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Determination of the Optimum Extraction Conditions of Carotenoid Pigment from Orange Peel by Response Surface Methodology

T. Al-idee [1*], H. Habbal [1], F. Karabet [2]

[1] *Department of Food Science-Faculty of Agriculture- Damascus University.*
[2]*Department of chemistry - Faculty of science- Damascus University.*

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tahane.alidee@yahoo.com
Phone:+963 949389043

Abstract

The objective of this study was to extract carotenoid pigments from orange peel (*Citrus sinensis*) by using three solvents (ethanol, acetone and hexane). The different temperatures (30-40-50°C) and times (90,120 and150 minutes) of each solvent were optimized by the statistical program Minitab and using statistical design Response Surface Methodology (RSM)**.** The results were showing that acetone was given high yield of carotenoids in all treatments followed by ethanol and hexane. The optimal conditions for the extraction of the highest yeild of carotenoids for each solvent have also been studied. The optimum conditions for the extraction of carotenoid (21.18mg/100g) using acetone were 30 °C for 90 min. Ethanol can extract the maximum yield of carotenoid (13.34 mg/100g) at 44°C for 105.2 minutes. While, the highest yield of the extracted pigment (5.73 mg /100 g) was obtained at the optimal conditions of 41.3°C for 120 minutes by using hexane.

1. Introduction

Sensory specification affects the way consumers perceive a product's quality and the preferences of consumers. Food colour is the first characteristic of food that attracts and satisfies the consumer when determining a quality and appearance of products, and therefore condition its acceptability [1]**.** There is a global trend towards to use the natural additives (food colorants) in food application [2].

Citrus is a major commercial and nutritional fruit crop in the world. Brazil is at the top of the list of the major producing countries, followed by the United States. The Mediterranean Basin accounts for around 20% of world citrus production and about 60% of world fresh citrus trade, according to CLAM data. [3]**.** In Syria, orange constitutes about 62.78 % of the total citrus production in 2017. During the orange juice production, great quantities of peels by-products are formed [4].

Orange peels are a source of many bioactive compounds such as essential oils, phenolic compounds and carotenoid pigments [5]. Carotenoids are C_{40} isoprenoids, consisting of eight isoprene units and the basic source of yellow, orange, or red color pigments synthesized by plants and microorganisms [6]. The carotenoids pigments are divided into two primary groups (i) carotenes (ß-carotene, lycopene) contain only hydrogen and carbon and can be cyclic or linear ; (ii) Oxy carotenoids (xanthophylls, lutein) contain hydrogen, carbon and oxygen in the form of hydroxy, epoxy or oxygen groups [7]. Carotenoids have excellent health effects due to their antioxidant and anticancer activity [8]. Presently, carotenoids have been used as natural food colorants, nutrient supplement and in the cosmetic sector [9]. Extraction of carotenoids from peels is affected by many factors such as type of solvents, ratio of solvents to peel and extraction time and temperature [10].

Response surface methodology (RSM) is a statistical method which is useful for process development, enhancement and optimization [11]. RSM has many advantages such as a reduction in the number of experiments needed and a 2D contour and 3D surface profiles of the interactive effects of the variables on the response [12]. The RSM was recorded as an appropriate method for optimizing the extraction of carotenoid and maximizing the antioxidant capacity of extracts from different plant resources [13 14]. The aim of this study was to optimize the extraction conditions of carotenoids pigments from orange peel using different solvents and by response surface methodology to get an information about its future food and pharmaceutical applications.

2. Material and Methods

2.1. Plant material

The peels of Sweet orange (*Citrus sinensis*) were collected from the local juice shop in Damascus city (2018).

2.2. Sample Preparation: orange peels were cleaned and dried in oven at 25°C for 48 h. The samples were ground using an electrical grinder (Starmix) and passed through a standard 500µm sieve.

2.3. Extraction and quantification of total carotenoids: The extraction of carotenoids from the orange peel was performed as described by [10] with some modifications. One gram of sample powder was mixed with 20 ml of different analytical grade solvents (hexane, ethanol and acetone). The mixture was left to stand at different temperature (30-40-50°C) in a water bath for (90-120-150minute), after that the extract was filtered through a filter paper and the absorbance of the filtrate was measured at 445nm.The carotenoid content was calculated using Equation(1):

$$\text{Total carotenoid}(\text{mg} / 100\text{g}) = \frac{A \times V(\text{ml}) \times 10^6}{A_{1\,cm}^{\%} \times 10^3 \times W} \ldots\ldots(1)$$

Where **A**: absorbance of extract at 445nm, **V**: volume of extract, **W**: weight of orange peel powder (g) and $^{\%}\mathbf{A_{1cm}}$: specific extinction coefficient of carotenoids in selective solvents (2559 for acetone,2592 for hexane and 2529for ethanol [15]).

2.4. Experimental Design and Statistical Analyses:

The influence of two independent variables -extraction temperature and time- for each solvent (ethanol,hexane and acetone) and total carotenoid as the response variable were studied. The response surface methodology (RSM) was employed to determine the optimum condition for total carotenoid extracted from orange peels using a central composite design (CCD). Each variable was coded at three levels -1, 0, +1 Table 1:

Table 1: Experimental variables at different levels used for the RSM approach:

Factors	Symbol	levels		
		Low (-1)	Medium (0)	High (+1)
Temperature (°C)	X_1	30	40	50
Time (minute)	X_2	90	120	150

The complete design consisted of 28 treatments and the results of carotenoid (mg/100g) were listed in the Table 2. The experimental design and statistical analysis were performed using Minitab 17.1.0 software (Minitab Inc., State College, PA, USA). Eq's. Parameters were determined by multiple regression analysis, using the RSM method. Eq 2 represents the relationship between carotenoid concentration (Y) and two test variables in coded units:

$$Y= a + bX_1 + cX_2 + dX_1^2 + eX_2^2 + fX_1X_2 \ldots..(2)$$

Y: response- a: constant- b, c: linear coefficient- d,e: square coefficient- f: interaction coefficient. X_1, and X_2 are the main variables (Table 1). The suitability of the model was evaluated by R^2 analysis -the regression analysis- and the ANOVA analysis. The response surface plots showed the relationship between the independent variables and the response variables (carotenoids). Many graphical and numerical optimizations of the experimental data were made to define the optimum extraction conditions for the maximum recovery of carotenoids.

3. Results and discussion

Effect of solvents on carotenoid concentration :

The results in Table 2 showed that acetone acetone (polar aprotic solvent) extracts in all treatments higher amounts of carotenoids than ethanol (polar protic solvent) and hexane (nonpolar solvent). The differences between the solvents' polarity resulted in a variation in their ability to extract different concentration of carotenoid from orange peel [16]. Lycopene and β-carotene are nonpolar carotenoids and soluble in non-polar solvent, whereas lutein or epoxy carotenoids are polar carotenoids and soluble in polar solvents [17]. Several studies have shown that violaxanthin (Fig.1) is the major carotenoid found in orange peels, comprising 52% [18] to 80% [19] of total carotenoid content. This carotenoid contains polar functional groups and can dissolve in polar solvents such as ethanol and acetone [14].

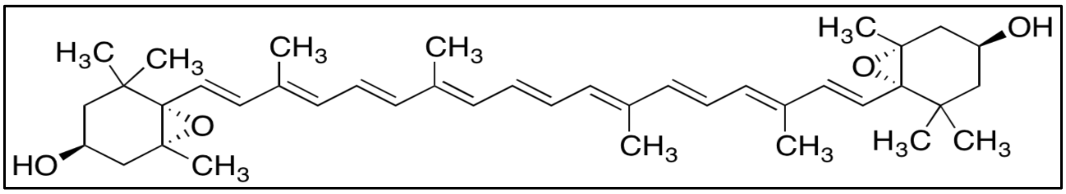


Figure 1: Violaxanthin chemical composition

Acetone is a useful solvent and a wetting material which penetrates the matrix more easily [20]. The superiority of acetone to ethanol ability for extraction of carotenoids is due to its better capacity in crossing plant cells to release carotenoids, carotenoids, and many researchers reported that acetone is a good solvent for extraction of carotenoids from tissues containing water. The superiority of acetone to ethanol to extraction carotenoids is may be due to its ability to release carotenoids when crossing plant cells [21]. According to [22] acetone has lower viscosity (0.32cp) than ethanol(1.2cp) which will have greater penetrability to plant cell and hence should produce the maximum yield of carotenoid.

1.1. Optimum condition for the carotenoid extracted by different solvents:

Based on the predictive models shown in Figure 2, the optimum conditions for the extraction of carotenoid compounds using acetone were 30 °C for 90 min. Strati and Oreopoulou [23] found that a temperature 70°C for 30 min were the optimum conditions for the acetone extraction of carotenoid from tomato waste. The results in Table 3 showed the influence of the examined factors separately, the square of factors and the relationship between factors in total carotenoid extract by acetone, *P*-value was less than 0.05 (P<0.05) for each extraction temperature and time, consequently each variable had a significant linear impact in carotenoid production. The quadratic term of temperature (X^2_1) was less than 5%. The interaction of extraction time and temperature was statistically significant ($p < 0.05$).

Table (2) carotenoid yield from orange peel using three different solvents were studied:

Block	Temperature (c)	Time (min)	Carotenoid yield (mg/100g)		
			Acetone	Ethanol	Hexane
1	30	90	21.99	8.84	4.48
2	50	90	13.1	12.58	4.48
3	30	150	15.03	6.85	4.06
4	50	150	13.69	2.29	4.02
5	40	120	12.58	12.45	5.28
6	40	120	13.72	11.77	5.08
7	40	120	12.52	13.8	5.25
8	30	120	16.14	6.59	4.31
9	50	120	11.822	12.44	4.79
10	40	90	11.75	10.33	4.65
11	40	150	11.47	7.63	5.34
12	40	120	11.75	11.78	5.47
13	40	120	13.12	12.79	5.59
14	40	120	13.72	12.78	5.67
15	30	90	21.89	10.59	4.26
16	50	90	14.94	11.94	5.07
17	30	150	14.69	7.58	4.2
18	50	150	17.06	8.54	4.91
19	40	120	13.57	14.33	5.17
20	40	120	13.71	11.47	6.54
21	40	120	12.5	14.3	6.2
22	30	120	17.35	7.76	5.05
23	50	120	11.65	12.77	5.54
24	40	90	13.25	11.03	5.14
25	40	150	11.74	8.28	5.46
26	40	120	15.24	12.58	6.36
27	40	120	14.36	14.4	6.49
28	40	120	14.21	12.43	5.64

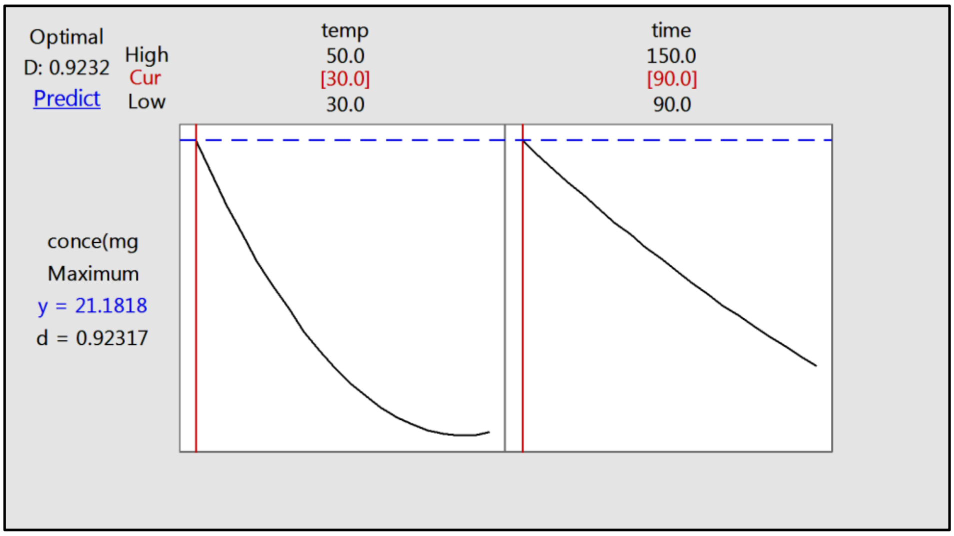


Figure 2: Optimal conditions for the extraction of carotenoids using acetone by RSM

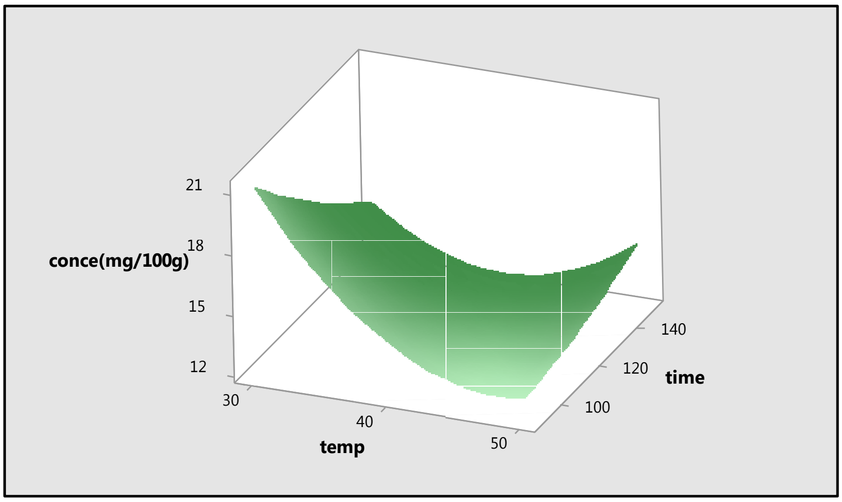
Table 3: Estimated Regression Coefficients for Total carotenoid using acetone:

Term	Coef	SE Coef	T-value	*P*-value
Constant	106.1	12.8	8.30	0.000
Temperature(X_1)	-3.092	0.508	-6.09	**0.000**
Time(X_2)	-0.415	0.169	-2.45	**0.023**
Temperature*Temperature($X^2{}_1$)	0.02552	0.00584	4.37	**0.000**
Time*Time($X^2{}_2$)	0.000404	0.000648	0.62	0. 539
Temperature*Time(X_1)*(X_2)	0.00703	0.00164	4.29	**0.000**

The R^2 =0.7822%, indicating that the model can predict 78.22% of the actual data for total carotenoid from orange peel using acetone. Depending on Table 3 the predictive equation for total carotenoid (Y) response was the following:

$$Y=106.1- 3.092\ X_1- 0.415\ X_2- 0.02552\ X^2{}_1- 0.000404\ X^2{}_2 +0.00703\ X_1X_2$$

Response surface plot for carotenoid extraction with different time and temperature using acetone are shown in Figure 3. Carotenoid significantly decreased along with the increase in the time and temperature of the extraction. There are several side effects -such as degradation and isomerization-of higher temperatures on carotenoid extraction [10].



Figur3: The 3D Response surface plot for the effects of the parameters on carotenoid yield using acetone.

The maximum carotenoid of 13.34 mg/100g was predicted to be obtained from orange peel using ethanol under the optimal conditions of 105.2 min and 44°C (Figure 4). Tao et al. [24] obtained high yield of carotenoids from pummelo peel with ethanol at 50°C for 40 min. Compared with carotenes (nonpolar), xanthophyll (polar substances) can be efficiently extracted by ethanol [25]**,** also ethanol is a short chain alcohol and has been recommended as a suitable solvent for the high carotenoids yield. In addition, ethanol is recognised as safe for use in the food industry [26]. The results showed that an increase in the extraction temperature positively increased the yield of carotenoid. Aflaki [27] reported the plant carotenoids contained are found in the cells and the cell diaphragm consists of a complicated structure, As the temperature increases, the cell wall breaks down and increases its solvent extraction.

Table 4 shows the influence of different parameters on the yield of carotenoids extracted from orange peel. There was a significant linear effect of the temperature and time on the amount of carotenoids extracted (*P*<0.05). The value of *P* for the effect of square factors of temperature and time was less than 5%, and this means that these factors have a significant effect on extraction , while the interaction between the temperature and time was not significant (*P*>0.05).

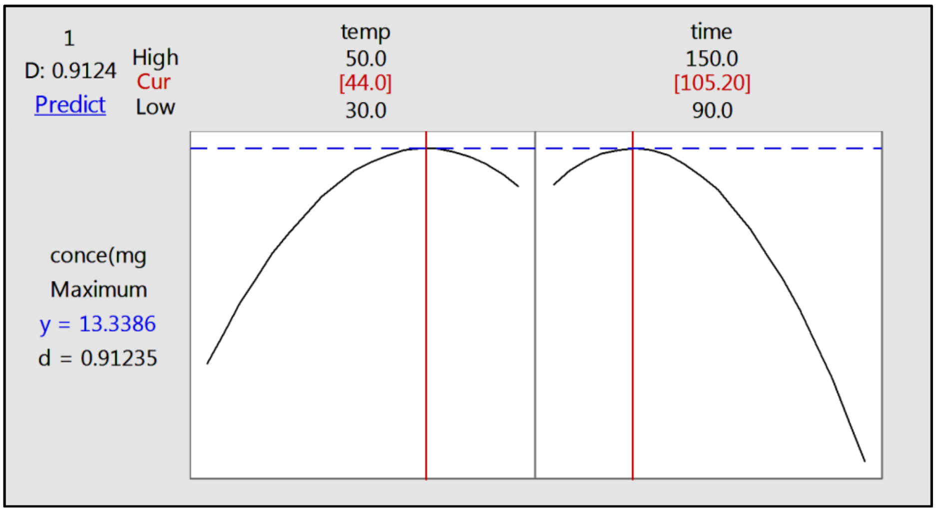


Figure 4: Optimal conditions for the extraction of carotenoids using ethanol by RSM.

Table 4: evaluated Regression Coefficients for carotenoid using ethanol:

Term	Coef	SE Coef	T-value	*P*-value
Constant	-71.1	15.5	-4.59	0.000
Temperature(X_1)	2.066	0.615	3.36	**0.003**
Time(X_2)	0.740	0.205	3.61	**0.002**
Temperature*Temperature(X^2_1)	-0019	0.007	-2.70	**0.013**
Time*Time(X^2_2)	-0.0028	0.00078	-3.51	**0.002**
Temperature*Time(X_1)*(X_2)	-0.0036	0.00198	-1.83	0.081

The coefficient of determination (R^2=0.7261) indicating that the model can predict 72.61% of the actual data for total carotenoids. Thus we can use the polynomial second order equation for the following two independent variables studied. The predictive equation for the response of carotenoids extracted from orange peel using ethanol (*Y*) was as follows:

$$Y=-71.1+ 2.066\ X_1+ 0.740\ X_2- 0.01911\ X^2_1- 0.002759\ X^2_2 - 0.00362\ X_1X_2$$

The response surface curve in Figure 5 also shows the influence of the independent factors and the mutual interaction on the extraction yield of carotenoids using ethanol**.** The curve shows that increase in extraction time and temperature significantly increased the concentration of carotenoids up to a maximum of 13.34mg/100g, then carotenoid concentration rapidly decreased with an increase in temperature and time of extraction to a minimum value at 50° for 150 min.

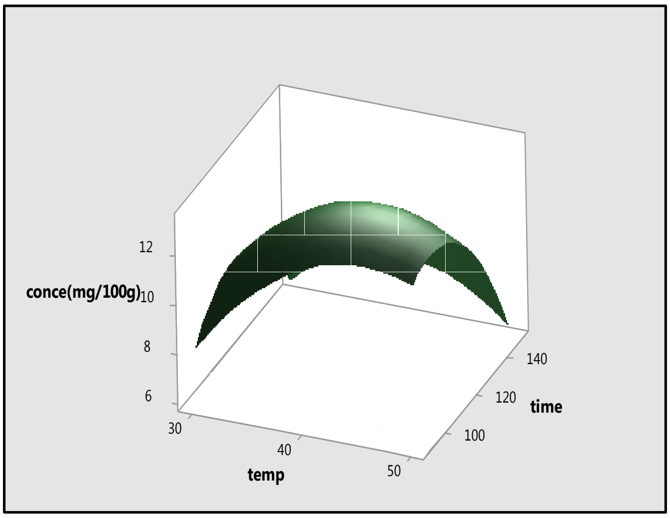


Figure 5: 3D Response surface plot for the effects of the parameters on carotenoids yield using ethanol.

Hexane presented the lowest ability to extract carotenoids compared to acetone and ethanol .The highest concentration of the extracted pigment (5.73 mg / 100 g) was obtained at the optimal conditions of 41.3°C for 120 minutes and the (Fig. 6).

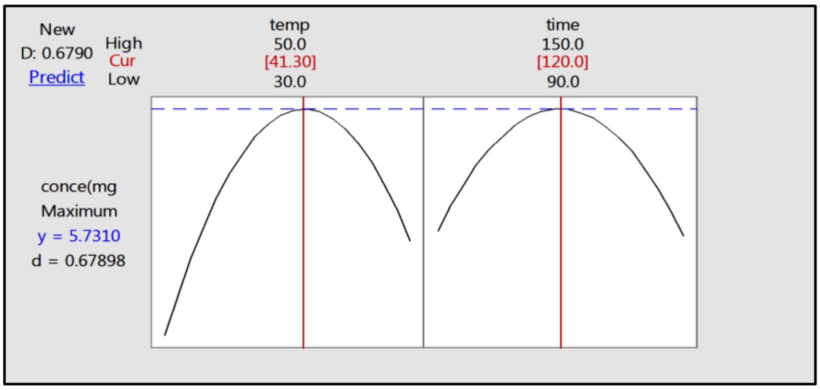


Figure 6: Optimal conditions for the extraction of carotenoids using hexane by RSM.

The statistical analysis in Table 5 shows the *P* value for the effect of individual and square factors was less than 0.05 ($P<0.05$), while the relationships between the extraction duration and temperature were not significant ($P>0.05$). The fitting of the model was calculated to be $R^2=0.6455$, which indicates that 64.55% of the variability in the response can be explained by the model. The following equation was derived depending on Table 5:

$$Y= -16.13 + 0.637\ X_1 + 0.1453\ X_2 - 0.00762\ X^2{}_1 -0.000597X^2{}_2 -0.000058X_1X$$

Table 5: Estimated Regression Coefficients for total carotenoid using hexane:

Term	**Coef**	**SE Coef**	**T-value**	***P*-value**
Constant	-16.13	4.31	-3.74	0.001
Temperature(X_1)	0.637	0.171	3.72	**0.001**
Time(X_2)	0.1453	0.0571	2.45	**0.019**
Temperature*Temperature($X^2{}_1$)	-0.00762	0.00197	-3.87	**0.001**
Time*Time($X^2{}_2$)	-0.00597	0.000219	-2.73	**0. 012**
Temperature*Time(X_1)*(X_2)	-0.00058	0.000552	-0.11	0.917

The 3D surface plot of the carotenoids (Figure7) shows that the concentration of pigments extracted from orange peel using hexane increased by increasing the temperature and extraction time up to a maximum of 5.73mg/100g at 41.3 °C for 120 minutes, then decreased as temperature and time increased.

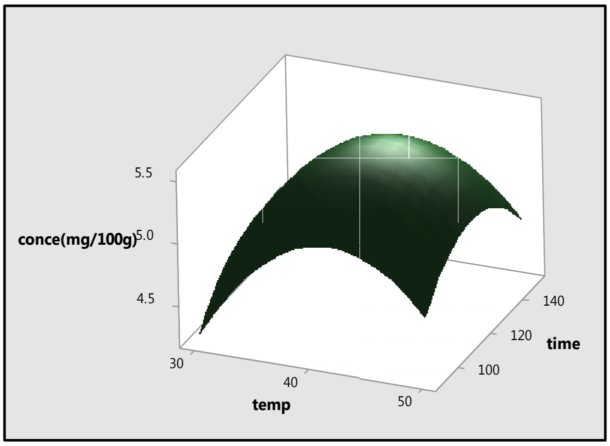


Figure 7: 3D Response surface plot for the effects of the parameters on carotenoids yield using hexane.

Conclusion

This research was conducted to extract carotenoids from orange peel by different solvents (acetone, ethanol and hexane) and to optimize the extraction parameters (extraction time and temperature) for maximum yield of each solvent using the statistical program and Response Surface Methodology (RSM) design. Among other solvents, acetone gave the highest yield of carotenoids (21.18 mg/100g) at 30 °C for 90 min, while ethanol can extract the highest yield of carotenoid at 44°C for 105.2 minutes. The highest yield of the extracted pigment (5.73 mg /100g) was obtained at the optimal conditions of 41.3°C for 120 minutes by using hexane.

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