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The industrial by-product of chili paste: optimized carotenoids extraction

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ABSTRACT/RESUME

Abstract: *Chili paste is a condiment that takes a typical place in our diet due to its innumerable culinary, nutritional and medicinal properties, but during its elaboration process, a considerable quantity of industrial by-product is rejected. This investigation aims to optimize the extraction solvent of carotenoids from the industrial by-product discarded during the production of chili paste (Harissa). In the first step, extraction kinetics using 15 solvents with variable polarities (5 alcohols, 4 polar aprotics, 4 hydrocarbons, chloroform, and water) were studied. In the first step, a mixture design following the simplex lattice type was applied so as to search the optimal combination of the three best previously selected solvents. The results showed that the three most efficient solvents for the recovery of carotenoids were respectively acetone, hexane and petroleum ether. The simplex lattice design applied for three best solvents indicated that the established model was accurate (R^2 = 0.89 with $p<0.0001$). The validation of the developed model manifests close results between the estimated optimal value and the experimental ones, which indicated its validation. The best combination of solvents was 60% acetone/40% petroleum ether/0% hexane. The obtained solvents mixture permits the amelioration of carotenoids extraction (42 mg kg^{-1} DM) from the by-product of chili paste.*

I. Introduction

Capsicum sp. (annuum, chinense, frutescens) a member of family Solanaceae is a major vegetable cum spice crop cultivated in tropical and subtropical regions of the world [1,2]. The world production of chili is estimated at 41 million tons in 2017 [3]. Chili is widely used for culinary and industrial purposes due to its characteristic flavor, taste, and color [4,5]. This vegetable is immensely valuable not only for its nutritional value but also for its efficient health functionality against various degenerative diseases caused by free radicals, that is due to its characterization by the presence of numerous carotenoids including β-carotene, α-carotene, β-cryptoxanthin, zeaxanthin, lutein, capsanthin, capsorubin, and cryptocapsin, which are the most abundant components. Chili contains many vitamins such as vitamins C, A, and E, and represents a potential source of trace minerals and phenolic compounds [6,7].

The paste of red chili is industrially processed from raw material, which continues to increase due to the increasing demand and consumer tastes for various chili products. The chili puree is composed by water (88%), total solids (11.82%), total soluble solids

(10°Brix), ash and fat (0.9%), protein (1%), titratable acidity (0.6%) with pH of 5, total sugars (4.9%), reducing sugars (3.7%), non reducing sugars (1.1%), fiber (1.6%), ascorbic acid (0.2%), carotenoids (0.74%) as well as other bioactive compounds such as phenolic compounds and capsaicinoids [8].

During the elaboration of chili paste, very considerable losses of up to 20% are discarded. The generated by-product consists of the various teguments–and seeds that remain after sieving the crushed chili. There is a great interest concerning the extracting of bioactive compounds, such as carotenoids from different natural resources, due to the high potential of these substances as functional ingredients in food formulations and nutritional supplements [9]. Therefore, we propose in this work the optimization of the extraction of carotenoids from the industrial by-product of chili paste (locally named "*Harissa*").

In this purpose, this study carried out the optimization of carotenoids extraction from the industrial by-product of chili paste. Firstly, by a screening of fifteen solvents with different polarities and, secondly, by the application of mixture design (simplex lattice) using the selected best solvents.

II. Materials and methods

II.1. Sample and sample preparation

This work performed on the industrial by-product rejected during the elaboration of red pepper concentrate "*Harissa*". The sample was obtained from the El-Kseur unit of CEVITAL Company in 2018. For the preparation of the by-product for the extraction of carotenoids, the sample was spread in a thin layer and then dried in an oven under a temperature of 40°C for 24 hours. The dried material was subjected to grinding using an electric grinder and then sieving through a sieve with a porosity of 500µm. The powder obtained was stored in a smoked bottle protected from light.

II.2. Optimization of carotenoids extraction

The extraction of carotenoids from the industrial by-product is carried out by maceration using a magnetic stir plate. The maceration is carried out using the ratio sample/solvent of 1/100 and to prevent the oxidation of carotenoids, the extraction is done in hermetically sealed bottles, to avoid oxygen, and coated with aluminum foil, in order to avoid light. The manipulations are carried out at an ambient temperature of approximately 25°C.

As a first step, the kinetics of carotenoids extraction were carried out using 15 solvents of different polarities. Then, from the three best solvents obtained, a mixing design is applied in order to find the best combination of solvents that maximizes the extraction of carotenoids.

II.2.1. Optimization of solvent type and time

In order to test the effect of the type of solvent on the extraction of carotenoids from the industrial by-product, solvents of different polarities were used (Tab. 1). The extraction was carried out from 1 g of sample powder per 100 ml of the various solvents for variable times (0, 2, 5, 10, 20 and 30 min). The carotenoids extract was obtained after centrifugation at 5000 rpm for 10 min.

Table 1. *Solvents used for the extraction of carotenoids*

N°	Solvent	Polarity	Solvent type
1	Water	10	Polar protic
2	Ethanol	5.2	Alcohols
3	Methanol	5.1	
4	1-Butanol	4	
5	1-Propanol	4	
6	n-Pentanol	2.2	
7	Dimethyl sulfoxide	7.2	Aprotic polar
8	Dimethylformamide	6.4	
9	Acetonitrile	5.8	
10	Acetone	5.1	
11	Chloroform	4.1	Organochloride
12	Toluene	2.4	Aprotc Apolar (Hydrocarbons)
13	Petroleum ether	0.1	
14	Heptane	0.1	
15	Hexane	0.1	

II.2.2. Optimization by mixture design

After studying the kinetics of carotenoids extraction for the fifteen solvents, the three most effective solvents (acetone, hexane and petroleum ether) were selected to study the effect of the mixture. For this purpose, a mixture design according to simplex lattice type was applied by using different combinations of the three levels (1, 1/3, 2/3 and 3/3) of the chosen solvents. For the mixture design in question, the composition points were regularly distributed in the studied triangle area [10].

The ten different solvent combinations were determined by the JMP software and indicated in Tab 2.

Hundred milliliters of each mixture were used for the extraction of carotenoids from 1 g of industrial by-product powder with magnetic stirring for a period of 10 min (optimal extraction time determined in the first step). After centrifugation, the carotenoid contents were determined according to the procedure indicated in the next section.

The desired response (carotenoid contents) was expressed according to the following Eq. (1):

$$y = b_1x_1 + b_2x_2 + b_3x_3 + b_{12}x_1x_2 + b_{13}x_1x_3 + b_{23}x_2x_3 \quad \text{Eq. (1)}$$

where y is the carotenoid content, b_1, b_2 and b_3 are the linear terms of the equation, b_{12}, b_{13} and b_{23} are the interaction terms of the equation, x_1, x_2 and x_3 are the factors (acetone, hexane, and petroleum ether, respectively).

II.3. Determination of carotenoids

For the determination of carotenoids, the supernatant obtained after centrifugation was recovered and the absorbance was measured at the maximal wavelength [11]. First, a spectral scan between 350 and 650nm is performed to determine the maximum absorption wavelength of carotenoids is performed on the extract of the chili by-product. The maximum wavelength obtained was 450nm. Then, to ensure that other compounds do not interfere with carotenoids, an examination of the extract is done by the Method of Standard Addition. This test consists of adding different concentrations of the standard (β-carotene) to the extract and plotting the absorbance as a function of the concentration to gives us a straight line that allows the determination of carotenoids concentration. The method of standard addition is a quantitative analysis technique used to minimize matrix effects that interfere with analyte measurement signals. The results indicate that interferents during the determination of carotenoids were negligible. The carotenoid concentrations were obtained according to the calibration curve plotted by different concentrations of β-carotene (y = 1882.7x + 0.0023, R^2=0.998) and results were expressed in milligram equivalent of β-carotene per kilogram of dry matter (mg Eβ-C kg^{-1} DM).

II.4. Statistical analysis

The results represent the average of the values of three replicates ± standard deviation with the exception of the mixture design where a single extraction was sufficient. Variance analysis (LSD test of Statistica 5.5 software) was applied to detect differences between means. JMP software was used for mixture design study. Microsoft Excel was used for calculating the means, standard deviations, and realization of graphs.

III. Results and discussion

Carotenoids represent a class of pigments responsible for various colors of fruits and vegetables [12,13]. Many parameters were important for extraction such as the solvent and the extraction time. The appropriate choice of these parameters was the one that allows the best extraction of a compound of interest in the shortest time possible.

The purpose of the drying step was to eliminate the main portion of water and to reduce the weight, volume and activity of the microorganisms and to allow better preservation of the sample. The influence of solvents and time on the extraction rate was studied by carrying out extractions with the fifteen solvents at six different times (0, 2, 5, 10, 20 and 30 min) at ambient temperature and with stirring. After having chosen the three best solvents, a mixing plan was established in order to have an optimal mixture for the extraction of these bioactive compounds. In order to maximize the recovery of carotenoids from the industrial by-product, a series of extraction cycles according to the ratio was also studied.

III.1. Optimization of solvent type and time

The carotenoids can be subdivided into two groups according to the chemical structure, the carotenes (apolar) which were formed by carbon and hydrogen (i.e., lycopene and β-carotene) and xanthophylls (less apolar) which presented in their structure oxygen in addition to carbon and hydrogen (i.e., lutein and zeaxanthin) [14]. Therefore, the choice of solvent and time for extraction were important parameters that have significant influences on the extraction efficiency of carotenoids.

The results of the kinetics of the extraction of these bioactive compounds were grouped in Fig. 1. The extraction of carotenoids by water was weak; it started with a content of 6.20 mg kg^{-1} DM and then progressed slowly to reach a rate of 9.88 mg kg^{-1} DM at 20 min (Fig. 1A). This was due to the low solvating power of water (very polar solvent) for carotenoids which were apolar molecules. The extraction of the carotenoids using chloroform (organochlorine solvent) began with the recovery of 15.13 mg kg^{-1} DM then increased rapidly during the first 5 min to stabilize at the value of 20.29 mg kg^{-1} DM.

The extraction of carotenoids by alcohols started with an average rate of 10.8 mg kg^{-1} (Fig. 1B). This amount represented the molecules that were extracted directly by the first contact of the solvent with the by-product powder. During the extraction, the accumulation of carotenoids in the solvent increased in the first phase of extraction (0-10 min) then the extraction decelerated to reach a tray where the majority of the carotenoids were extracted after around 10 min. The maximum levels were reached at 10 min for methanol and pentanol, at 20 min for ethanol and butanol and at 30 min for propanol. The extraction efficiency of carotenoids by alcohols varied according to the following decreasing order: Methanol> 1-Butanol = 1-Propanol> Ethanol> n-Pentanol.

Fig. 1C summarized the results of polar aprotic solvents extraction as a function of time. Acetone exhibits a strong ability for carotenoids extraction of the industrial by-product powder. As soon as the solvent was brought into contact with the sample, the majority of compounds (80%) were extracted. Subsequently, the extraction continues linear progression to stabilize at 10 min to reach 37.5 mg kg^{-1} DM. However, the other three solvents were relatively less effective with an initial concentration of only 6.3 mg kg^{-1} DM. The two solvents

acetonitrile and dimethylformamide follow the same extraction pattern and stabilized after 20 min at a concentration of 19 mg kg^{-1} DM while dimethyl sulfoxide was the least effective polar aprotic solvent and has a maximum extraction of only 10, 23 mg kg^{-1} DM.

The analysis of 25 chili genotypes by Dubey et al. [5] showed that the carotenoid contents considerably varied between genotypes ranging from 0.9 to 77.2 mg kg^{-1} DM. A content of 49.8 mg kg^{-1} DM of carotenoids was obtained from the chili pods [15].

The results of the extraction of carotenoids by the four hydrocarbon solvents were showed in Fig. 1D.

follows: petroleum ether> hexane> toluene> heptane.

Statistical analysis showed that all solvent types used in this study have significant differences and a variable carotenoids extraction powers; they were classified as follows: Hydrocarbons> Organochlorine> Alcohols> Polar aprotic (in the exception of acetone)> water. According to the statistical analysis, the extraction power varies greatly between the solvents tested, which were given in the following order: Acetone> Petroleum ether> n-Hexane> Methanol> Toluene = Chloroform> Butanol= Heptane= Propanol> Ethanol> n-Pentanol> Dimethyl-formamide= Acetonitrile> Dimethyl sulfoxide> Water.

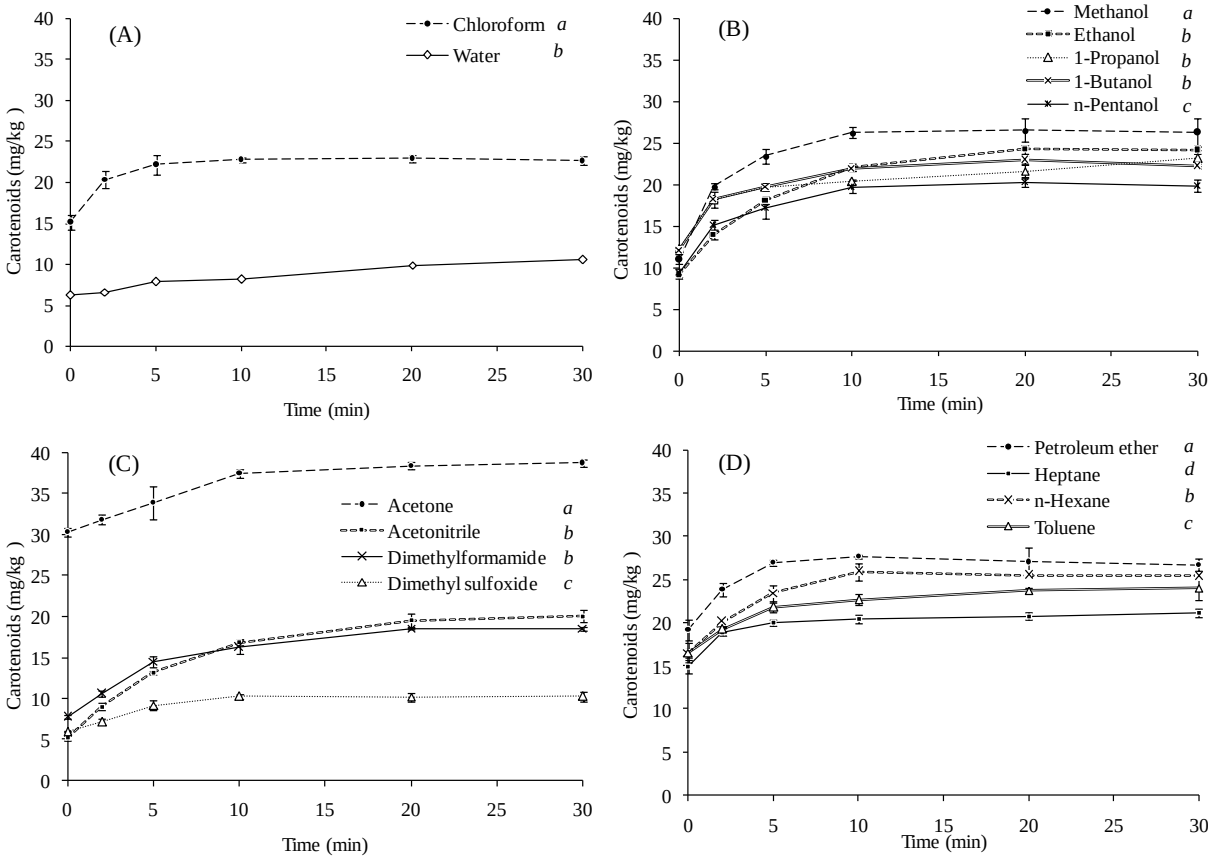

Figure 1. *Kinetics of carotenoids extraction by different solvents, (A) chloroform and water, (B) alcohols, (C) polar aprotics, and (D) hydrocarbons. For each group of solvents, different letters are statistically different at $p<0.05$ (ANOVA-LSD test)*

The initial carotenoid content obtained by this type of solvent was evaluated at 17.60 mg kg^{-1} DM and which increases to stabilize at 5 min for petroleum ether and heptane, 10 min for hexane and toluene to reach the values of 27.02, 20.02, 25.92 and 22.69 mg kg^{-1} DM, respectively. The statistical analysis showed that the four solvents were significantly different extraction powers and were classified as

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III.2. Optimization by mixture design

The results obtained with various solvent combinations and the predicted ones for carotenoid contents of the analyzed by-product were summarized in Tab. 2. The obtained concentrations were ranged from 32.62 to 41.26 mg kg^{-1}. It was established that experimental and calculated values were close and this was supported by the high value of the determination coefficient that was 0.89.

Table 2. *Simplex lattice mixture design for combination of solvents and experimental and predicted values of carotenoids extracted from industrial by-product*

N°	Acetone (x_1)	Petroleum ether (x_2)	Hexane (x_3)	Carotenoids (mg kg^{-1} DM)	
				Measured	Predicted
1	0.67	0.33	0	41.26	41.66
2	0.33	0	0.67	36.81	38.61
3	0.67	0	0.33	40.86	39.82
4	0	0	1	35.65	35.15
5	1	0	0	38.45	38.68
6	0	0.33	0.67	34.12	33.80
7	0	0.67	0.33	32.62	33.69
8	0.33	0.67	0	39.98	40.34
9	0	1	0	35.31	34.83
10	0.33	0.33	0.33	40.01	38.47

III.2. Adjustment of the model

Tab. 3 presented the variance analysis of the carotenoids extraction model. The Fisher value of the model abstained was 1071.59 corresponding to a probability less than 0.0001 indicating that the model adjustment was very highly significant (p<0.001).

Table 3. *Variance analysis of the model for carotenoid extraction from industrial by-product*

Source	Degrees of freedom	Sum of squares	Mean Square	F Ratio
Model	6	14141.67	2356.94	1071.59
Error	4	8.80	2.20	**Prob > F**
Total	10	14150.46		<0.0001*

** indicates a significant value.*

III.3. Effect of factors

The effects of the three solvents (acetone, petroleum ether, and hexane) and their interactions on the response were shown in Tab. 4. The larger the difference between the value of the coefficient (term) and the standard error, the greater the factor was considered powerful, and this can be expressed by the *t*-ratio. The value of the probability indicates the significance of the parameter, it was considered statistically significant when it was less than 0.05. Tab. 4 showed that the three solvents have very significant effects on the extraction of carotenoids as well as a significant interaction between acetone and

petroleum ether. However, the interaction terms between acetone-hexane and petroleum ether-hexane have no effects on the extraction of carotenoids.

Table 4. *Regression coefficient, standard error, and Student's t-test results of mixture design for carotenoids extraction from industrial by-product*

Term	Estimate	Std Error	*t* Ratio	Prob>|*t*|
Acetone	38.68	1.39	27.75	<0.0001*
Petroleum ether	34.83	1.39	24.99	<0.0001*
Hexane	35.15	1.39	25.22	<0.0001*
Acetone*Petroleum ether	19.20	6.22	3.09	0.0367*
Acetone*Hexane	10.40	6.22	1.67	0.1698
Petroleum ether*Hexane	-5.61	6.22	-0.90	0.4179

** indicates a significant value.*

The mathematical model of the mixture design for carotenoids extraction can be presented as the first-order polynomial. This model takes into account the linear and interaction effects of factors. The mathematical model of response by considering the terms of significant influences can be presented as following Eq. (2):

$$Y = 38.68x_1 + 34.83x_2 + 35.15x_3 + 19.20x_1x_2$$ Eq. (2)

where Y is the response (carotenoid contents), x_1 is acetone, x_2 is petroleum ether, and x_3 is hexane.

III.3. Experimental determination and validation of optimal extraction conditions

The mixture required to maximize the extraction of carotenoids from the industrial by-product was determined using the prediction profiler module (JMP software) that was estimated by acetone and petroleum ether ratio of 0.6/0.4 (60/40%, v/v). This mixture predicted a maximum carotenoids value of 41.75 mg kg^{-1} DM.

In order to confirm the optimal theoretical values, experimental validation was carried out. For this, the optimal solvent mixture for the extraction of carotenoids was tested. The experimental result obtained was 42.05 ± 1.37 mg kg^{-1} DM which was very close to the theoretical value; indicating that the established mathematical model is valid.

Among vegetables, red capsicum had the highest carotenoids content (30 mg $100g^{-1}$ FW), which far exceeds that of any other preferred vegetables such as tomato and carrots with 4-8 mg $100g^{-1}$ and 8-10 mg $100g^{-1}$, respectively. Considering its elevated carotenoid level, it is a suitable candidate vegetable for the extraction of natural pigments. For this, we were interested in the industrial by-product of this vegetable [16].

As this work is the first to evaluate the carotenoids content of the industrial by-product of chili puree, a comparison with the literature is not available. However, many works were performed on the chili puree itself. Kaur and Kaur [17] found that carotenoid contents of different purees prepared using red, yellow, and green peppers were 734.36, 640.28, and 10.44 mg $100g^{-1}$ FW, respectively. High levels of carotenoids varying between 99.98 and 232.46 mg $100g^{-1}$ DM were also obtained by Civan and Kumcuoglu [18]. The reduced amount of carotenoids obtained in this study compared to previous authors can be explained by the composition of chili paste by-product that contains only the remains of the skin, integuments, and seeds of the vegetable which relatively less concentrated in carotenoids.

IV. Conclusion

In conclusion, the study of the kinetics of carotenoids extraction from the by-product rejected by the industry of hot chili paste showed that the fifteen tested solvents have variable extraction capacities. The best solvents were acetone, hexane, and petroleum ether; and the concentrations obtained were 37.50, 25.92 and 27.71 mg kg^{-1} DM, respectively. The time required for extraction was considered at 10 min. The mixture design demonstrated that the mathematical developed model was very significant and indicated that the best combination of the three most effective solvents acetone/petroleum ether/hexane was 60%/40%/0%. Using this mixture of solvents, an optimum carotenoids content of 42 mg kg^{-1} was obtained.

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