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Optimisation of Encapsulation by Complex Coacervation of Phenolic Compounds from Propolis with *Triumfetta cordifolia* Gum and Kinetic Study

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ABSTRACT

Phenolic compounds from propolis have pharmacological properties such as antioxidant, antidiabetic, antiulcer, anticancer. However, they are unstable and oxidize in contact with ultraviolet rays and humidity. In order to limit their oxidation, phenolic compounds need to be protected through a polymeric membrane. The aim of this work is to optimize the encapsulation of phenolic compounds from propolis by complex coacervation using a polymer matrix consisting of gelatin and a Cameroonian tropical gum named *Triumfetta cordifolia*. A second order regression using Central Composite Design (CCD) was used to determine the optimal conditions of encapsulation as follows: pH (X_1) 4.2, gelatin concentration (X_2) 54 % and active ingredient concentration (X_3) 25 %. The use of *Triumfetta cordifolia gum* as an anionic polymer led to a considerable encapsulation rate of total polyphenols and flavonoids with the values of 85.75 % and 88.24 % respectively. Concerning the study of the release kinetic of phenolic compounds from propolis, it appeared that the zero-order model better fitted the release in the gastric environment with a high correlation coefficient of R^2 = 0.99. While in the intestinal environment it was the first-order model which better explained their slowed diffusion into the solvent (R^2= 0.97).

Keywords: Encapsulation, phenolic compounds, propolis, *Triumfetta cordifolia* gum

Introduction

Propolis (bee glue) is a sticky resinous substance produced by bees from different plant sources such as leaves, flowers and bud exudates modified with bee secretions and wax.[1] In Cameroon propolis is used traditionally to treat diseases such as: coughs, diabetes, hypertension, dental infections, appendicitis and various skin infections.[2] Propolis contains several secondary metabolites such as: flavonoids, polyphenols, triterpenes, saponins, sterols and tannins.[3] In addition, it has several biological properties including antifungal,[4] anti-inflammatory,[5] antimicrobial,[6] antiviral,[7] antioxidant,[8] antibacterial,[9] and antidiabetic.[10] Chemical interactions and oxidation lead to the conversion of hydroxyl groups of phenolic to ketones and produce peroxides which are free radicals responsible for oxidative stress.[11] Nowadays, encapsulation has become one of the most attractive methods of immobilisation and protection of active ingredients.[12] The microcapsules protect the substance from ultraviolet rays, humidity or oxygen.[13] Complex coacervation is a very interesting technique allowing high encapsulation yield and easy release of compounds from microcapsules.[14] Complex coacervation process consists of simultaneous desolvation of two polyelectrolyte polymers carrying opposite charges caused by adjustment of pH induced electrostatic attraction of polymers.[14] Proteins and polysaccharides are widely used to make coating membranes.[12]

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Triumfetta cordifolia was chosen as polysaccharide due to its availability as tropical plant whose gum extract is negatively charged and has good stabilising, thickening and texturising functions. *T. cordifolia* gum was associated with gelatin which is a protein positively charged and characterised by its hydrophilic and hydrophobic properties.[15] The phenolic compounds encapsulated must be released into the body in simulating gastric and intestinal conditions. To understand the mechanism that occurs during the release of phenolic compounds from microcapsules, it was necessary to study kinetic models with three computational models such as: zero-order, first-order and Higuchi's models. The aim of this work was to optimize the encapsulation of phenolic compounds contained in MeOH/H_2O extract of propolis using *Triumfetta cordifollia* gum and gelatin as polymeric matrices and also study the release kinetic in physiological conditions.

Materials and Methods

Materials

Propolis was collected in April 2018 during the rainy season in a village called Nyambaka located in the Vina division in the Adamawa region of Cameroon. The propolis was dried at room temperature for one week and then stored in hermetically sealed plastic bags to be used for further work. *T. cordifolia* was collected in May 2021 in a village called Dang located in Ngaoundere which is the capital of the Adamawa region. The barks were cut into pieces of 10 cm of length and dried at 50 °C during 48 hours in an electric drier according to the method of Kamdem *et al*.[18] with slight modifications.

Propolis extraction

Propolis extract was obtained under optimal microwave assisted extraction with conditions as follows: time 74 s, power 400 W, ratio solid/liquid 1:20 g/mL and water-methanol polarity 23/87 %. The filtrate was concentrated using a rotary evaporator and stored to determine polyphenols and flavonoids content.

T. cordifolia gum extraction

The gum was extracted from *T. cordifolia* using the method described by Kamdem et al.[18] The ratio of 4:150 (g/mL) of barks/water was used. The barks were infused in distilled water at 50 °C during 1 hour under agitation with a magnetic stirrer. The gum solution was centrifuged at 3600 rpm for 30 minutes and the supernatant represented the extract.

Microcapsules production

The formulation of microcapsules with active propolis extract, gelatin and *T. cordifolia* gum was carried out according to the protocol of Siow and Ong.[12] with slight modifications. A solution of gelatin (1%) was introduced into a beaker placed in a water bath at 50°C and propolis extract (0.1g) was added. The mixture was stirred at 1200 rpm for 4 minutes. Then a solution of *T. cordifolia* gum (2%) was added dropwise. The mixture was kept under stirring and the volume of the medium reaction was made up to 100 mL by adding distilled water. A solution of HCl at 1 N concentration was used to adjust the pH. After that, 0.35 g of D (-) ribose was added to the mixture in order to form the covalent bond between gelatin and *T. cordifolia gum*.

Optimization of phenolic compounds encapsulation using Central Composite Design (CCD) and Response Surface Methodology (RSM)

In order to optimize the microencapsulation of phenolic compounds from propolis extract, a Central Composite Design (CCD) approach was applied. This method was based on Response Surface Methodology (RSM) analysis with 3 factors at 3 levels. The parameters selected as independent variables were: pH (X_1), gelatin concentration (X_2) and active ingredient concentration (X_3). Four central repetitions were realised, resulting in 18 experiments with the value of orthogonality equal to 1.607 as shown in Table 1.

Mathematical model of CCD

Total polyphenols (TP) and Total Flavonoids (TF) encapsulated were selected as dependent responses, and the regression coefficients were obtained by adapting the experimental results to a second-order polynomial model according to equation (1):

$$Y_i = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \beta_3 x_3 + \beta_{12} x_1 x_2 + \beta_{13} x_1 x_3 + \beta_{23} x_2 x_3 + \beta_{11} x_1^2 + \beta_{22} x_2^2 + \beta_{33} x_3^2 \quad (1)$$

Where Y_i is the expected response, β_1, β_2, β_3 are the linear coefficients, $\beta_{12}, \beta_{13}, \beta_{23}$ are the interaction coefficients, β_{11}, β_{22}, β_{33} the square coefficients, x_1, x_2, x_3 are the levels of the independent variables.

Statistical analysis

Analysis of Variance (ANOVA) was used to determine the statistical significance of each factor and their effect. The Fisher test was used to determine the significance of each factor and the correlation coefficient (R^2). Model *p*-value and the construction of response surfaces (3D) graphs were determined using the software Statgraphics Centurion XV.I 15.1.2 and Sigmaplot.

Encapsulation rate

The encapsulation rate (E_R) was calculated as the ratio between the amount of Total Polyphenols or Flavonoids encapsulated over Total Polyphenols or Flavonoids introduced. E_R expressed as a percentage (%) according to the formula (2):

$$E_R = \frac{Qencapsulated}{Qintroduced} X100 \quad (2)$$

In vitro release kinetics of polyphenols and flavonoids from microcapsules

To study the release of phenolic compounds from microcapsules, *in vitro* simulations of gastric (pH of 1.2) and intestinal (pH of 6.8) fluids were performed using the protocol of Adejero *et al*.[16] with slight modifications. The study was carried out over 8 hours and the different steps according to physiological conditions are distributed as follows: from 0 h to 2 h, 2 g of microcapsules were dispersed in 125 mL of HCl at pH of 1.2 and temperature of 37 °C (gastric fluid condition). The solution was kept under agitation (50 rpm). From 2 h to 6 h, 42 ml of phosphate buffer (Na_2HPO_4) at pH of 6.8 was added to the previously acidic solution. Every 30 minutes, 5 mL of supernatant was withdrawn with syringes followed by centrifugation at 10000 rpm to remove suspended particles of the withdrawn solution during 20 minutes. After that, the Total Polyphenols and Flavonoid released were calculated from the microcapsules in the supernatant. Three computational models were applied: zero-order model, first-order model and Higuchi's model.[17]

zero-order model: $C_{(t)} = C_0 + K_0 t$ (3)

first-order model: $C_{(t)} = C_0 (1\text{-}e^{-kt})$ (4)

Higuchi model: $C_{(t)} = K_H t^{1/2}$ (5)

Where k_0 is the zero-order constant, t is the time of release, $C_{(t)}$ is the released concentration of phenolic compounds at time t, C_0 is the initial concentration of phenolic compounds within solutions, k is the first-order rate constant, and k_H is the diffusion constant.

Table 1: Experimental conditions for the CCD of phenolic compounds encapsulation

Factors (unit)	**Range and levels**					
	Level	**-1.607**	**-1**	**0**	**1**	**1.607**
pH	X_1	2.5	3	3.6	4.2	4.6
Gelatin (%)	X_2	21.47	30	42.5	55	63.5
Active ingredient (%)	X_3	8.18	15	25	35	41.81

Results and Discussion

Total Polyphenols content was determined using the method of Folin Ciocalteu, while total flavonoids content was based on a colorimetric test using aluminium chloride according to the method described by Sakava *et al*.[6] Total polyphenols and flavonoids present in MeOH/H_2O extract from propolis were determined before encapsulation. After calculation, TP and TF values were 990.34 mg G.A.E/100g DM and 48.67 mg Q.E/100g DM respectively.

The method of Central Composite Design (CCD) was applied on a matrix of 18 experiments with 3 factors. The effect of gelatin concentration, active ingredient concentration and pH variation on the polyphenols and flavonoids content are reported in Table 2. The experimental values are between 702.30 and 817.07 mg GAE/100 gP for TP encapsulated. And between 37.04 and 42.10 mg EQ/100 gP for TF encapsulated. The maximum values of 817.07 mg G.A.E/100 gP and 42.10 mg Q.E/100 gP are obtained at experimental conditions of pH = 4.6, gelatin concentration of 42.5 % and active ingredient concentration of 25%. The linear, quadratic and also interaction coefficients of the models were calculated using the least square technique and the significance of parameter effects are reported in Table 3. It was shown that all the linear parameters of pH (X_1), gelatin concentration (X_2), active ingredient concentration (X_3) and also two interactions (X_1X_2) and (X_2X_3) were highly significant at the level of *p*-value < 0.05 according to Table 3.

Some mathematical standards were used such as: the linear regression coefficient (R^2); the Absolute Analysis of Average Deviation (AADM), the Bias factor (Bf) and the Accuracy factor (Af). According to Table 4, all the models are valid to predict the encapsulation of polyphenols and flavonoids from propolis with high correlation coefficient of $R^2 = 0.96$ and $R^2 = 0.99$ respectively.

The equations of the predictive model are as follows:

$$Y_{TP} = 931.13 + 53.45*X_1 + 7.91*X_2 + 1.58*X_3 + 1.67*X_1X_2 + 1.07*X_1X_3 - 0.92*X_2X_3 - 7.20*X_1X_1 - 0.95*X_2X_2 - 0.01*X_3X_3 \quad (6)$$

$$Y_{TF} = 157.01 + 46.37*X_1 + 0.96*X_2 + 0.39*X_3 + 1.33*X_1X_2 + 1.19*X_1X_3 - 0.03*X_2X_3 - 5.21*X_1X_1 - 1.07*X_2X_2 - 0.02*X_3X_3 \quad (7)$$

Table 2: Experimental data of the CCD for the optimisation of encapsulation of total polyphenols (Y_{TP}) and total flavonoids (Y_{TF})

N°	Real values			Experimental responses	
	pH X_1	Gelatin (%) X_2	Active Ingredient (%) X_3	Y_{TP} (m G.A.E/100gP)	Y_{TF} (mg Q.E/100gP)
1	2.5	42,5	25	788.98	37.20
2	4.2	55	15	808.94	41.81
3	4.2	55	35	779.35	38.02
4	3	30	15	729.84	37.32
5	3.6	21.47	25	702.30	37.04
6	4.2	30	35	785.48	40.72
7	3	55	35	711.52	39.56
8	3	30	35	707.92	37.49
9	3.6	63.5	25	783.99	38.46
10	3.6	42.5	25	806.14	40.07
11	3.6	42.5	25	806.13	40.09
12	3.6	42.5	25	806.12	40.05
13	3.6	42.5	41.81	724.57	38.41
14	4.2	30	15	801.63	40.42
15	3.6	42.5	25	796.27	40.11
16	3.6	42.5	8.18	727.99	38.28
17	4.6	42.5	25	817.07	42.10
18	3	55	15	719.87	37.62

Table 3: Significance of parameters and the analysis of variance (ANOVA)

Factors	Y_{TP}		Y_{TF}	
	Coefficients	*P*-value	Coefficients	*P*-value
X_1	53.455	0.000	46.377	0.000
X_2	7.912	0.019	0.960	0.004
X_3	1.587	0.002	0.391	0.000
X_1X_2	1.671	0.015	1.331	0.000
X_1X_3	1.007	0.070	1.195	0.068
X_2X_3	-0.921	0.001	-0.030	0.000
X_1X_1	-7.208	0.080	-5.218	0.061
X_2X_2	-0.953	0.340	-1.007	0.231
X_3X_3	-0.011	0.675	-0.024	0.564

Bold values indicate that the corresponding independent variables are significant on the responses ($p < 0.05$)

In both equations (6) and (7), the pH effect is predominant over the other factors because it determines the charge of polymers. The interaction X_1X_2 (pH-gelatin) is the strongest and contributes significantly, according to Table 3, to increase the rate of polyphenols and flavonoids encapsulated.

To observe the link between the responses and experimental levels of independent variables, 3D response surfaces were constructed through the polynomial models corresponding to equations (6) and (7). By fixing the pH at the centre value of 3.6, the responses surfaces of Figure 1-A and B were obtained. For a gelatin concentration of 55 % and active ingredient concentration of 15% the content of polyphenols and flavonoids encapsulated are respectively 780 mg G.A.E/100 g.P and 38.5 mg Q.E/100 g.P. The gelatin and *T. cordifolia gum* have a good emulsifying and stabilising power which lead to the formation of a good protective matrix for the active ingredient.[18] When increasing the active ingredient concentration to 35 % and decreasing the concentration of gelatin to 30 %, the TP and TF decrease also and the values are 710 mg G.A.E/100 g.P and 36.5 mg Q.E/100 g.P respectively. Indeed, the active ingredient incorporated into the system affects the encapsulation yield because when it is in excess, part of it remains in solution and there is saturation at the membrane binding sites.[19] When the gelatin concentration value is fixed at the centre value of 45% the responses surfaces of Figure 1-C and D are obtained. For the minimum value of active ingredient of 15% and a value of pH of 4.2 the yields of encapsulated TP and TF increased respectively with the values of 798 mg G.A.E/100 g.P and 42.5 mg Q.E/100 g.P. The best capsules are formed with a few active ingredient concentrations at pH value between 4-4.5. Figure 1-E and F illustrated the interaction pH-gelatin, it was observed that the TP and TF encapsulated increase with gelatin concentration and pH because the pH is lower than isoelectric point of gelatin and there is electrostatic attraction between the two polymers.[15]

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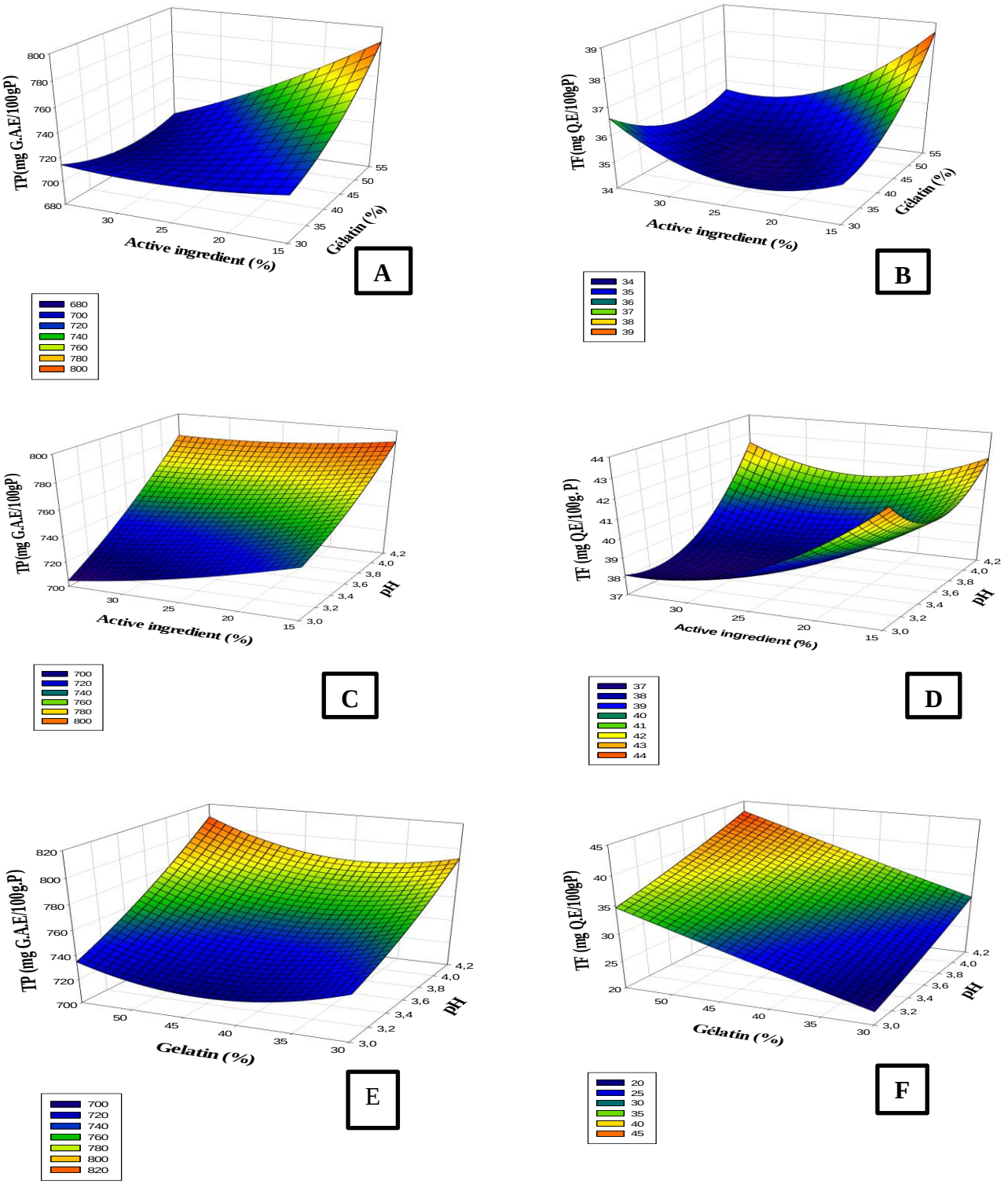

Figure 1: Response surfaces showing influence of interactions between gelatin and active ingredient (A, B), active ingredient and pH (C, D), gelatin and pH (E, F) on the Total Polyphenols and Flavonoids encapsulated

The superposition of iso-responses curves of TP and TF obtained from X_1X_2 (pH-gelatin) interaction allowed to identify where the optimal area will be reached. Figure 2 shows the compromise area to have the maximum of TP and TF encapsulated. The optimal conditions indicate a pH of 4.2 and gelatin concentration of 54%.

The encapsulation rate (τ) of TP and TF obtained under optimal conditions are:

$$\tau_{TP} = \frac{849.43}{990.34} X\ 100 = 85.75\ \%;\ \tau_{TF=}\frac{42.95}{48.67} X100 = 88.24\ \%$$

Where τ_{TP} is encapsulated rate of Total Polyphenols and τ_{TF} is encapsulate rate of Total Flavonoids.

These results are superior to those of Maroof *et al.*[19] who obtained a rate of 76.86 % for the encapsulation of phenolic compounds from Malaysian propolis by spray drying method using gum Arabic as

anionic polymer. In fact, the use of *Triumfetta cordifolia* gum in this work leads to a better encapsulation rate.

Mathematical modelling is interesting to understand how the sustained release formulation works and what impact the process parameters have on the release rate of the active ingredient. In order to explain the release mechanisms of encapsulated phenolic compounds from propolis, three models were applied at different pH levels: zero order, first order and Higuchi's model. The results are reported in Table 5. According to the results, the release mechanism of polyphenols and flavonoids at acidic pH (1.2) is better described by the zero-order model (R^2 = 0.99) than the first-order and Higuchi models. This result is related to the rapid leaching of compounds present on the membrane which is porous and hydrophilic. When the particles switch to intestinal conditions (pH=6.8), the release follows first-order (R^2=0.97) and even Higuchi-model (R^2=0.95) this observation is due to Fick's first law which explains the slow diffusion of the active ingredient in the solvent.[20] Once a global modelling of the whole gastrointestinal tract is done, the first-order model (R^2 = 0.96) explains the mechanism better than zero-order (R^2=0.84) and Higuchi models (R^2 = 0.92). The slowed diffusion observed is related to the high viscosity of *Triumfetta cordifolia* gum (ŋ = 18.33 dl/g) and its high molecular weight (4076 KDa) which slows down the release of compounds. Higuchi's model focuses on diffusion through the pore network created by the solvent, which is the only limiting step in the mechanism. These results are different to those of Azevedo *et al.*[17] who showed that the first-order mechanism controls the release of encapsulated phenolic compounds from Indian red propolis with higher kinetic constants (R^2 = 0.98 and K=2.75 h^{-1}) compared to the value obtained in Table 5 (R^2 = 0.96 and k = 0.33 h^{-1}). The gum arabic has a low molecular weight (300 kDa) and low viscosity (ŋ = 0.24 dl/g) compared to *Triumfetta cordifolia gum* that is the reason why they found a high diffusion constant. Moreover, Tamfu *et al.*[21] showed that brown propolis from Cameroon contains many complex phenolic compounds and the intermolecular interactions slow down their release in physiological conditions.

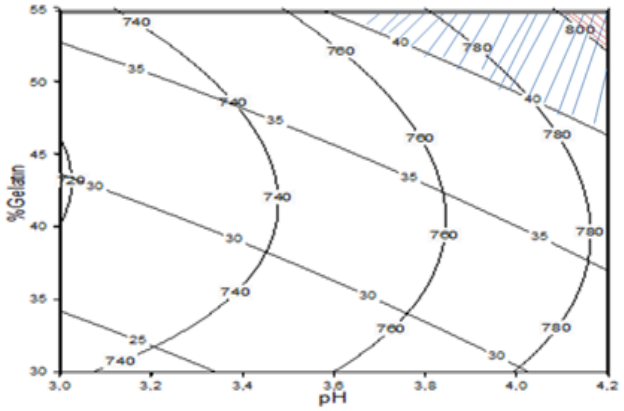

Figure 2: Optimal encapsulation area of Total polyphenols and Flavonoids encapsulation

Table 4: Validation of polyphenols and flavonoids models

Validation indicators	Y_{TP}	Y_{TF}	Standard values
R^2	0.96	0.99	> 0.95
R^2 adjusted	0.93	0.98	> 0.80
AADM	0.02	0.03	0.00 <AADM < 0.30
Bf	0.90	0.89	0.75 < Bf < 1.25
Af	1.11	1.12	0.75 < Af < 1.25

Table 5: *In vitro* release kinetic of TP and TF with zero-order, first-order and Higuchi models

Environment	Responses	Zero-order model		First-order model		Higuchi-model	
		K_0 (h^{-1})	R^2	K (h^{-1})	R^2	K_H ($h^{-1/2}$)	R^2
pH= 1.2	TP	0.88	0.991	0.34	0.96	0.44	0.90
gastric	TF	0.89	0.992	0.36	0.96	0.45	0.91
pH= 6.8	TP	0.63	0.77	0.22	0.97	0.31	0.95
intestinal	TF	0.68	0.79	0.25	0.97	0.34	0.95
pH= 1.2/6.8	TP	0.47	0.84	0.33	0.96	0.63	0.92
Gastro-intestinal	TF	0.49	0.87	0.35	0.96	0.66	0.93

Conclusion

The release mechanism of phenolic compounds from propolis encapsulated with gelatin/*T. cordifolia* gum is controlled in the gastric environment by the rapid leaching of compounds present on the matrix. Conversely, in the intestinal environment, the mechanism is controlled by slowed diffusion of compounds trapped in the heart of the membrane. Propolis capsules formulated with gelatin/*T. cordifolia gum* have prolonged release kinetic and can be used as potential antioxidants due to the high rate of Total Polyphenols and Flavonoids encapsulated.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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