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Avaliação do tempo, temperatura e concentração de solvente, na extração de compostos bioativos da cenoura (*daucus carota*)

Evaluation of time, temperature and concentration of solvent, in the extraction of bioactive compounds from carrot (*daucus carota*)

Evaluación de tiempo, temperatura y concentración de disolvente, en la extracción de compuestos bioactivos de la zanahoria (*daucus carota*)

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Resumo

O objetivo deste trabalho foi analisar o teor de compostos bioativos extraídos da cenoura, através de delineamento experimental de 3 fatores, 3 níveis e dois pontos axiais, incluindo tempo de extração (17- 37- 57 min), temperatura (30-50-70ºC) e concentração de etanol (50-65-80%). O resultado foi expresso em porcentagem. Com base nos resultados, pode-se dizer que o tempo não influenciou significativamente a extração dos compostos antioxidantes ($p > 0,001$), e que a temperatura é diretamente proporcional ao rendimento. Em relação aos compostos fenólicos apenas a temperatura foi significativa, e para os flavonóis, apenas o tempo e a proporção. O uso de etanol como solvente apresentou bons resultados quando comparado aos solventes comumente utilizados, além de ter a qualidade de ser economicamente viável e de grau alimentício. Outro fator importante foi o pré-tratamento da amostra, que permitiu bons valores de rendimento quando comparado à literatura.

Palavras-chave: Alimentos funcionais; Compostos bioativos; Otimização.

Abstract

The objective of this work was to analyze the content of bioactive compounds extracted from the carrot, through an experimental design of 3 factors, 3 levels and two axial points, including extraction time (17-37-57min), temperature (30-50-70ºC) and ethanol concentration (50-65-80%). The result was expressed as a percentage. Based on the results, it can be said that time did not significantly influence the extraction of antioxidant compounds (p> 0.001), and that the temperature is directly proportional to the yield. Regarding phenolic compounds, only temperature was significant, and for flavonols, only time and proportion. The use of ethanol as a solvent showed good results when compared to the commonly used solvents, in addition to having the quality of being economically viable and food grade. Another important factor was the pre-treatment of the sample, which allowed good yield values when compared to the literature.

Keywords: Functional foods; Bioactive compounds; Optimization.

Resumen

El objetivo de este trabajo fue analizar el contenido de compuestos bioactivos extraídos de la zanahoria, a través de un diseño experimental de 3 factores, 3 niveles y dos puntos axiales, incluido el tiempo de extracción (17-37-57 min), temperatura (30-50-70ºC) y concentración de etanol (50-65-80%). El resultado se expresó como un porcentaje. Con base en los resultados, se puede decir que el tiempo no influyó significativamente en la extracción de compuestos antioxidantes (p> 0.001), y que la temperatura es directamente proporcional al rendimiento. Con respecto a los compuestos fenólicos, solo la temperatura fue significativa, y para los flavonoles, solo el tiempo y la proporción. El uso de etanol como solvente mostró buenos resultados en comparación con los solventes comúnmente utilizados, además de tener la calidad de ser económicamente viable y de grado alimenticio. Otro factor importante fue el pretratamiento de la muestra, que permitió buenos valores de rendimiento en comparación con la literatura.

Palabras clave: Alimentos funcionales; Compuestos bioactivos; Mejoramiento.

1. Introduction

The growing search for healthy foods by the current generation arouses the interest of the scientific community for foods commonly known as functional foods (Delgado-Andrade, 2017). These foods are known to promote health benefits acting in the prevention of diseases, they must remain natural and have a functionality beyond the basic nutritional function. (Nazir et al., 2019).

One of the reasons responsible for this growth is in the reliability of the consumer regarding the product, since the amount of nutrients and bioactive compounds present helps in various functions of the body, such as stomach and intestinal problems, for example. (Kraus, Annunziata, & Vecchio, 2017).

In relation to bioactive compounds, which exert antioxidant, anti-inflammatory and anti-atherogenic activities through various mechanisms (Manach et al., 2017), there are phenolic compounds, a very important natural antioxidant in foods of plant origin, in addition to being one of the most interesting phytochemicals in recent times, as they help in the better functioning of the organism (Delgado-Andrade, 2017) and flavonoids, which have a cardioprotective, anticancer, anti-diabetic, anti-aging and neuroprotective effect (Song et al., 2019).

The market for the extraction of bioactive compounds grows every day, aiming at the improvement of methods, cost reduction, and mainly the increase of yield, thus, several studies have been carried out, using different matrices (Klein, Santos, Palú, Vieira, & Silva, 2018; Lima, Ribeiro, Cardozo-filho, Vedoy, & Alves, 2019; Mena-García, Ruiz-Matute, Soria, & Sanz, 2019; Rodrigues, Melo, & Silva, 2018; Saini & Keum, 2018; Zhang, Wen, Zhang, Duan, & Ma, 2019).

Among the matrixes used for the extraction of bioactive compounds, is the carrot (*Daucus Carota*). It is a highly nutritious vegetable and known to be a source of beta-carotene (Haq, Kumar, & Prasad, 2016), it also has a considerable amount of fibers, minerals, phenolic compounds and vitamins C and E. Its intake has some beneficial effects on the body, such as reducing DNA damage and increasing plasma antioxidant levels (Owolade et al., 2017). Such effects are mainly attributed to the antioxidant power present, which “eliminates free radicals and singlet oxygen, produced during the metabolic pathway” (Haq et al., 2016).

Therefore, the objective was to analyze the content of bioactive compounds extracted from the carrot, through an experimental design of 3 factors, 3 levels and two axial points,

including extraction time (17-37-57 min), temperature (30-50-70ºC) and ethanol concentration (50-65-80%).

2. Materials and Methods

This work consists of qualitative laboratory research (Pereira, Shitsuka, Dorlivete Moreira Parreira, & Shitsuka, 2018), through physical-chemical analyzes based on the methodologies cited below.

The raw material used was obtained from local businesses in the city of Maringá-PR, and the analyzes carried out in the laboratory of the Department of Food Engineering and Chemical Engineering, at the State University of Maringá - UEM.

The carrot was cut into 1 cm^3 cubes and dried in an oven at 50 ºC for 24 hours. After drying, it was ground in an industrial blender until a flour was formed, thus proceeding to the extraction, which was conducted in a jacketed reactor, using 1g of solute with 50ml of Ethanol solution, where the time, temperature and percentage of solvent in the solution were varied.

The antioxidant activity was evaluated according to the DPPH method. The DPPH free radical capture method was carried out as described by Brand-Williams; Cuvelier; Berset (1995) with some modifications. The DPPH radical at 30mg / L was prepared in methanol. The spectrophotometric reading was performed after 1h, from the mixture of 4ml of the radical with 0.5ml of the extract and 5ml of methanol at a wavelength of 517nm. The results were expressed in percentage of Antioxidant Activity (AA%), and calculated using the formula below.

$$AA\% = \frac{[Aa - (Ab - Ac)]}{Aa} \; x \; 100$$

Aa: Absorbance of the DPPH solution without extract; Ab: Absorbance of the mixture of the DPPH solution containing extract; Ab: Absorbance of the blank solution without the DPPH solution.

The quantification of phenolic compounds was performed with the Folin-Ciocalteu reagent, using the standard curve of gallic acid as reference (Larrauri, Rupérez, & Saura-Calixto, 1997). For analysis, 1mL of the extract (or water for the white) was mixed with 5mL of the 10% Folin-Ciocalteu solution and 4mL of a 7.5% sodium carbonate solution. The mixture was kept in a water bath for 5 minutes at 50ºC, after which the reading was made at

760nm. The results were expressed in mg of gallic acid equivalent (EAG) per 100 mL of extract.

To quantify the flavonoid content, the methodology described by Hung e Duy (2012) was used, where 0.5 ml of extract was mixed with 1.5 ml of 95% ethanol, followed by 0.1 ml of 10% aluminum chloride, 0.1 ml of 1M potassium acetate and 2.8 ml of distilled water . After incubation at room temperature for 30 min, the absorbance of the reaction mixture was recorded at 415 nm.

A Central Rotational Composite Design (CRCD) 2^3 (Table 1), was performed in order to find a real interval for the factors (Time, Temperature and Proportion), which presented the maximum point of the analyzed compounds. The factors were defined according to the literature (Swamy, Sangamithra, & Chandrasekar, 2014), with intervals of 17 to 57 minutes, 30 to 70ºC and 50 to 80% solvent. The levels of antioxidant, phenolic and flavonoids were the observed responses variables.

Table 1 - Factors, interval and independent levels use on CRCD.

	Coded levels				
Factors	**-1.68**	**-1**	**0**	**1**	**1.68**
Time (min)	3,4	17	37	57	70,6
Temperature (ºC)	16,4	30	50	70	83,6
Proportion (%)	39,5	50	65	80	90,2

Source: Authors.

The tests were performed at random, thus reducing the effects of unexplained variability in responses, due to external factors, and the contour curves were used to assess the impact of the three independent variables on the compounds.

The statistical analysis was performed using the software Protimiza Experiment Design (http://experimental-design.protimiza.com.br), and the significance of the model was tested by ANOVA.

3. Results and Discussion

The results of the content of antioxidants, phenolic compounds and flavonoids obtained in the DCCR for the 3 variables under study are shown in Table 2.

Table 2 - CRCD for the two independent variables used in the process and the experimental responses obtained.

Run	Time (minutes)	Temperature (ºC)	Proportion (%)	Antioxidant Activity	Phenolic Compound	Flavonoids
1	17	30	50	66,58	193,72	136,572
2	57	30	50	62,60	186,70	147,3324
3	17	70	50	74,78	204,01	191,3093
4	57	70	50	74,90	199,80	205,3445
5	17	30	80	74,54	185,76	159,4962
6	57	30	80	72,25	191,85	172,5957
7	17	70	80	83,12	222,37	205,3445
8	57	70	80	82,26	233,95	241,3682
9	3,4	50	65	76,47	192,78	162,7711
10	70,6	50	65	72,61	179,68	159,4962
11	37	16,4	65	80,45	189,51	159,0284
12	37	83,6	65	90,10	271,38	248,8536
13	37	50	39,8	68,03	199,80	158,5605
14	37	50	90,2	75,75	229,74	107,0981
15	37	50	65	73,94	179,21	159,4962
16	37	50	65	74,30	171,73	156,6892

Source: Authors.

According to the Central Rotational Composite Design (CRCD), the condition that provided the highest extraction rate of antioxidants from carrots, 90.10%, was that with a time equal to 37 minutes, temperature of 83.6 ºC and solvent ratio 65% ethanol. For phenolic compounds and flavonoids, the test12 was the one with the highest values.

Tables 3, 4 and 5 show the regression coefficients of studied compounds. It can be seen, in Table 3, that for the antioxidant activity, only the linear terms of temperature and proportion were significant ($p < 0.1$), and the quadratic term of the temperature. Emphasizing that temperature is a factor of great importance in the extraction of antioxidants.

Table 3 - Regression coefficients for antioxidant activity.

Factors	Coef	Std. Error	t- calc	p-value
Média	34,535	4,976	6,939	0,0002
x1 (L)	0,3477	2,337	0,148	0,8859
x1 (Q)	1,5118	2,572	0,587	0,5751
x2 (L)	10,376	2,337	4,439	0,0030
x2 (Q)	5,6986	2,572	2,215	0,0622
x3 (L)	6,5935	2,337	2,821	0,0257
x3 (Q)	-3,186	2,572	-1,238	0,2553
x1:x2	0,8552	3,053	0,280	0,7875

Source: Authors.

Na Tabela 4, é possível observar que apenas os termos de temperatura linear e quadrático foram significativos para compostos fenólicos.

Table 4 - Regression coefficients for phenolic compounds.

Factors	Coef	Std. Error	t- calc	p-value
Média	157,282	13,325	11,803	0,000007
x1 (L)	5,009	6,257	0,800	0,4497
x1 (Q)	4,546	6,8874	0,660	0,5302
x2 (L)	27,710	6,257	4,428	0,0030
x2 (Q)	19,681	6,887	2,857	0,0244
x3 (L)	0,856	6,257	0,1368	0,8949
x3 (Q)	-5,460	6,887	-0,7928	0,4539
x1:x2	3,274	8,175	0,4005	0,7006
x1:x3	3,0409	8,175	0,3719	0,7209
x2:x3	0,2339	8,1759	0,0286	0,9779

Source: Authors.

For flavonoids, observing Table 5, the linear terms of time and proportion, and the quadratic term of proportion were significants.

Table 5 - Regression coefficients for flavonoids.

Factors	Coef	Std. Error	t- calc	p-value
Média	1,2076	0,2169	5,5665	0,00084
x1 (L)	0,2812	0,1018	2,7610	0,02805
x1 (Q)	-0,1243	0,1121	-1,1091	0,30401
x2 (L)	0,1532	0,1018	1,5039	0,17630
x2 (Q)	0,0160	0,1121	0,1432	0,89012
x3 (L)	0,5180	0,1018	5,0848	0,00142
x3 (Q)	0,5309	0,1121	4,7354	0,00211
x1:x2	0,1884	0,1331	1,4155	0,19983
x1:x3	0,1731	0,1331	1,3007	0,23452
x2:x3	-0,2393	0,1331	-1,7980	0,11520

Source: Authors.

With the removal of statistically non-significant parameters, and addition of residues, the model follows the following form:

$$\text{Antioxidant Activity} = 32{,}88 + 10{,}38\ x_2 + 6{,}08\ x_2^2 + 6{,}59\ x_3$$

$$\text{Phenolic Compound} = 156{,}38 + 27{,}71\ x_2 + 19{,}89\ x_2^2$$

$$Flavonoids = 1,10 + 0,28\ x_1 + 0,52\ x_3 + 0,56\ x_3^2$$

It is also possible to observe, by estimating the coefficients, that all factors have positive effects on the response variables, that is, the largest content is extracted in higher values, however, some have a greater effect than the other.

ANOVA for the three compounds, considering only the significant terms, is shown in Table 6. The coefficient of determination (R^2) was 75.13%, 75.55% and 77.79% for antioxidant activity, phenolic compounds and flavonoids, respectively. However, as the analysis of the coefficient of determination (R^2) measures the reduction of the variability of the response using the regressor variables in the model, a high value does not necessarily imply a good fit (Bas & Boyaci, 2007).

Table 6 - ANOVA for antioxidant activity (A), phenolic compounds (B) and flavonoids (C).

Variation source	SS	FD	MS	Fcal	p-value
Regression	2545,907	3	848,635	13,0887	0,00031
Residues	842,878	13	64,8368		
Lack of fit	842,562	11	76,5965	484,2772	0,00206
Pure Error	0,31633	2	0,1581		
Total	3388,786	16			
R^2	75,13%				
PHENOLIC COMPOUND					
Variation source	SS	FD	MS	Fcal	p-value
Regression	15640,905	2	7820,452	21,632	0,00005219
Residues	5061,105	14	361,507		
Lack of fit	5056,873	12	421,406	199,189	0,005005
Pure Error	4,231	2	2,115		
Total	20702,010	16			
R^2	75,55%				
FLAVONOIDS					
Variation source	SS	FD	MS	Fcal	p-value
Regression	8,769	3	2,923	15,176	0,000154
Residues	2,504	13	0,193		
Lack of fit	2,502	11	0,228	276,994	0,00360
Pure Error	0,001	2	0,00082		
Total	11,273	16			
R^2	77,79%				

$$^{A}F_{3;13;0,1} = 2,56\ ;\ ^{B}F_{2;14;0,1} = 2,73\ ;\ ^{C}F_{3;13;0,1} = 2,56$$

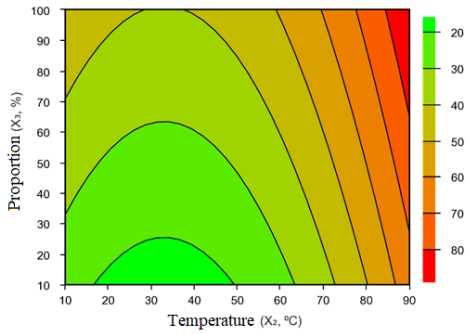
Source: Authors.

Analyzing the value of F, it can be said that all compounds were statistically significant (f-calculated> f-tabulated), showing the adequacy of the models and enabling the demonstration of the results through the contour curves.

With the results obtained, contour graphs (Figures 1 and 3) were constructed for the antioxidant activity and flavonoids, respectively, in order to visually verify the interaction between the factors analyzed.

Watching Figure 1, it can be reaffirmed what was previously analyzed, that temperature is a factor of great influence in the extraction of antioxidants, that is, the higher it is, the greater the extraction yield.

Figure 1 - Contour plot for antioxidant activity by extraction conditions.

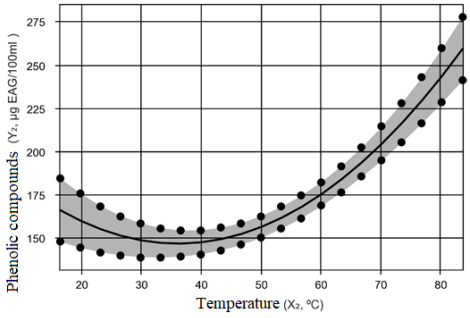


Source: Authors.

Regarding the proportion, it can be observed that in larger quantities, it was possible to obtain a higher percentage of the compound, however, it would be necessary to carry out another experiment to affirm with greater property the optimal extraction intervals.

For phenolic compounds, as only the temperature was significant, it was possible to construct a curve that shows the amount of compound obtained according to the temperature levels analyzed (Figure 2).

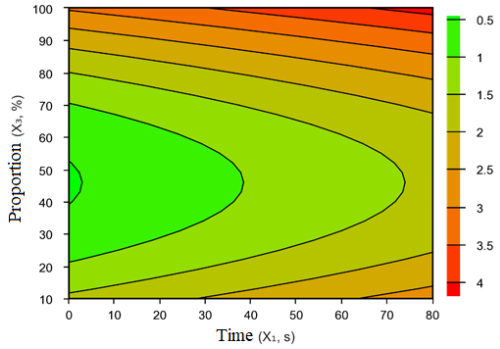
Figure 2 – Curve of temperature variation for phenolic compounds.



Source: Authors.

Regarding the phenolic compounds (Figure 2), it was observed that the time and the proportion were not statistically significant, therefore, only the temperature caused an effect on the extraction, having an exponential increase from 40ºC.

Figure 3 - Contour plot for flavonoids by extraction conditions.



Source: Authors.

A similar result can be seen by Prakash Maran, Manikandan, Vigna Nivetha, & Dinesh (2017) in their extraction of anthocyanins and polyphenols by ultrasound, using water as a solvent, where it was possible to obtain a higher concentration when the temperature was high 30 to 50 °C.

Flavonoids behaved differently than other compounds. It presented a high yield when combined with a long period and a high proportion, however, it would also be necessary to perform another experiment to affirm the optimal extraction intervals with greater property.

4. Final Considerations

The present study revealed that it is possible to extract bioactive compounds from the carrot using a direct extraction methodology and ethanol as the only solvent, but only in high concentrations, the same being true for the temperature, which must be high. Regarding time, it was not possible to observe significant influence during the extraction. The use of ethanol as a solvent showed good results when compared to the commonly used solvents, in addition to having the quality of being economically viable and food grade.

The methodology used for the extraction as for the quantification of the compounds is simple, facilitating the analysis. Another important factor was the pre-treatment of the sample, which allowed good yield values when compared to the literature.

Further studies should be carried out in order to improve the extraction, aiming to find an interval where it is possible to obtain a larger amount of the compound.

Conflicts of interest

All authors declare no competing interests.

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