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Scientific Research

Microencapsulation of canthaxanthine with electrospray and optimizaing processing papameters toward efficiency encapsulation

Fatemeh Karimi[1], Mohammad Hossein Azizi[2*], Sohrab Moeini[3], Mohammad Karimi[4] and Roxana Moogouei[5]

1.Department of Food Science and Technology, Islamic Azad University North Tehran branch,Tehran, Iran
2.Department of food science and Technology, Tarbiat Modares university, Tehran, Iran
3.Department of Food Science and Technology, Islamic Azad University North Tehran branch,Tehran, Iran
4.Department of Textile Engineering, Amirkabir University of Technology, Tehran, Iran
5.Department of Enviromental planning, Management and Education, Islamic Azad University North Tehran Branch, Tehran, Iran

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*Corresponding Author E-
azizit_m@modares.ac.ir

This study showed the potential of the electrospinning (in this case electrospraying) technique to produce whey protein concentrate (WPC) micro and nanocapsules for applications in the encapsulation of canthaxanthin. The results showed that the solution concentration, feed solution flow rate and applied voltage had a direct effect on the encapsulation efficiency. by increasing of the solution concentration, feed solution flow rate and applied voltage, the encapsulation efficiency increased. The results showed that solution concentration was the most effective factor in electrospraying, because its scale estimate was highest. According to the results of analysis of variance (ANOVA) for this model, the regression model suggested a significant value for both linear and quadratic terms at $P < 0.05$. Also in order to obtain 0.93% encapsulation efficiency, optimum point was found at the emulsion concentration of 39.4%, feed solution flow rate of 12.2 ml/min, applied voltage of 17.5 kV, and 17.1cm distance between needle tip and collector.

1- Introduction

The role of food in the modern lifestyle has gone beyond providing basic and essential nutrients for the consumer, and in the field of preventing food-related diseases as well as improving the physical and mental health of the consumer, it is making extensive progress [1]. Today, carotenoids are produced as food coloring, food supplements, cosmetics, and for the purpose of human health [2]. Among the carotenoids, we can mention beta-carotene, alpha-carotene, canthaxanthin, astaxanthin, lycopene, etc. [3]. Canthaxanthin is used in some countries today as a permitted additive in all kinds of sauces, soups, sweets, soft drinks, ice cream, dairy products, meat products, and seafood products [4, 5] The development of micro or nano encapsulation is one of the solutions that biological compounds It protects the active against unfavorable environmental conditions. In a research, corn protein was used as a wall material for beta-carotene microcoating by electrospray method. In this way, after preparing a solution containing beta-carotene and corn protein (zein), a very strong magnetic stirrer was used in an alcoholic environment to produce microcapsules. Microscopic investigations showed the production of microcapsules containing beta-carotene by this method, and the results of the experiments also showed the stability of these microcapsules against light. [6] Takarotinon et al. (2009) used ethyl cellulose, PB4 (polymethoxyvinyl 4-cinnamate (PCPLC) polymethoxyamyl phthalyl chitosan) as wall material and freeze drying method. They observed that ethyl cellulose was unable to form capsules and PB4 had a very low efficiency while the microcapsules prepared with PCLC had good solubility in water and had good thermal stability so that after two hours exposure at 70 degrees Celsius no reduction of astaxanthin was observed in the microcapsules prepared with this coating [7]. Microcoating using electrospray is one of the new methods for microcoating food ingredients. The purpose of this research is to provide a model for the efficiency of canthaxanthin electrospray based on the effective variables in the efficiency of canthaxanthin microcoating using the response surface method and to provide the optimal points of independent variables in canthaxanthin microcoating. In order to optimize The efficiency of canthaxanthin electrospray is based on utility functions.

2- Materials and methods

In this study, whey protein from Pentair Leben company with the characteristics of protein 80%, lactose 9%, lipid 8% and water 2.8% and canta xanthine from Merck company were used.

The electrospray machine used in this study was made by the textile faculty of Amirkabir University of Technology. In order to produce canthaxanthin nanocapsules by electrospray method, the emulsion prepared in a 9cc syringe with a 69-gauge needle (inner diameter 0.90 mm) was poured. The syringe was placed horizontally on the feeding pump, and the positive pole of the machine was connected to the syringe containing the solution and the negative pole was connected to the collecting plate, which was an aluminum foil plate (with dimensions of 90 x 90 cm). A diagram of the electrospray device is shown in Figure 1.

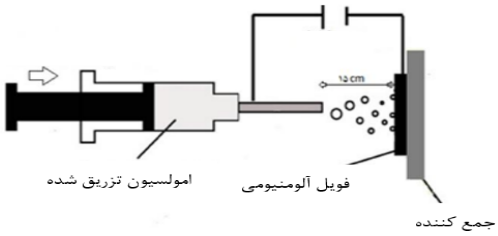

Fig 1. Electrospray machinery

2-1-preparation of canthaxanthin emulsion

Canthaxanthin emulsion and water-soluble coating materials are an oil-in-water emulsion in which the canthaxanthin pigment forms its oily phase and the protein used as a wall to cover the pigment forms its aqueous phase. The preparation of this emulsion includes three separate stages of each phase and

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mixing them with an ultrasonic device. At first, whey protein samples were prepared in concentrations (20%, 25%, 30%, 35% and 40%) in distilled water, then the canthaxanthin solution was prepared by dissolving 1.25% w/v in corn oil. Tween-20 surfactant solution was used to prepare the emulsion and the emulsion was completely homogenized with the high pressure laboratory homogenizer of Albertslund company, made in Denmark.

2-2-Microencapsulation Efficiency:

By measuring the amount of total carotenoid and surface carotenoid and using the following equation, the efficiency (ME) of microcovering is calculated:

$$ME\% = \frac{TC - SC}{TC} \times 100 \qquad \text{Equation 1}$$

Microcoating efficiency: ME
TC: total carotenoid content
SC: surface carotenoid content
To calculate the amount of total carotenoid, 50 mg of finely coated canthaxanthin is dissolved in 25 ml of distilled water, then 25 ml of hexane is added, and its absorbance is measured using a spectrophotometer at a wavelength of 476 nm. We get [9,8]. bTo calculate the amount of carotenoid on the surface of the particles, we dissolve 50 ml of the prepared powder with 25 ml of hexane, and then we read its absorbance at the wavelength of 476 nm [10,9].

In order to statistically analyze the effect of test variables on microcoated canthaxanthin by electrospray method, the response surface method was used in this research. By using the response level method, it is also possible to study the relationships between test and response variables (dependent variable). The independent variables in the electrospray method are the concentration of the resin, the applied voltage, the input rate and the working distance. The desired answer is the efficiency of electrospraying. Based on the central composite design, the levels of the independent variables are selected based on the coded values of 0, ±1, and ±α. α is equal to the square of the number of independent variables.

The equation of the model that will be obtained from the implemented plan for the desired answer is in the form of equation 2:

$$y = a_0 + \sum_{i=1}^{4} a_i x_i + \sum_{i=1}^{4}\sum_{j=i}^{4} a_{ij} \qquad \text{Equation 2}$$

where y, predicted response (electrospray efficiency), x_i and x_j Coded independent variables and a_0، a_i and a_{ij} are the coefficients of the model.
Data analysis was done using SAS9.1 software and based on the plan of response level method and utility functions[11].

3-Results and discussion

3-1- Canthaxanthin microcapsule modeling

The design used in this study is the central composite design. The central compound design is obtained by using the central points and several central points of the factorial design.
As can be seen in Table 1, the four independent variables of this design include whey protein concentration (C), applied voltage (V), input rate (F) and the distance between the syringe head and the collector (D). This table shows the coded levels and the actual (uncoded) values of the test points for these four independent variables. The variables are coded in five ranges +2, +1, 0, -1 and -2. Tests have been conducted at 30 points. The electrospray efficiency of cantha-xanthine in whey protein is considered as the answer.

Table. 1 Experimental design of independent variables in the central composite design for canthaxantine capsule

Independent variable		Range and level				
Symbol		-2	-1	0	+1	+2
Voltage	X1	9	12	15	18	21
Distance between needle tip and collector	X2	7	10	13	16	19
Feed rate	X3	0.5	1	1.5	2	2.5
Concentration of whey protein	X4	20	25	30	35	40

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After the regression coefficients were obtained and the electrospray efficiency was modeled based on the test variables, the studied response (electrospray efficiency) was estimated using the model equation for the test points (Table 2), usually the behavior of the system is unknown; As a result, it should be evaluated whether the model covers the experimental data well or not. Due to the need to analyze and validate the obtained data and model statistically, variance analysis of the first and second order expressions and the mutual effects of the regression model were performed. Then the variance analysis of the coefficients of the model terms and the explanatory coefficient (R^2) model was also obtained. To ensure the proper fit of the curvature of the model, the lack of fit test is done in the form of variance analysis table using the software. SAS9.1 done

Table. 2 Responses of dependent variables for efficiency encapsulation to changes in the independent variables. X1: concentration of whey protein (wt.%), X2: feed rate (mLh^{-1}), X3: voltage (kV), X4: distance between needle tip and collector (cm).

Exp. No	Variable levels				Efficiency y	Efficienc
	X1	X2	X3	X4		
1	-1	-1	-1	-1	32	32
2	-1	-1	-1	+1	61	58
3	-1	-1	+1	-1	30	29
4	-1	-1	+1	+1	61	66
5	-1	+1	-1	-1	30	29
6	-1	+1	-1	+1	59	55
7	-1	+1	+1	-1	30	36
8	-1	+1	+1	+1	73	73
9	+1	-1	-1	-1	37	40
10	+1	-1	-1	+1	65	66
11	+1	-1	+1	-1	37	37
12	+1	-1	+1	+1	74	74
13	+1	+1	-1	-1	32	36
14	+1	+1	-1	+1	63	62
15	+1	+1	+1	-1	40	44
16	+1	+1	+1	+1	82	80
17	-2	0	0	0	45	44
18	+2	0	0	0	60	59
19	0	-2	0	0	55	57
20	0	+2	0	0	57	61
21	0	0	-2	0	41	42
22	0	0	+2	0	60	57
23	0	0	0	-2	20	13
24	0	0	0	+2	70	75
25	0	0	0	0	66	59
26	0	0	0	0	61	59
27	0	0	0	0	60	59
28	0	0	0	0	58	59
29	0	0	0	0	60	59
30	0	0	0	0	63	59

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The results of variance analysis of canthaxanthin electrospray efficiency model with whey protein ester are presented in Table 3. This table shows general models and predictions for whey protein electrospray.

Table. 3 Analysis of variance (ANOVA) for response of the dependent variable

Source	Predicted model			Total model		
	d.f	F	p-value	d.f	F	p-value
regression	8	43.37	0.0001	14	33.37	0.0001
Linear				4	104.22	0.0001
Squar				4	7.57	0.0027
interaction				6	3.34	0.0355
Residual error	18			12		
Lac-of-fit	16	2.13	0.36	10	1.63	0.4377
Pure error	2			2		
Total	26			26		

The F values in variance analysis of the general and predicted models were 33.38 and 43.37, respectively. The p-value for the models was less than 0.05, which shows that these models are statistically capable of predicting the effects of independent variables on electrospray efficiency. After confirming the validity of the model, it is necessary to measure each expression of the equation. Since the p-value for all expressions (first order, second order and interaction effect) related to the studied equation was less than 0.05, it can be concluded that all three types of expressions (first order, second order and interaction effect) were statistically valid for entering the equation so that this equation has the ability to predict the second order effects and the interaction effects of independent variables on the efficiency of canthaxanthin electrospray. Because the response surface method is only capable of providing first and second order models and cannot provide other models other than these two models for predicting the dependent variable, the lack of fit test is also needed to ensure the appropriateness of the model in the curves. As it is clear in Table 4, the p-value for the test of lack of fit was determined to be 0.36, which indicates the rejection of the hypothesis of lack of proper fit of the equation. In other words, the quadratic equation provided by the response surface method can well predict effects other than the first order on the efficiency of canthaxanthin electrospray with whey protein ester.. Models and expressions whose P value is less than 0.05 can statistically predict the data with an error of less than 5%. In this study, beta-carotene was microcoated by gum arabic and the results showed that the linear, quadratic and interaction coefficients in the equation were significant and the mathematical model was obtained with acceptable confidence coefficients. In this study, the ANOVA table shows that the lack of fit test $P \geq 0.05$ was not significant at the 95% level and the expected error of the model is also predicted at the 7.2% level [12].

2-3-Evaluation of the efficiency model of Xanthine electrospray

After confirming the models, the response surface method examines the variance analysis of all model terms. The results of the variance analysis of the expressions of the equation related to canthaxanthin electrospray using whey protein ester are presented in Table 4. In the studied equation, the p-value for all the first and second order terms, except for the second order factor of thickness and also the term D×F, was less than 0.05. In other words, it can be said that the effects of these expressions on the efficiency of canthaxanthin electrospray with whey protein ester were significant. However, the effects of the rest of the interaction terms on electrospray efficiency were not significant. Expressions that did not have a significant effect on performance were not included in the prediction model. R values[2] and R^2 Adjusted for the prediction model was 95.33 and 92.86 percent respectively. The equation below provides the prediction model of canthaxanthin electrospray efficiency with whey protein ester based on the actual (encoded) numbers of the test variables.

$$If = -1.98 + 3.75 \times V - 0.91 \times D - 3.83 \times F + 1.66 \times C - 1.95\, V^2 + 2.5\, D \times F - 2.45 \times F^2 - 3.83 \times C^2$$

Equation 3

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Ef is the efficiency of canthaxanthin electrospray (%), V is the applied voltage (kV), D is the distance between the syringe tip and the collector (cm), F is the input rate (mL/h) and C is the whey protein concentration (%). The positive sign of the coefficients shows that the corresponding term had a positive effect on the electrospray efficiency, and the negative sign indicates an inverse effect on the canthaxanthin electrospray efficiency.

Table. 4 Estimated coefficients of the fitted quadratic polynomial equation for the response of efficiency encapsulation based on t-statistic. X1: Concentration of whey protein (wt.%), X2: feed rate (mLh^{-1}), X3: voltage (kV), and X4: distance between needle tip and collector (cm).

parameter	Coefficient	SS	p-value	F
V	3.75	337.5	0.0005	21.31
D	0.916	20.16	0.0028	1.27
F	3.833	352.66	0.0004	22.27
C	1.666	5890.88	0.0001	372.04
V^2	-1.958	133.33	0.0132	8.42
D^2		56.33	0.0863	3.55
F^2	2.45	192	0.0045	12.12
C^2	3.833	408.33	0.0002	25.87
V×D		0.25	0.92	0.015
V×F		56.65	0.083	3.55
V×C		9	0.46	0.56
D×F	2.5	100	0.027	6.31
D×C		42.25	0.128	2.66
F×C		110.25	0. 216	6.96

High value of R^2 It shows that there is a high correlation between the observed and predicted values of canthaxanthin electrospray efficiency with whey protein. It also shows that the presented model can describe and predict 86.92% of the total changes in the electrospray efficiency of canthaxanthin with whey protein ester in the tested conditions.. In the study They microencapsulated β-carotene by gum arabic and obtained a quadratic model of independent variables, which showed that when the concentration of the wall (gum arabic) increases and the time decreases, an increase in the efficiency of carotenoid microencapsulation is seen [12]. Also, the microcoating efficiency of beta-carten was 32.5%, and this difference can be due to the better performance of electrospraying compared to spray drying, due to the absence of heat and as a result of not damaging the remaining carotenoids. In another study, they achieved 25.4% carotenoid yield [13]. Also, the optimization of astaxanthin microencapsulation using spray drying was studied by Shishir et al. and the results showed that the efficiency of carotenoid microencapsulation ranged from 48.73 to 83.65% [14]

Fig 2. a) the contour plots for the effect of whey protein concentration and the distance between needle tip and collector electrospray efficiency of canthaxanthin , b) the effect of voltage and the distance between needle tip and collector electrospray efficiency of canthaxanthin by whey protein macromolecule, and c) the effect of changing whey protein concentration and the feed rate on the electrospray efficiency of canthaxanthin by whey protein macromolecule

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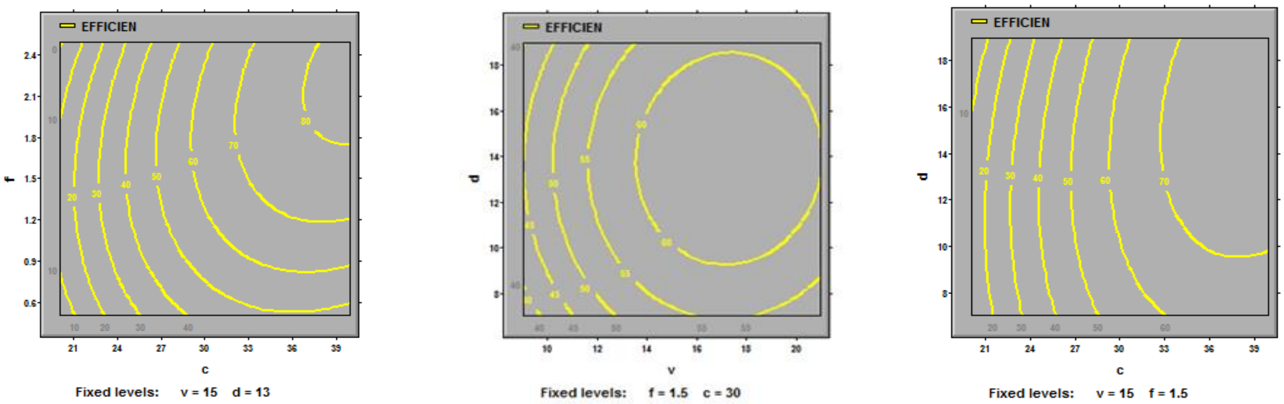

Parallel response plots are a two-dimensional graphical image that can depict the specific and cumulative effects of the variable as well as the interaction of the independent variables on the dependent variable. The response plot shows the geometric properties of the surface using parallel lines. [15].

The parallel plots for the model of electrospray efficiency of canthaxanthin with whey protein ester are shown in Figure 1. In the conditions of constant voltage and input rate, at the lowest concentration of whey protein, the possibility of microcoating of canthaxanthin pigment decreases, and when the distance between the nozzle and the collector increases, the effect of the electric field strength decreases and the possibility of spraying decreases, therefore, the efficiency of electrospray decreases. In fact, in very low concentrations, the application of voltage leads to the formation of grains due to instability. In fact, in low concentrations, entanglement of polymer chains is not enough. In fact, in high concentrations, the efficiency of electrospraying increases due to the lower tension of the solution [16]. As can be seen in the figure, in the condition of whey protein concentration and constant input rate at the coded zero point, with increasing applied voltage, there was an increasing trend in the efficiency of canthaxanthin electrospray, but at values higher than the optimal value of applied voltage, the efficiency of canthaxanthin electrospray decreased. In fact, for the capsule to form, we need time for the solution to separate from the nozzle head. At high voltages, the solution is not given the opportunity to separate from the nozzle head in the form of smaller particles, so it causes coarse particles to form, in other words, larger microcoatings, which reduces the efficiency of electrospraying at higher than optimal voltages [17].

3-3 Optimizing the microcoating process

Optimization is one of the most important steps in experiment design and analysis. In this research, utility functions have been used to optimize (independent) test variables in order to achieve the maximum point of the dependent variable (electrospray efficiency of canthaxanthin with whey protein ester).

Table5. Prediction profile for the trends in efficiency encapsulation at the maximum desirability

Desirability	Feed rate	Voltage applied	Distance	Concentration
93%	12/2	17/5	17/1	39/4

As can be seen in Table 5, the most favorable value was determined with a value of 0.93 in whey protein concentration 39.4, voltage 17.5, input rate 12.2, nozzle and collector distance 17.1. Based on the results of utility functions, at this optimal point, the electrospraying process of canthaxanthin with whey protein experiences the highest spraying efficiency.

4- General conclusion

Today, carotenoids are produced as food coloring, food supplements, cosmetics and for human health. Since carotenoids are unsaturated and unstable compounds, microencapsulation is one of the solutions that protect bioactive compounds against unfavorable environmental conditions. Electrospray is one of the new methods for microcoating of food ingredients. In this study, a model for the efficiency of canthaxanthin electrospray based on the effective variables (concentration of canthaxanthin, input rate, applied voltage and nozzle to collector) in the efficiency of canthaxanthin microcoating using response surface method was presented. The p-value for the models was less than 0.05, which shows that these models are statistically capable of predicting the effects of independent variables on electrospray efficiency. The presented model predicts the efficiency of micro-covering with an explanation coefficient of 95.33%. The most favorable value was determined with a value of 0.93 in whey protein concentration 39.4, voltage 17.5, input rate 12.2, nozzle and collector distance 17.17.

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بهینه سازی فرایند ریزپوشانی کانتا گزانتین به روش الکترواسپری

فاطمه کریمی[۱]، محمدحسین عزیزی[۲*]، سهراب معینی[۳]، محمد کریمی[٤]، رکسانا موگویی[٥]

۱-دانش آموخته دکتری، گروه علوم و صنایع غذایی، دانشکده علوم زیستی، واحد تهران شمال، دانشگاه آزاد اسلامی، تهران، ایران

۲-عضوهیات علمی گروه علوم و صنایع غذایی دانشکده کشاورزی دانشگاه تربیت مدرس، تهران، ایران

۳-عضو هیات علمی گروه علوم و صنایع غذایی دانشکده علوم و فنون دریایی دانشگاه آزاد تهران شمال، تهران، ایران

٤-عضوهیات علمی گروه نساجی دانشکده نساجی، دانشگاه امیرکبیر، تهران، ایران

٥-عضو هیات علمی گروه مدیریت، برنامه ریزی و آموزش محیط زیست، دانشکده علوم و فنون دریایی، دانشگاه آزاد اسلامی واحد تهران شمال، تهران، ایران

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* مسئول مکاتبات:

azizit_m@modares.ac.ir

چکیده

کانتاگزانتین مولکول کتوکاروتنوئید غیراشباع بوده که نسبت به عوامل محیطی حساس است. توسعه میکرو-نانو کپسولاسیون یکی از راهکارهایی است که ترکیبات زیست فعال را در برابر شرایط نامناسب محیطی محافظت می‌کند. در این مطالعه کانتاگزانتین با استفاده از پلیمر پروتئین آب پنیر با روش الکترواسپری ریزپوشانی شدند و با استفاده روش سطح پاسخ تاثیر چهار متغیر مستقل غلظت پروتئین آب پنیر، ولتاژ به کار رفته، نرخ ورودی و فاصله سر سرنگ و جمع کننده بر روی کارایی الکترواسپری کانتاگزانتین ارزیابی شد. بر اساس نتایج به دست آمده توابع مطلوبیت بیشترین مطلوبیت با مقدار ۰/۹۳ در غلظت پروتئین آب پنیر ۳۹/٤%، ولتاژ kV۱۷/٥، نرخ ورودی mL/h۲/۲ ، فاصله سرسرنگ و جمع کننده ۱۷/۱cm تعیین شد.