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NANOENCAPSULATION BY IONIC GELATION OF POLYPHENOLS FROM ARTICHOKE (*CYNARA SCOLYMUS* L.) RESIDUES USING ULTRASOUND

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ABSTRACT

Background. Artichoke (*Cynara scolymus* L.) residues are a rich source of phenolic compounds, but these compounds are susceptible to external factors. Therefore, nanoencapsulation by ionic and ultrasound-assisted gelation techniques can be used as an alternative to preservation. This work aimed to determine the effects of the interaction of the following variables: chitosan (Ch) concentration, sodium tripolyphosphate (TPP), Ch/TPP ratio, pH, and sonication time to ensure high encapsulation efficiency (%EE).

Materials and methods. Optimal nanoencapsulation conditions were evaluated using a 2^{5-1} fractional factorial design to maximize nanoencapsulation efficiency (%EE) using multivariate regression analysis.

Results. The model was adequate with $R^2 = 0.998$. The optimum conditions for nanoencapsulation were Ch (0.28%), TPP (0.29%), Ch/TPP (5/1), pH (4.9) and sonication time (4.79 min). Under these conditions, a %EE of 69.9 ±0.67%, a particle size between 72.3 nm and 460.7 nm, a polydispersity of 0.458, and a charged Z potential of +15.73 mV were determined. In addition, the results showed a good loading of DPPH radical cutting activity 24.21 mM TE and Trolox equivalent antioxidant capacity (TEAC) of 16.45 mM TE in the nanocapsules, which allowed the antioxidant activity of polyphenols to be maintained.

Conclusions. The 2^{5-1} fractional factorial design was successfully applied to optimize the individual and interactive effects of the variables during the gelation nanoencapsulation process of artichoke waste polyphenols. Experimental and predicted values showed closely related values. Finally, the nanocapsules obtained with the highest %EE were characterized by particle size, Z-potential and polydispersity index (PDI) and showed good DPPH radical scavenging antioxidant activity loading and Trolox equivalent antioxidant capacity (TEAC).

Keywords: nanoencapsulation, efficacy, antioxidant capacity, Z-potential, particle size

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INTRODUCTION

The food industry generates tons of food waste that is released into the environment daily. Much of this waste may contain important sources of nutrients, proteins, polyphenols, and others (Bittencourt et al., 2018). For example, industrial processing of artichoke (bracts, stems, and leaves) generates a huge amount of waste (40–50% is discarded) (Zeaiter et al., 2019). It has been reported that artichoke residues are rich in polyphenols such as 1-O-caffeoylquinic acid, 1,5-O-di-caffeoylquinic acid, catechins, curcumin, resveratrol, chlorogenic acid, and apigenin-7-O-glucoside, among others, which predominate in bracts and stems (Mena-Garcia et al., 2020). Polyphenols are very beneficial to the human body and help in the fight against different diseases such as blood pressure and cardiovascular conditions, diabetes, and cancer (Mustafa et al., 2020; Prabhu et al., 2021; Suleman, 2018).

However, these compounds are very susceptible to different conditions such as temperature, oxygen, and light exposure and do not have good long-term stability, thus producing oxidation, as well as undesirable odors, and colors (Sharma et al., 2019). In addition, they have low bioavailability due to their poor water solubility and have a very bitter and astringent taste (Faridi Esfanjani et al., 2018). Based on these limitations, the food industry is looking for alternatives to take advantage of and conserve polyphenol-rich waste and residues.

In recent years, encapsulation techniques have gained great interest in the food and pharmaceutical fields as they offer efficient protection and preservation of bioactive compounds (mainly phenolic enzymes and antioxidants, micronutrients, and nutraceuticals) against degradation or evaporation of volatile compounds (Fangmeier et al., 2019; Wang et al., 2022). In addition, the nanoencapsulation technique can enclose bioactive compounds (BACs) in gaseous, solid, or liquid states, enhancing the stability of BACs by regulating their release at physiologically active sites (Atilgan and Bayraktar, 2022; Pateiro et al., 2021).

Currently, several methods allow nanoencapsulation of active materials, but they depend on the type of material and the desired release system. For example, ionic gelation (induced gelation) is a simple and gentle method for the preparation of chitosan nanoparticles in an aqueous medium without chemical modification of the chitosan surface molecule.

This method consists of extrusion-dripping the nanoencapsulation material and core in an ionotropic solution, which generates a spherical structure through the interaction between low molecular weight ions with a polymeric solution (Aman et al., 2021). Li et al. (2017) said that this is a simple, efficient, and low-cost encapsulation technique that does not require specialized equipment, organic solvents, or high temperatures, making it suitable for both hydrophobic and hydrophilic compounds. Nanoencapsulation by ionic gelation with chitosan contains reactive amino groups, which allows it to be easily modified to create nanoparticles or porous hydrogels. In addition, the positive charge allows the preparation of nanoparticles by ionic gelation with polyvalent anions such as tripolyphosphate (TPP) (Pedroso-Santana and Fleitas-Salazar, 2020; Singh et al., 2021). Xavier-Junior et al. (2018) applied a two-level fractional factorial design to optimize the nanoencapsulation efficiency of polyphenols from artichoke residues. This design showed fast and low-cost optimization because the variables and results were obtained using a reduced number of experiments.

Thus, this study aimed to contribute to the conservation of polyphenols obtained from artichoke residues through the formation of nanoparticles. Therefore, this work had the following objectives: a) to optimize the parameters of the 2^{5-1} fractional factorial design for the nanoencapsulation of artichoke waste polyphenols in chitosan particles; b) to establish the physical and physicochemical characteristics of the nanocapsules with the highest polyphenol nanoencapsulation efficiency (%EE) of artichoke waste.

MATERIALS AND METHODS

Chemicals and reagents

Potassium persulfate, 1,1-diphenyl-2-picrylhydrazyl radical, sodium carbonate, methanol, Folin-Ciocalteu, 2,2-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid), chitosan (49419). Sodium tripolyphosphate (TPP) (T9656), and acetic acid (818755) were purchased from Sigma-Aldrich (Lima, Peru). Sodium persulfate, Trolox (6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid), and gallic acid were purchased from Fluka Chemie AG (Buchs, Switzerland).

Plant material

Artichokes (*Cynara Scolymus* L.) were collected from a producer that supplies companies for export, located in Alayo – Province of Concepcion (Longitude 74°10'25.14"W; Latitude 13°6'43.6"S) Junin Region, Peru. Between May and July 2020, the bracts were washed, disinfected (0.2% sodium hypochlorite), and cut to obtain fresh bracts (approximately 0.5 kg) which were then freeze-dried (Biobase model, BK-FD10PT, Shandong, China), ground (model M20-S000, KIKA WERKE, Argentina), sieved (500 μm mesh) to obtain a fine powder, and stored in plastic tubes for analysis in the laboratory.

Extracts

The fine flour of artichoke bracts was mixed in a 1:10 ratio (bract flour:water:ethanol extraction solution (1:1, v/v)) and placed in amber colored jars and covered with aluminum foil. Ultrasound-assisted extraction (UAE) was carried out using Kisker 053275 ultrasound device (Kisker Ultrasonic, Steinfurt, Germany) with 1.4 L capacity, 42kHz frequency, and 230 V at 97% radiation amplitude, 53% ethanol concentration, and 9.7 min extraction time, as described in a previously published work (Quispe et al., 2021). The obtained solution was centrifuged in Falcon tubes at 400 rpm for 15 min, filtered with Whatman No. 1 paper, and placed under vacuum pressure between 100–200 mbar. The solution obtained was stored at 5°C for preservation and analysis.

Preparation of artichoke-loaded chitosan nanoparticles (ANPs)

ANPs were produced using the ionic gelation method described by Tzeyung et al. (2019). For this purpose, chitosan (CS) was dissolved in aqueous acetic acid (1% v/v) and this solution was mixed with polyphenolic extract in a 1:1 ratio under continuous stirring. Sodium tripolyphosphate (TPP) was then added dropwise with constant stirring (100rpm) at room temperature for 1 hr. Concentration, ratio, and pH (graded with NaOH at 1N) were adjusted according to the conditions of the 2^{5-1} fractional factorial design. Finally, the resulting nanoparticles formed were pelleted by centrifugation for about 45 min at 10 251 rpm to extract the hydrogel from the suspension (Saravanakumar et al., 2018). The treatments were evaluated by sonication on the suspensions using an ultrasound system with an amplitude of 70% and for 5 min. Then, the obtained granules were freeze-dried at a pressure of 1 Pa and a temperature of –54°C until they reached a content of 8% humidity (model BK-FD10PT, Biobase, China) for further characterization, and the supernatant solution was used to evaluate the encapsulation efficiency (%EE).

Determination of Total Phenolic Compounds (TPC)

TPC was quantified following the Folin-Ciocalteu method described by (Singleton and Rossi, 1965). Briefly, 40 μL of extract sample and 500 μL of Folin-Ciocalteu reagent were added to a 10 ml volumetric flask and covered with aluminum foil. After 15 min rest, 0.5 mL of 10% Na_2CO_3 was added, and the flask was filled to 10mL with ultrapure water. Absorbance was measured in a spectrophotometer (Model GEN10S, Thermo Fisher, UK) at 755 nm wavelength against a blank prepared with ultrapure water. Quantification was performed with a nine-point calibration curve (0.1 mg/mL to 0.9 mg/mL) prepared with standard solutions ($R^2 = 0.994$) of gallic acid. The TPC was expressed as mg GAE/g gallic acid equivalents of sample. All measurements were performed in triplicate (n = 3).

Antioxidant activity

The 2,2,2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay was used to determine the antioxidant activity according to (Brand-Williams et al., 1995). The DPPH solution was dissolved with 80% ethanol mixture and radical stock was performed daily. One milliliter of DPPH solution was mixed with 3 mL of ethanol and 1mL of polyphenol extract. The mixture was stirred and allowed to stand for 20 min at room temperature. The absorbance of the solution was determined at a wavelength of 517 nm. Quantification was performed with eight points (0.1–0.8 mM) of the Trolox standard curve ($R^2 = 0.998$). The results were expressed as mM Trolox/100 g DW. All measurements were performed in triplicate.

Trolox-equivalent antioxidant capacity (TEAC)

The TEAC assay is based on free radical scavenging activity and was determined using the ABTS

(2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) radical method according to (Rice-Evans et al., 1996) with some modifications. The absorbance was measured at 734 nm for 6 min with a UV-VIS spectrophotometer (model GEN10S, Thermo Fisher Scientific, UK). Quantification was performed with an eight-point (0.1–0.8 mM) standard calibration curve (R^2 = 0.994) of Trolox. All experiments were performed in triplicate and the results were expressed in mM Trolox equivalents (TE)/g dry sample.

Characterization of the nanoparticles

Encapsulation Efficiency (EE%). The initial concentration of the polyphenolic compounds in the artichoke bract extract was set at 24.45 mg GAE/g. For those that were taken to encapsulation in which the percentage of encapsulation efficiency was evaluated, the synthesized nanoparticles were separated from the suspension by centrifugation at 12 000 g for 15 min obtaining supernatant. These were filtered using sterile 0.45 µm Milliporey nylon (Milinčić et al., 2019). Once the nanoparticles loaded with the polyphenolic extract were obtained, they were sonicated for 5 min at a wave amplitude of 70%. Polyphenols were quantified using a spectrophotometer at a wavelength of 755 nm (Kisker Ultrasonic, model 053275). All measurements were performed in triplicate. Percentage %E-Cap was calculated according to equation 1, which was proposed by (Gopalakrishnan et al., 2014; Madureira et al., 2016).

$$\%EE = \frac{\text{(Total amount of polyphenol) – (Total amount of polphenols in supernatant)}}{\text{(Total amount of polyphenol)}} \times 100 \quad (1)$$

Mean particle size, size distribution, and zeta potential (ZP). The particle size and zeta potential of the nanoparticles (NPs) were measured using the dynamic light scattering (DLS) technique (Model 90Plus, NanoBrook, India) (Md et al., 2019). The size distribution of the NPs is indicated by the polydispersity index (PDI). Zeta potential of NPs (suspended-diluted in distilled water (1:10, v/v)) was performed using the electrophoretic light scattering (ELS) method. The NPs were diluted in saline phosphate buffer and placed in an electrophoretic cell at 22°C. Measurements were carried out at room temperature with a laser wavelength of 673 nm, a scattering angle of 90°, and in triplicate expressed as mean ±standard deviation (SD) (Dima and Dima, 2018).

Morphological characterization. Particle morphology was performed using scanning electron microscopy (SEM) analysis using a HITACHI microscope model SU8230 (Japan). For this purpose, a drop of NP was carefully adhered onto a metal slab on which the particles were coated with a thin monolayer of gold (45 nm) using the sputtering technique (Dima and Dima, 2018) and examined using an accelerating voltage of 15.0 keV.

EXPERIMENTAL DESIGN

The encapsulation efficiency of phenolic compounds from artichoke (*Cynara scolymus* L.) residues (bracts) was analyzed using a 2^{5-1} fractional factorial design, as it allows the number of experiments to be reduced when there are many factors included in the study. Therefore, this design is a way to simplify the full factorial design as it reduces the number of experiments needed (Elazazy et al., 2018). The encapsulation efficiency variables were chitosan (Ch) concentration, sodium tripolyphosphate, Ch/TPP ratio, pH, and sonication time. Table 1 presents the variables and the levels studied for each variable. Design-Expert software version 12.0 (trial version, Stat-Ease, Minneapolis, MN, USA) was used.

Table 1. Proposed factors and levels for obtaining chitosan nanoparticles using a 2^{5-1} fractional factorial design

Independent variables	Coded variable level		
	symbol	level	
		–1	+1
Chitosan (%)	A	0.1	0.2
TPP (%)	B	0.2	0.3
Ch/TPP	C	3	5
pH	D	3	5
Sonication	E	0	5

Optimization

Optimization was performed using Derringer's desired function method. This method depends on whether a particular response (Y_i) tends to be minimized, maximized, or assigned another value according to the desired process, while the independent variables are kept within the range (Derringer and Suich, 1980). The general approach is to first convert each response (Y_i) into a dimensionless individual desirability function (di), as shown in equation 2.

$$di = h_n(Y_i) \quad (2)$$

Statistical analysis

Statistical and experimental analyses were performed with the 12.0 trial package of the Design expert statistical software. All experiments were performed in three replicates and expressed as means ±standard deviation (SD). The statistical significance of the regression model coefficients, as well as the lack of fit, and the optimality of the extraction conditions were evaluated by analysis of variance (ANOVA).

RESULTS AND DISCUSSIONS

Determination of ionic nanoencapsulation parameters and model fitting

The number of experiments (or combinations, n = 16) and nanoencapsulation conditions based on the 2^{5-1} fractional factorial experimental design and their respective experimental %EE response (expressed as mean ±SD) are presented in Table 2. Here, five factors (chitosan (Ch), TPP, Ch/TPP ratio, pH, and sonication time) and two levels were evaluated to be optimized by combining these factors and levels in the ionic gelation nanoencapsulation process. These combinations aimed to

Table 2. Fractional 2^{5-1} factorial experimental design and responses under different experimental conditions

Number of experiments	Nanoencapsulation conditions					Response mean (n = 3)		
	A	B	C	D	E	Final polyphenol content mg GAE/g	%EE	SD
	Ch %	TPP %	Ch/TPP v/v	pH	Sonication min			
1	0.5	0.3	3/1	5	0	10.09	58.71	±0.01
2	0.2	0.1	5/1	3	0	8.08	66.94	±0.02
3	0.2	0.1	3/1	3	5	9.90	59.52	±0.02
4	0.5	0.3	5/1	5	5	9.24	62.19	±0.02
5	0.2	0.3	3/1	5	5	10.99	55.05	±0.03
6	0.5	0.1	5/1	5	0	11.72	52.04	±0.02
7	0.2	0.1	5/1	5	5	7.81	68.06	±0.01
8	0.5	0.1	5/1	3	5	11.90	51.32	±0.01
9	0.5	0.1	3/1	5	5	12.86	47.42	±0.02
10	0.2	0.1	3/1	5	0	13.41	45.15	±0.01
11	0.5	0.1	3/1	3	0	8.68	64.48	±0.03
12	0.2	0.3	5/1	5	0	7.68	68.59	±0.03
13	0.5	0.3	3/1	3	5	10.36	57.61	±0.02
14	0.2	0.3	5/1	3	5	7.60	68.93	±0.01
15	0.5	0.3	5/1	3	0	11.40	53.35	±0.02
16	0.2	0.3	3/1	3	0	9.43	61.44	±0.01

SD – standard deviation, A – chitosan concentration, B – TPP concentration, C – Q/TPP ratio, D – pH, E – sonication time; %EE – encapsulation efficiency.

evaluate the effect on the nanoencapsulation efficiency (%EE) of polyphenols obtained from artichoke (*Cynara scolymus* L.) bracts extracted using the ultrasound-assisted method (initial loading of 24.45 mg GAE/g).

In Table 2, the final polyphenol content loadings after nanoencapsulation ranged from 13.41 mg GAE/g to 7.60 mg GAE/g, and the nanoencapsulation efficiency (%EE) of polyphenols ranged from 45.15% to 68.93%, respectively. Similar results were reported using chitosan with chlorogenic acid (48%–59%) (Dima and Dima, 2018), gallic acid (59%–85%) (Lamarra et al., 2016), curcumin (62%–73%) (Asabuwa Ngwabebhoh et al., 2018), and ferulic acid (14.7%–56.5%) (Panwar et al., 2016). The differences in EE% observed in each compound can be attributed to the different polarities. Polarity influences the associative-repulsive equilibrium forces between chitosan chains and polyphenolic compounds.

Table 3 shows all the variables significantly affecting the efficiency of nanoencapsulation using the 2^{5-1}

Table 3. ANOVA test for encapsulation efficiency (%EE) of total polyphenol content

Source	Sum of squares	DF	Mean square	F-Value	*p*-value	Prob > F
Nanoencapsulation efficiency of polyphenols						
Model	862.91	12	71.91	147.78	*0.0008*	***
A-Ch	135.48	1	135.48	278.41	*0.0005*	***
B-TPP	59.80	1	59.80	122.89	*0.0016*	**
C-Ch/TPP	110.51	1	110.51	227.11	*0.0006*	***
D-pH	43.50	1	43.50	89.39	*0.0025*	**
E-sonication	0.02	1	0.02	0.05	*0.8357*	ns
AC	230.16	1	230.16	472.99	*0.0002*	***
AD	11.53	1	11.53	23.69	*0.0166*	**
AE	23.77	1	23.77	48.85	*0.0060*	**
BD	67.23	1	67.23	138.16	*0.0013*	**
CD	138.38	1	138.38	284.38	*0.0005*	***
CE	24.38	1	24.38	50.10	*0.0058*	**
DE	18.15	1	18.15	37.31	*0.0088*	**
Residual	1.46	3	0.49			
Cor Total	864.37	15				
Std. Dev.	0.70					
Mean	58.80					
C.V. %	1.19					
PRESS	41.52					
R-square	0.998					
Adj R-square	0.992					
Pred R-square	0.952					
Adec Precisión	38.521					

A – chitosan concentration, B – sodium tripolyphosphate concentration, C – relationship with the crosslinking agent, D – pH, E – sonication time.

Significance level: ***$p \leq 0.001$, **$p \leq 0.01$, *$p \leq 0.05$, $0.05 \leq {}^{fcs}p \leq 0{,}1$ (factor considered significant (Rezende et al., 2017)), nsp > 0,1 (not significant).

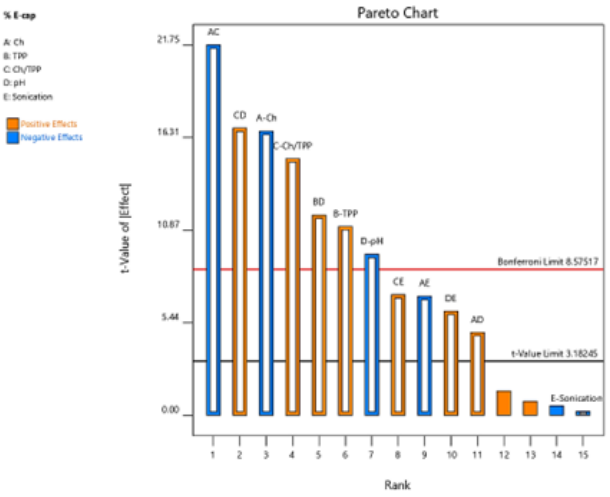

Fig. 1. Pareto plot for the percentage of polyphenol encapsulation of artichoke residues

fractional factorial design. A-Ch, B-TPP, and C-Ch/TPP were the intrinsic variables, while ultrasound application (E-sonification) was the process variable used to reduce the number of experiments, time, and process cost, as well as to obtain a better response (Mi et al., 2021). The main factors affecting nanoencapsulation were determined using ANOVA analysis of variance.

Normal curves show the main significant factors in nanoencapsulation. Prob > F less than 0.05 indicates that the model terms are significant (Table 3) and show a high coefficient of determination $R^2 = 0.998$.

The significant effects of the factors evaluated can be seen in the normalized Pareto diagram (Fig. 1).

In the analysis of variance, non-significant variables (AB, BC, and BE) were discarded. Variable E showed an interaction with the significant variables, which was used to determine the regression coefficient (equation 3).

$$\%E - Cap = 58.80 - 2.91A + 1.93B + 2.63C - 1.65D - 0.0394E - 3.79AC + 0.8489AD - 1.22AE + 2.05BD + 2.94CD + 1.23CE + 1.07DE \quad (3)$$

Effect of variables on nanoencapsulation by ionic gelation

Figure 2 shows a positive effect on nanoencapsulation when the concentration of sodium tripolyphosphate

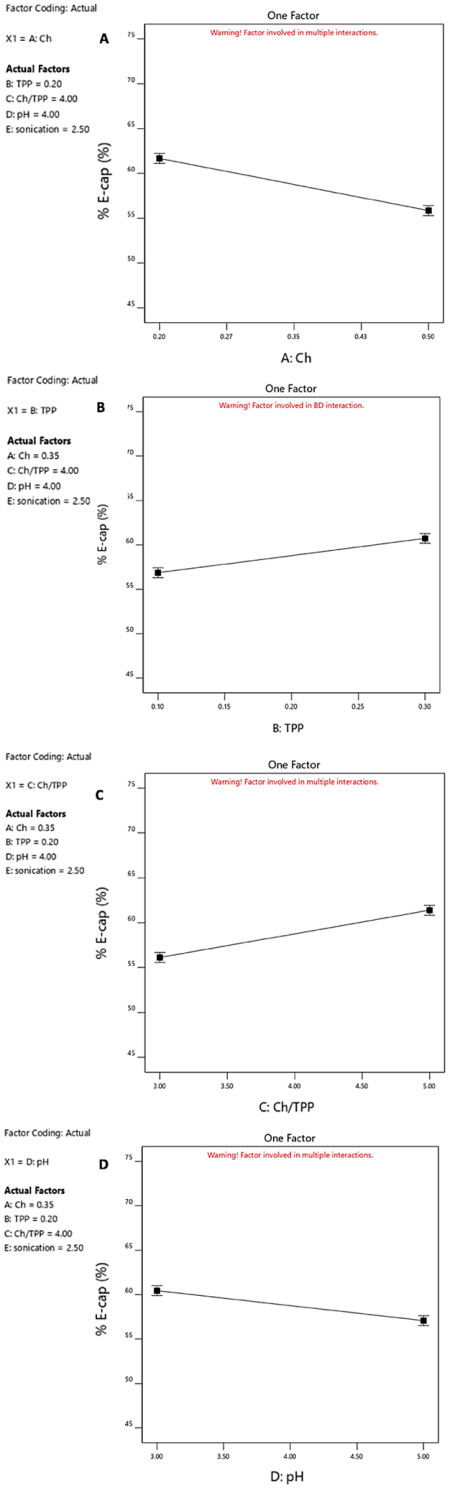

Fig. 2. Interaction plots (2D) of the %E-cap based on the significant differences between the factors. A – chitosan concentration, B – TPP concentration, C – Ch/TPP ratio, D – pH

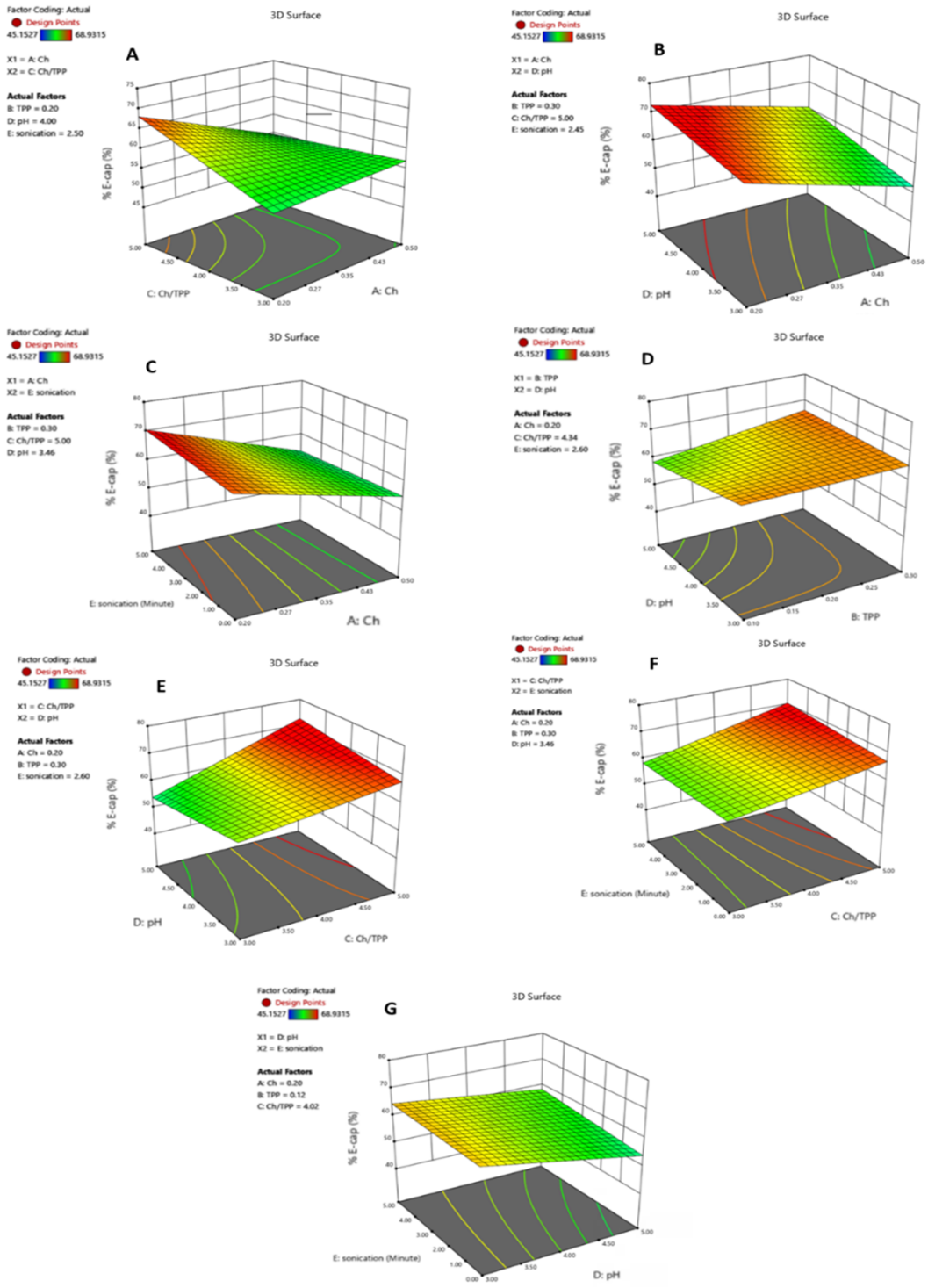

Fig. 3. 3D Response Surface plots for the interactions of %EE in function of factors: A – Ch and Ch/TTP, B – Ch and pH, C – Ch and sonication, D – TPP and pH, E – Ch/TTP and pH, F – Ch/TTP and sonication, G – pH and sonication

(Factor B) ranged from 0.1% to 0.3% and the ratio of chitosan/sodium tripolyphosphate (Factor C) was 3/1–5/1, and an increase in sonication from 0 to 5 min increased the encapsulation efficiency of polyphenols (%E-cap).

The interactions of the factors (A) chitosan concentration, (B) TPP concentration, (C) Ch/TPP ratio and (D) pH are presented in Figure 3. It was observed that chitosan concentration (factor A) and pH (factor D) have negative effects. This behavior indicates that if the chitosan concentration (0.2–0.5%) and pH (from 3 to 5) are increased, the %EE decreases. From Figure 3, it was observed that decreasing the initial chitosan concentration (factor A) and increasing the chitosan/TPP ratio (factor C) yields better results in terms of the nanoencapsulation efficiency of artichoke polyphenols. A similar finding was reported by (Lamarra et al., 2016), who, using Gallic Acid (GA), reduced chitosan concentration (factor A) and pH (factor D) and found better yields at %EE conditions. This finding may be attributed to the fact that the pH of the TPP solution could influence the properties of the nanoencapsulation by changing the conformation of the polymer chain (Lino et al., 2021). Furthermore, Wong et al. (2020) found that the influence of pH on the TPP crosslinker solution showed that the nanoencapsulates prepared with serum had larger size and higher yield, which was different for the case studied with artichoke residue polyphenol extracts.

Determination and validation of optimum conditions

Table 4 presents the experimental and predicted values of the responses under optimum conditions, where it can be observed that there are no significant differences between the predicted and experimental values for %EE. Therefore, the suitability and validity of the model is established within the specified ranges of the process parameters. These values were close to the values obtained in the studies of gallic acid polyphenols %EE of 59–85% (Lamarra et al., 2016) and curcumin %EE of 62.36–72.99% (Asabuwa Ngwabebhoh et al., 2018).

Physical and physicochemical characterization of nanoencapsulation by ionic gelation

Tables 5 and 6 show the results of the physical and physicochemical variables of the optimization for the nanoencapsulation process of polyphenols by ionic gelation using the treatment with a higher %E-cap. From Tables 5 and 6, it can be observed that the ionic gelation method was successfully applied to obtain polyphenol-loaded nanocapsules using SEM and DLS methods, with an average diameter of 72.3 nm (Fig. 4)

Table 4. Predicted and experimental values of responses under optimal conditions

Optimal levels of process parameters	Optimized values (predicted values)	Experimental values
	%E-cap	%E-cap
A = 0.28%	71.05a	69.9% ±0.67a
B = 0.29%		
C = 5/1		
D = 4.9%		
E = 4.79 min		

%EE have been expressed as a mean of three determinations ±standard deviation (SD).

Table 5. Physical characterization of the nanocapsules

Physical characteristics	Results	
	mean (n = 3)	SD
Average particle size (SEM)	72.3 nm	±10.20
Average particle size (DLS)	460.7 nm	±0.012
Zeta (Z) potential	+15.73 mV	±2.54
Polydispersion index (IPD)	0.458	±0.078

SD – standard deviation.

Table 6. Physicochemical characterization of the nanocapsules

Physicochemical characteristics	Result	
	mean (n = 3)	SD
Radical scavenging activity (DPPH)	24.21 mM	±0.029
Trolox Equivalent Antioxidant Capacity (TEAC)	16.45 mM	±0.013

SD = standard deviation.

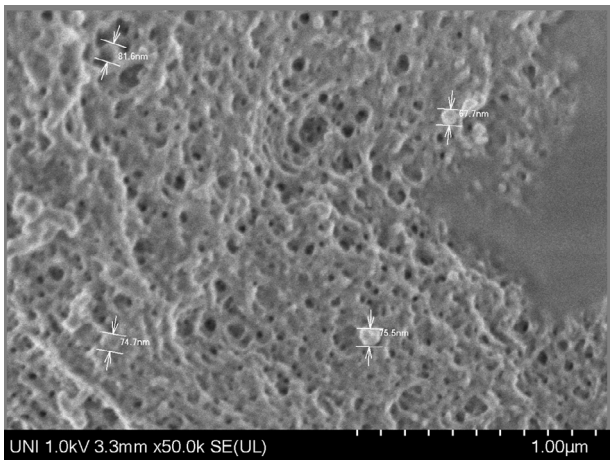

Fig. 4. Particle size distribution, shape, and morphology of the coated chitosan (Ch) visualized with scanning electron microscopy (SEM) taken from HITACHI equipment model SU8230

and 460.7 nm for each method, respectively. The difference in particle size between SEM and DLS lies in the sample treatment conditions; the former analyzes the dry sample and the latter the hydrated sample (Cuadros-Moreno et al., 2014). A similar size diameter was reported by (Lamarra et al., 2016). It was also reported that the ionic gelation method with mechanized agitation or ultrasound produced chitosan nanoparticles with different sizes (from 84 nm to 600 nm) and encapsulation efficiency from 23% to 97% (Singh et al., 2021). These results match our findings.

Nanoparticle properties, such as size, polydispersity index, and potential charge Z, are related to each other. These properties usually affect stability, biological properties, cellular uptake, and nanoparticle accumulation and distribution (Al-Nemrawi et al., 2018). However, Ch/TTP nanoparticles are usually polydisperse and have low stability, which limits their use. Thus, the size and distribution of nanoparticles depend on factors such as Ch/TPP ratio, Ch concentration, degree of deacetylation, molecular weight, ionic strength, and pH (Echeverri-Cuartas et al., 2020). These problems must be overcome before they move to the level of clinical trials and then become commercially available. (Jain et al., 2021) stated that the determination of the potential Z (PZ) is a measure of the thickness of the diffuse layer. Thus, the higher the value of PZ, the thicker the diffuse layer tends to be and, therefore, the more stable the diffuse layer becomes for suspension.

Low molecular weight surfactants and pure electrical stabilization, absolute PZ values above +30 mV indicate good suspension stability, and above +60 mV provide excellent stability. A Z potential of +15.73 mV indicates low stability. Furthermore, this author states that high molecular weight stabilizers with a Z potential of +20 mV or much lower values can provide sufficient stabilization. This behavior is because the adsorbed layers of the high molecular weight stabilizer shift the shear plane farther away from the particle surface and consequently lead to a decrease in the PZ value (Cano-Sarmiento et al., 2018). Chitosan is a compound that combines electrostatic stabilization due to its positive charge and steric stabilization due to its polymeric nature. In addition, the Z-potential of the nanoparticles was always positive, indicating the presence of chitosan amino groups on the surface (Lu et al., 2019). The antioxidant activity of the polyphenol extracts of artichoke bracts had an initial loading of 38.71 ±0.01 mM TE antioxidant capacity (DPPH) and 32.97 ±0.03mM TE antioxidant capacity Trolox (TEAC). After nanoencapsulation, this became 24.21 ±0.029 mM TE and 16.45 ±0.013 mM TE, respectively, with an efficiency of 69.9 ±0.67%. This result shows a good loading of antioxidant capacity in the nanocapsules coinciding with that reported by (Lopez-Polo et al., 2021) and reaffirming that chitosan maintains the antioxidant activity of polyphenols.

CONCLUSION

The 2^{5-1} experimental factorial design was successfully applied to optimize and study the individual and interactive effects of variables during the nanoencapsulation of polyphenols from artichoke residues by ionic gelation to encapsulate polyphenols efficiently (EE%).

A coefficient of determination (R^2) of 0.998 was obtained, suggesting the satisfactory fit of the developed models. The optimum values for polyphenol nanoencapsulation were chitosan concentration (0.28%), tripolyphosphate concentration (0.29%), 1/5 ratio for a cross-linking agent, a pH of 4.9, and a sonication time of 4.79 min. The %E-cap was 69.9%. The physical and physicochemical characterization was

successfully carried out, demonstrating the efficiency of the ionic gelation method to obtain polyphenol-loaded nanocapsules. The properties of the nanoparticles had an average diameter of 72.3 nm and 460.7 nm, which resulted in SEM and DLS methods, a polydispersity of 0.458, and a charge Z potential of +15.73 mV. Furthermore, the results showed a good loading of radical cutting activity (DPPH – 24.21 mM TE) and antioxidant equivalent to TROLOX (TEAC – 16.45 mM TE) in the nanocapsules while maintaining the antioxidant activity of polyphenols. Thus, these results indicate that nano encapsulates from artichoke (*Cynara scolymus* L.) residues are a promising potential source of phenolic compounds that can be used for the formulation of food and pharmaceutical products.

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