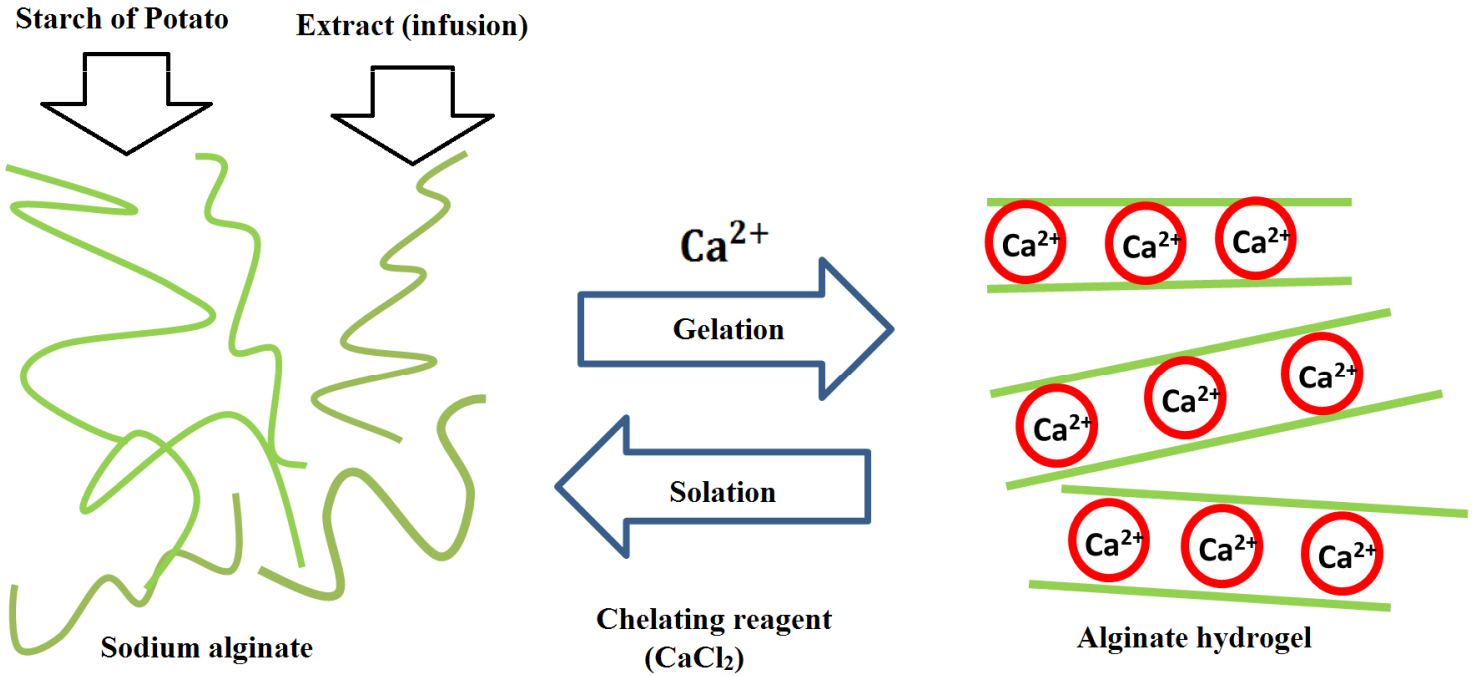
Materials and methods

- Plant materials were collected in June 2014 from countryside of Faro in Algarve-Portugal.

Aerial Photography of where tested plants picked up

Methods

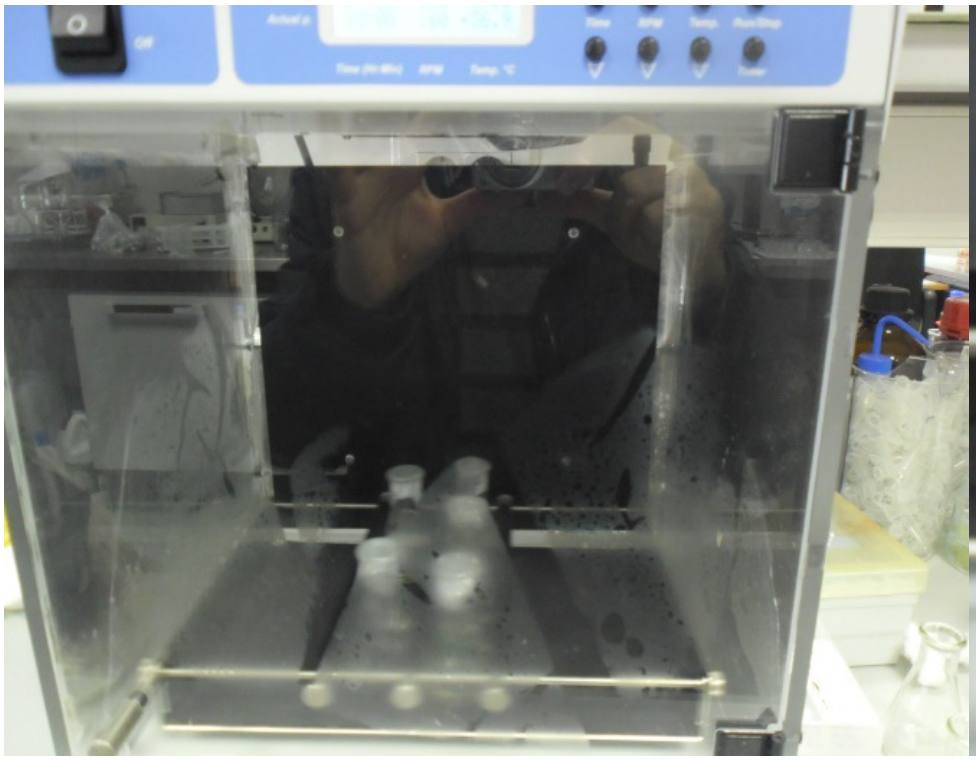
- Extraction of olive leaves were performed by distilled water using microwave assisted extraction (MAE) under 300 W microwave power for 120 s.
- Infusions were concentrated, frozen and preserved under -20 °C by the day of encapsulation and further analyses.
- The hydrogel beads prepared by ionic gelation obtaining a calcium alginate matrix according to the method explained previously by (López Córdoba, Deladino, & Martino, 2013).

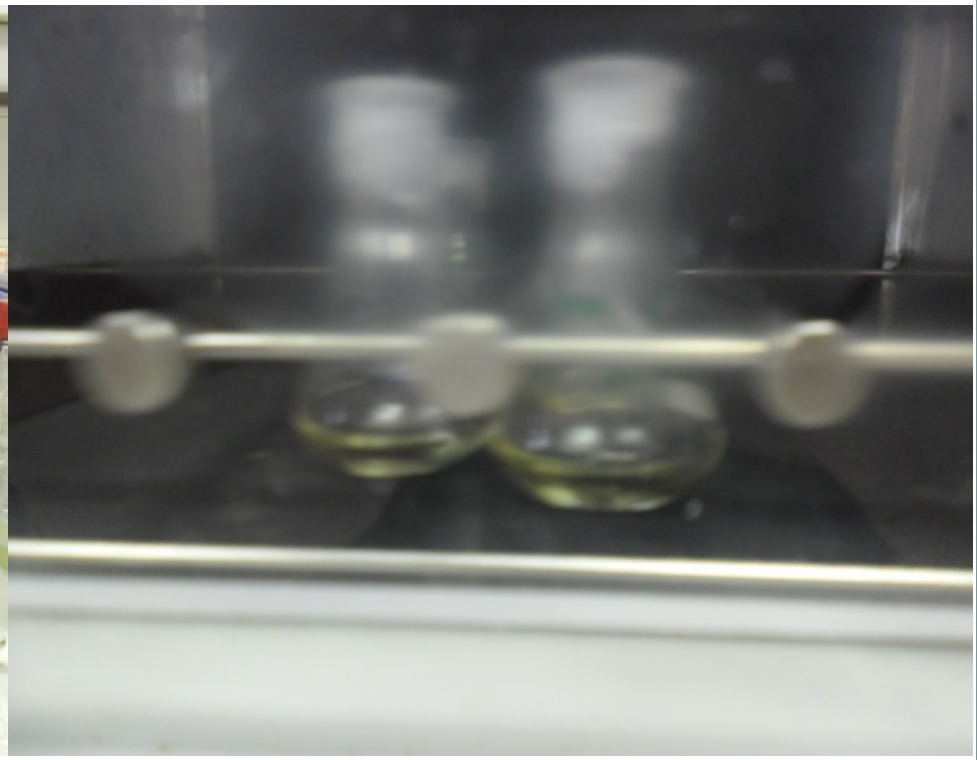


A schematic of encapsulation mechanism of sodium alginate and calcium chloride

Methods

- Three various concentrations of potato of starch including 0.5, 1 and 1.5% were considered for preparing of beads.
- Concentration of the extract in the beads (3 g/100 ml)
- Concentration of soidum alginate was 2%
- Extraction of the beads were performed within three different period of times including 60, 120 and 180 min.





Sodium alginate beads

Among polynomic polymers, alginate has been widely investigated and applied for its possibility to adjust the release, due to the following reasons

- Availability of carboxyl groups
- Biodegradable properties
- Absence of toxicity

Chitosan also has spread applications in pharmaceutical technology as tablet disintegrant, for the production of controlled release solid dosage or for promotion of drug dissolution

Examined tests

- Encapsulation efficiency on TPC, TAA and FRAP values were determined.

- Solvent for extraction of beads was selected citrate sodium (5 $g.ml^{-1}$).

Experimental design and statistical analysis

- The extraction procedure was carried out in 10 different runs regarding our design.
- On face Central Composite Design (CCD) of Response Surface Methodology (RSM) is exploited using JMP Pro program version 11.
- Starch concentration (*X1*) of the beads as well as extraction time of bioactive compounds from beads (*X2*) were considered as independent variables.
- Three levels for each of independent variables are shown in Table 1:

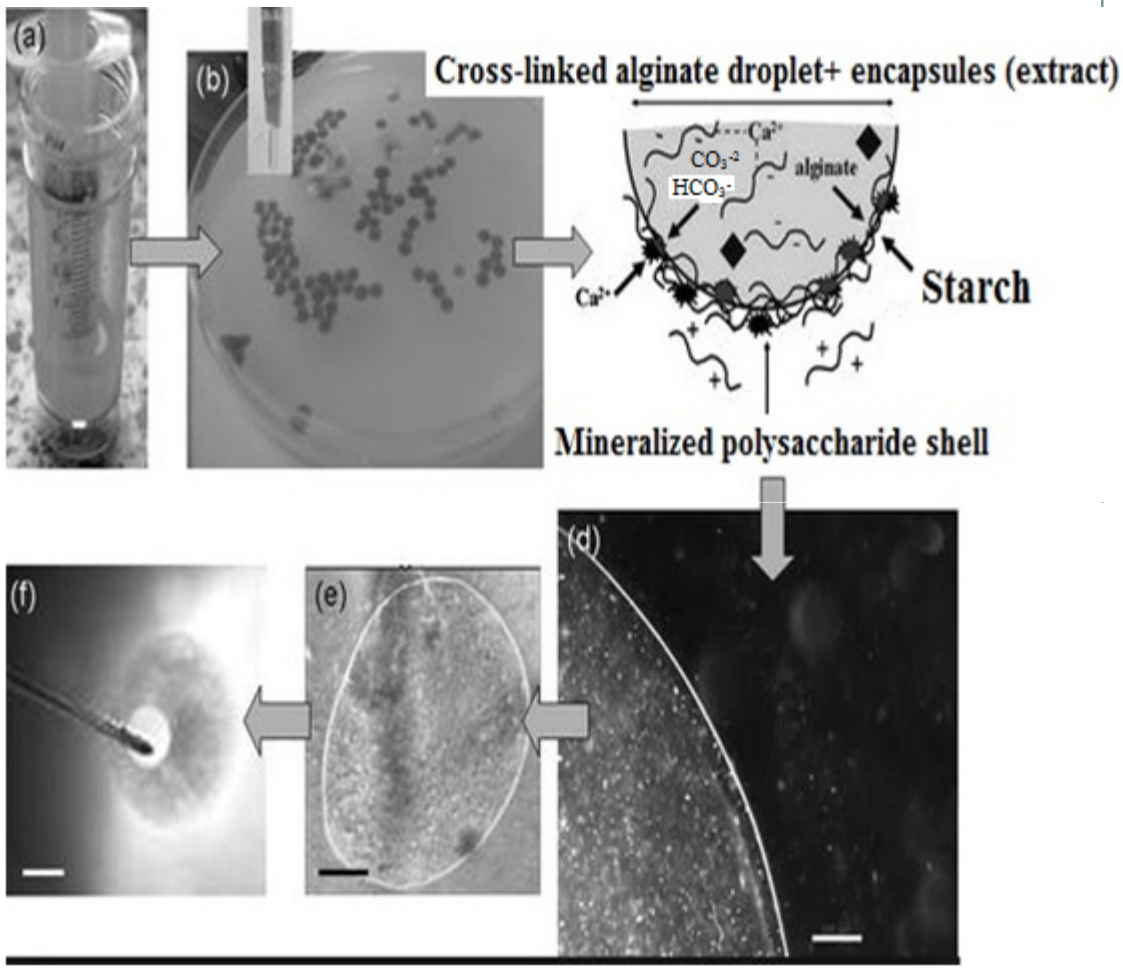
Table 1
Independent variables and their coded and actual values used for optimization of encapsulation efficiency

Independent variables	Units	Symbol	Code levels		
			-1	**0**	**1**
Starch of patato	%	X_1	0.5	1	1.5
Extraction time	min	X_2	60	120	180

Results

Visual characteristics of calcium alginate-starch (CAS) hydrogels beads

- Starch addition affected the optical properties as such the diameter and colour of the prepared capsules.
- An enhancement in the quantity of applied starch in encapsulation of *Olea europaea* aqueous infusion, created bigger and darker capsules.
- This finding is not in agreement with the finding of (López Córdoba et al., 2013). (Starch addition did not affect the average diameter, sphericity factor, bulk density, moisture content and water activity with respect to calcium alginate beads).
- Some other researchers reported that starch improves the physico-mechanical properties of the produced beads (Chan et al., 2011; Santagapita et al., 2012).



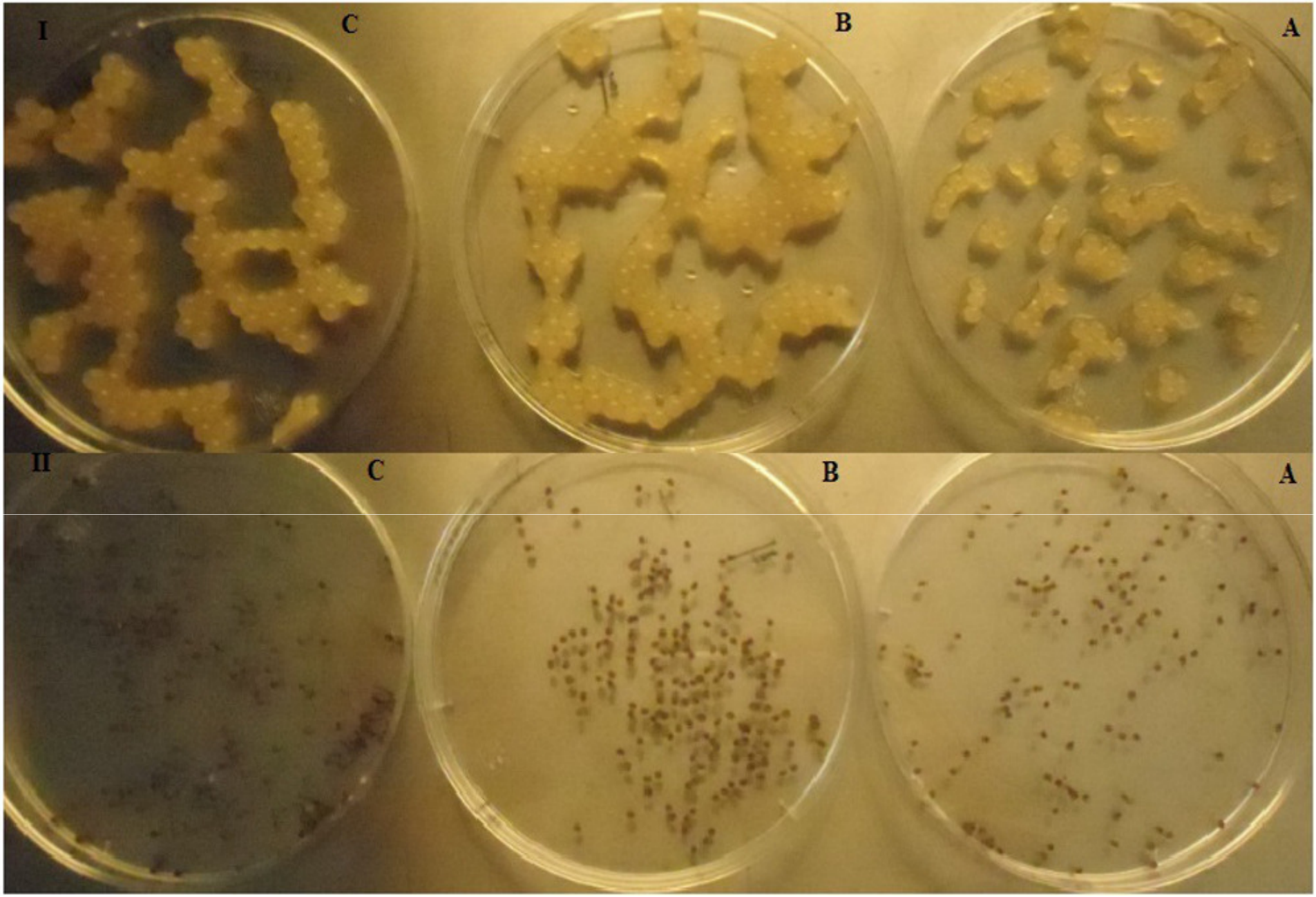


Figure 1- *Olea europaea* starch filled calcium alginate beads, in three different concentrations, A (0.5%), B (1%) and C (1.5%) in optical (I) and wizen (II) macrographs.

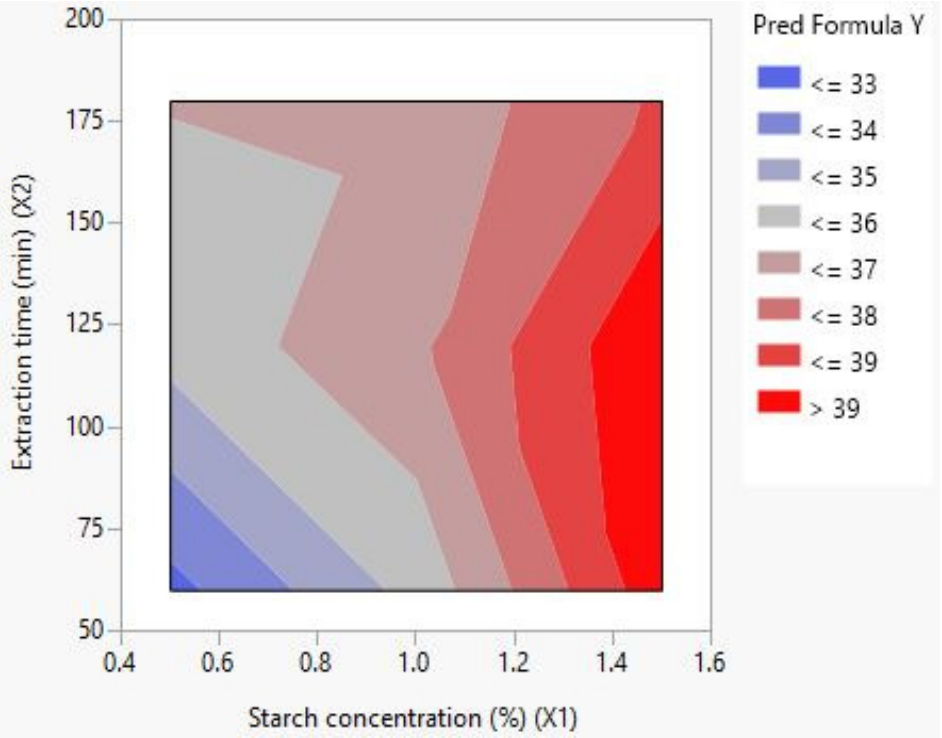
Table 2
Central composite design matrix with observed and predicted values of response variables

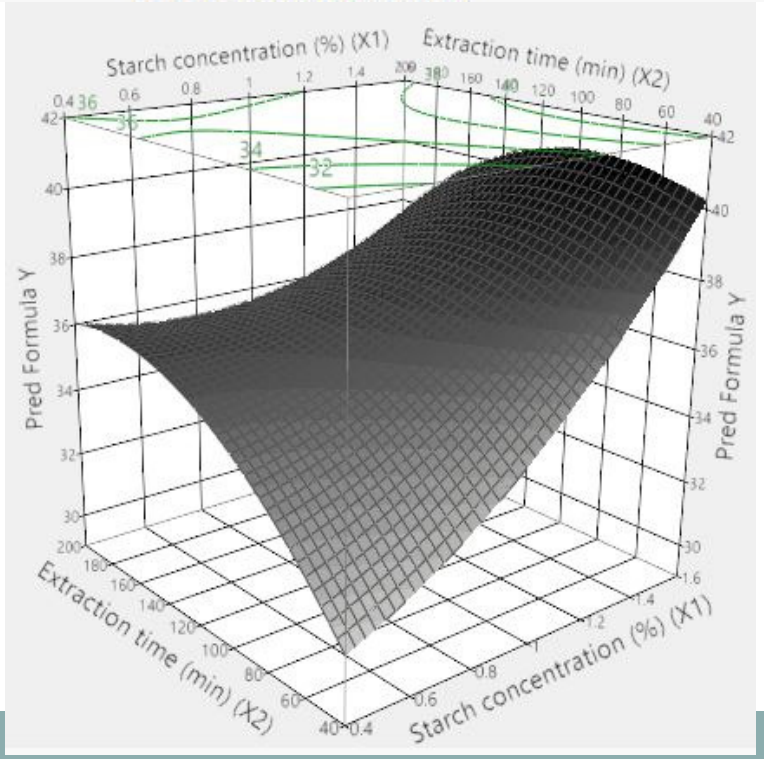
Run	Coded variable levels		Encapsulation efficiency of TPC (%)		Encapsulation efficiency of TAA (%)		Encapsulation efficiency of FRAP (%)	
	X_1	X_2	EXP	Pred	EXP	Pred	EXP	Pred
1	0.5	60	**33.562**	32.668	**57.695**	57.267	2.609	2.505
2	0.5	120	34.193	35.373	62.789	63.093	**2.388**	2.409
3	0.5	180	36.334	36.046	64.303	64.426	2.688	2.770
4	1	60	34.185	35.324	60.745	61.943	2.843	3.003
5	1	120	37.43	36.815	68.115	67.404	2.688	2.726
6	1	120	37.268	36.815	67.988	67.404	2.713	2.726
7	1	180	36.347	36.273	**68.274**	68.372	3.119	2.907
8	1.5	60	39.893	39.646	61.444	60.672	**3.841**	3.784
9	1.5	120	**40.037**	39.922	64.775	65.767	3.401	3.327
10	1.5	180	37.806	38.166	66.590	66.370	3.198	3.327

Encapsulation efficiency of TPC

Table 3
ANOVA results for the effect of *X1* (starch concentration (%)) and X_2 time of extraction (min) on response variables ($p<0.05$).

Source	Encapsulation efficiency of TPC (%)			
	DF	Sum of Squares	F Ratio	Prob>F
X_1	1	0.234	0.215	0.666
X_2	1	6.053	5.546	0.078
$X_1 \times X_2$	1	5.902	5.408	0.080
X_1^2	1	1.617	1.482	0.290
X_2^2	1	2.409	2.208	0.211
Model	5	41.759	7.652	0.035*
Lack of fit	3	4.352	110.567	0.069
Error	4	41.759		
C. Total	9	46.125		
		R^2= 0.905		
		Adj, R^2= 0.787		





Encapsulation efficiency of TAA

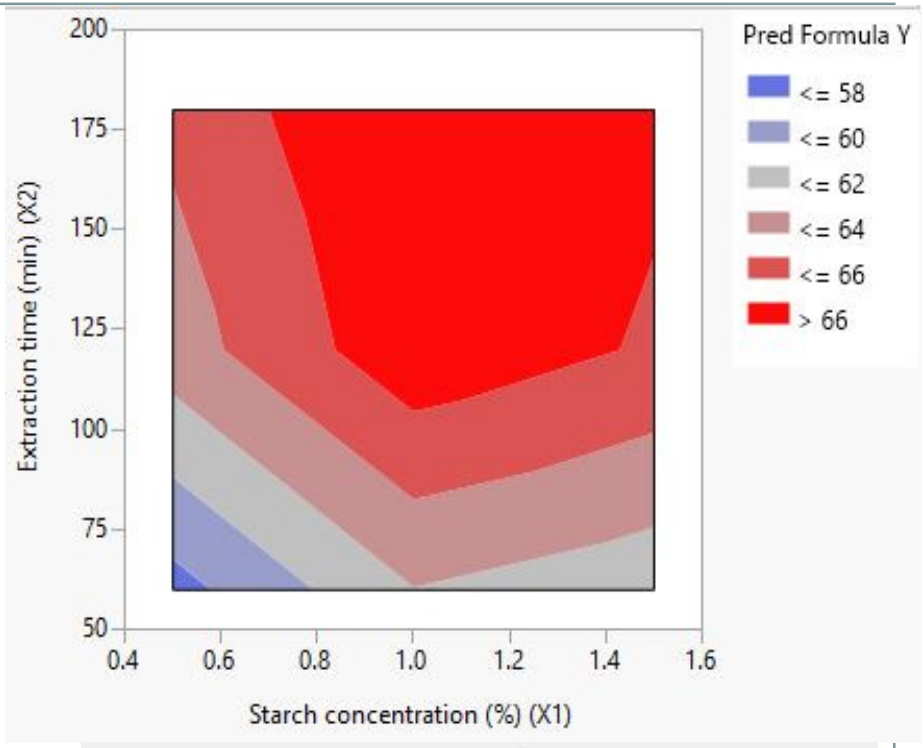
Table 4

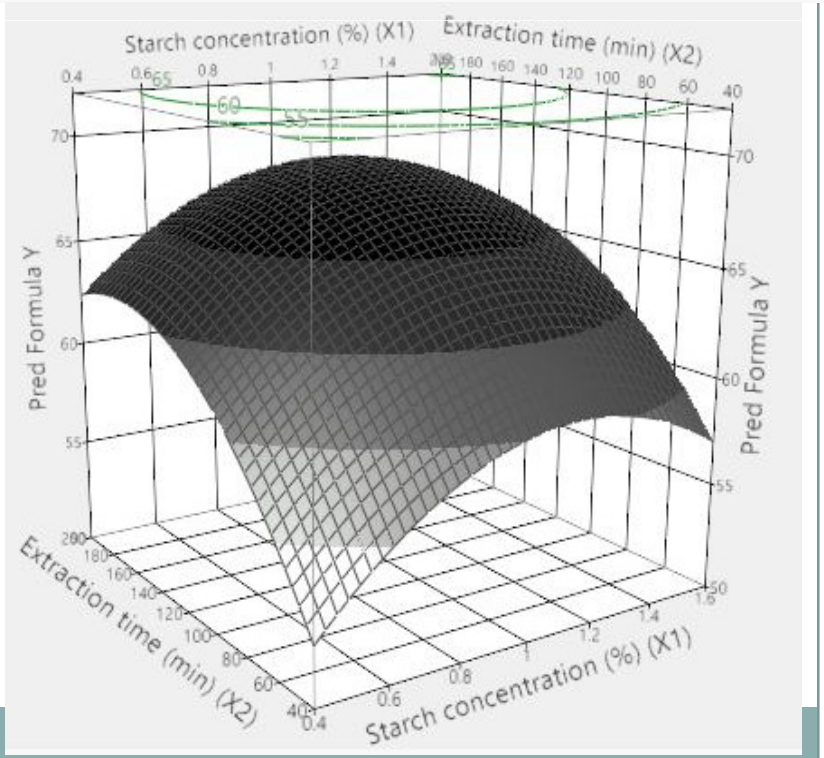
ANOVA results for the effect of *X1* (starch concentration (%)) and X_2 time of extraction (min)) on response variables ($p<0.05$).

Source	Encapsulation efficiency of TPC (%)			
	DF	Sum of Squares	F Ratio	Prob>F
X_1	1	24.297	23.064	0.0086*
X_2	1	20.834	19.777	0.011*
$X_1 \times X_2$	1	0.534	0.507	0.515
X_1^2	1	20.633	19.586	0.011*
X_2^2	1	11.772	11.175	0.028*
Model	5	111.918	21.248	0.0056*
Lack of fit	3	4.205	173.795	0.055
Error	4	4.213		
C. Total	9	116.132		

$R^2 = 0.963$

Adj, $R^2 = 0.918$





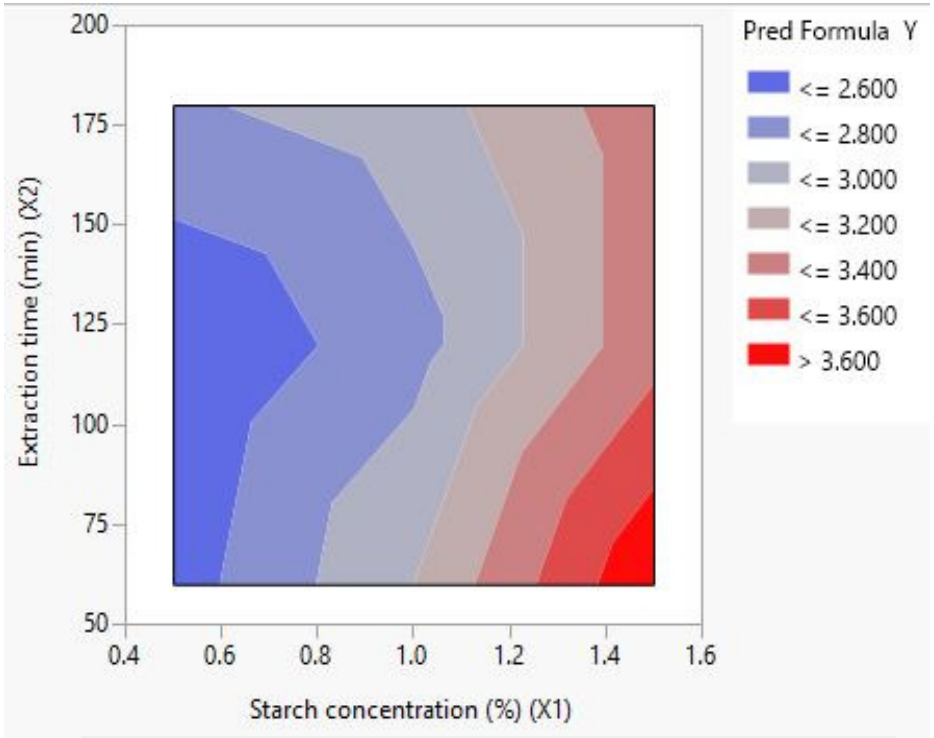
Encapsulation efficiency of FRAP

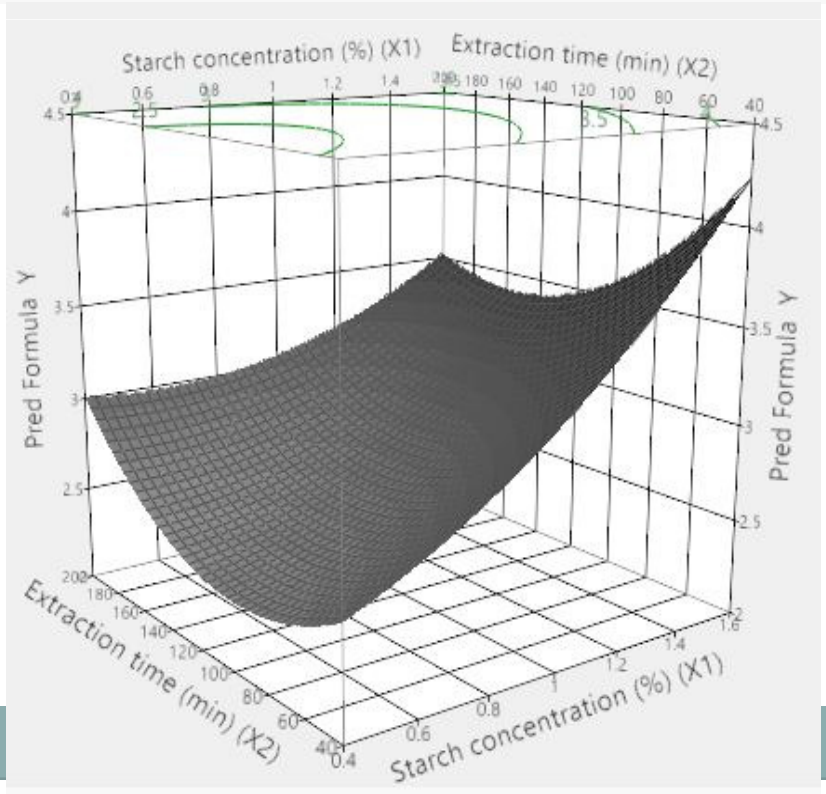
Table 5
ANOVA results for the effect of *X1* (starch concentration (%)) and X_2 time of extraction (min) on response variables ($p<0.05$).

Source	Encapsulation efficiency of TPC (%)			
	DF	Sum of Squares	F Ratio	Prob>F
X_1	1	0.007	0.274	0.628
X_2	1	0.045	1.558	0.280
$X_1 \times X_2$	1	0.130	4.511	0.100
X_1^2	1	0.047	1.628	0.271
X_2^2	1	0.121	4.217	0.109
Model	5	1.608	11.138	0.0183*
Lack of fit	3	0.1152	122.924	0.0662
Error	4	0.1155		
C. Total	9	1.724		

R^2= 0.932

Adj, R^2= 0.849





Analysis of the model for encapsulation efficiency

Table 6
Regression coefficient, standard error, and student's t-test results of response surface of the determined parameter ($p<0.05$).

Source	Encapsulation efficiency of TPC (%)				Encapsulation efficiency of TAA (%)				Encapsulation efficiency of FRAP (%)			
	Estimate	Std. error	t Ratio	Prob > |t|	Estimate	Std. error	t Ratio	Prob > |t|	Estimate	Std. error	t Ratio	Prob > |t|
Intercept	25.724	3.865	6.65	<0.0026*	35.960	3.797	9.47	0.0007*	2.664	0.628	4.24	0.0133*
X_1	2.746	5.918	0.46	0.666	27.925	5.814	4.80	0.0086*	0.504	0.962	0.52	0.628
X_2	0.116	0.049	2.360	0.078	0.215	0.048	4.45	0.011*	-0.010	0.008	-1.25	0.28
$X_1 \times X_2$	-0.040	0.017	-2.33	0.080	-0.012	0.017	-0.71	0.515	-0.006	0.002	-2.12	0.100
X_1^2	3.330	2.735	1.220	0.290	-11.894	2.687	-4.43	0.011*	0.568	0.445	1.28	0.271
X_2^2	-0.0002	0.0001	-1.49	0.211	-0.0006	0.0001	-3.34	0.028*	6.3472e-5	0.000031	2.05	0.109

Concluding remark

- Starch addition not only, affects the antioxidant capabilities of encapsulated *Olea europaea* of hydrogel capsules; but also it affects the optical morphology and physico-chemical specifications of the produced beads.
- The optimum conditions for encapsulation efficiency of the extract are obtained as follows:

Table 5
Optimum conditions of the variables on the selected responses

Parameter	Starch of potato, concentration (%)	Time of extraction (min)	Encapsulation efficiency (%)	Desirability
TPC	1.5	98.537	40.057	0.866
TAA	1.091	161.620	68.651	0.888
FRAP	1.5	60	3.784	0.862

Thanks a lot for your attention