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Short communication

SHORT COMMUNICATION

Accelerated solvent extraction of bioactive compounds from carrot – Optimization of response surface methodology

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Abstract: Carrot is considered to be rich in bioactive antioxidants, both lipophilic (carotenoids) and hydrophilic (phenolic compounds). In the present study, the conditions for accelerated solvent extraction (ASE) of bioactive compounds from carrots (*Daucus carota* L.) were optimized using response surface methodology (RSM). Box–Behnken design was employed for the experimental design to obtain the optimized combination of extraction temperature, time, and number of extraction cycles. Total carotenoid content (*TCar*), total polyphenol content (*TPh*), free radical scavenging activity (*SA*) and reducing power (*RP*) of the obtained extracts were used as responses for the optimization. Considering the four quality indicators, the ideal extraction conditions were found to be: 120 °C, 60 min and three extraction cycles. Under these conditions, predicted values of 28.84 mg *β*-carotene/100 g for *TCar*; 530.81 mg GAE/100 g for *TPh*; 2572.29 μmol *TE*/100 g for *SA* and 1336.26 μmol TE/100 g for *RP* were obtained with high desirability (0.975) and no significant difference ($p < 0.05$) with the experimental values.

Keywords: carotenoids; polyphenols; scavenging activity; reducing power; extraction.

INTRODUCTION

Bioactive compounds in plants have gained popularity for their beneficial effects on human health.[1] Storage root of carrot (*Daucus carota* L.) provides an important source of carotenoids and polyphenols in human diets. The extraction process is the first step towards recovery of natural antioxidants from plants.

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Optimum extraction methods depend on the characteristics of the bioactive compounds and the diversity of the tissue plant structures.[2] Therefore, optimization of the extraction conditions is a starting point in obtaining the highest yields of target compounds. Accelerated solvent extraction (ASE) is an automated extraction technique that uses elevated pressure and temperature to achieve efficient extraction in a very short time, using lower solvent volume and resulting in higher extraction yields.[3] Mustafa *et al.* reported that both time and temperature and the interaction between these two factors significantly affect the extraction yield of carotenes.[4] The aim of this study was to optimize the extraction conditions, *i.e.*, time, temperature and number of extraction cycles, for isolation of bioactive compounds from carrots using the ASE technique, taking into account the total carotenoid and polyphenol contents, free radical scavenging activity and reducing power.

EXPERIMENTAL

Plant material

Fresh carrots (*Daucus carota* L.) were purchased from a local supermarket. After washing, fresh carrots were chopped in a kitchen blender (B 800 E, Gorenje, Slovenia), freeze-dried at –40 °C (Martin Crist Alpha 2-4, Osterode, Germany), ground and stored at –20 °C until use.

Experimental design

The optimization of the extraction conditions was established by response surface methodology (RSM). The experimental plan was based on three variables at three levels, referred to as Box–Behnken design. The design consisted of 15 experimental runs, including three replicates at the central point.[5] The independent variables were extraction time (X_1: 20–60 min), temperature (X_2: 40–120 °C) and number of extraction cycles (X_3: 1–3). The coded values of the independent variables were –1, 0 and 1. The actual values were chosen from the preliminary studies, and the corresponding coded values of three independent variables are given in Table I.

Accelerated solvent extraction procedure

A Dionex ASE 350 (Thermo Scientific, Waltham, MA, USA) system was used for the extraction of the carrots using 100 % ethanol. For this purpose, a stainless-steel Dionex cell was filled with a diatomaceous earth (to reduce the volume of the extraction solvent) and the carrot sample (0.5 g) in the ratio 4:1. To prevent the collection of suspended particles in the extract, a cellulose filter was placed at the bottom of the cell. Finally, the cell was placed in the cell tray and used for extraction under the conditions obtained from the RSM guided experimental design. Glass vials were used to collect the extracts, which were stored at −20 °C until further use.

*Total carotenoid content (*TCar*)*

The contents of carotenoids in the carrot extracts were analyzed spectrophotometrically by the method of Nagata and Yamashita[6] using the extraction solvent as blank. The content of total carotenoids was calculated using the equation:

$$TCar \text{ (mg } \beta\text{-carotene per100 ml)} = 0.216A_{663}–1.22A_{645}–0.304A_{505}+0.452A_{453} \quad (1)$$

where A_{663}, A_{645}, A_{505} and A_{452} represent the absorbance measured at 663, 645, 505 and 453 nm, respectively. The total carotenoid content is expressed as mg of β-carotene equivalents per 100 g sample.

*Total polyphenol content (*TPh*)*

The total polyphenol content in carrot extracts was determined spectrophotometrically by Folin–Ciocalteau method adapted to microscale.[7] The results are expressed as gallic acid equivalents (GAE) per 100 g sample.

*Radical scavenging activity by DPPH assay (*SA*)*

The levels of free radical scavenging activity (*SA*) of the carrot extracts on the 2,2--diphenyl-1-picrylhydrazyl (DPPH$^{\bullet}$) radical were measured spectrophotometrically in a 96--well microplate, according to Girones-Vilaplana *et al.*[8] The *SA* values were calculated using the following equation:

$$SA = 100(A_C - A_S)/A_C \quad (2)$$

where A_C is the absorbance of the control and A_S is the absorbance in the presence of extracts. The results are expressed as μmol trolox equivalents (TE) per 100 g of sample.

*Reducing power (*RP*)*

The reducing power of the extracts was determined by the method of Oyaizu[9] adapted for a 96-well microplate. A calibration curve was made with trolox and the results are expressed as μmol TE per 100 g of sample.

RESULTS AND DISCUSSION

In order to understand the effect of the extraction parameters on the efficiency of carotenoids and polyphenols extraction, and antioxidant activity of carrot extracts, experimental design was prepared and evaluated by RSM (Table I and Figs. S-1 and S-2 and Table S-I of the Supplementary material to this paper).

The medium time (40 min), lowest temperature (40 °C) and one extraction cycle (experiment 9) were the least suitable for the isolation of carotenoids, while the longest extraction (60 min), medium temperature (80 °C) and 3 cycles (experiment 8) yielded the highest amount of *TCar*. Mustafa *et al.* obtained the highest yield of α- and β-carotene in extracts of carrot by-products using the following ASE extraction conditions: 60 °C and 10 min extraction time (5 cycles of 2 min each).[4] However, the carrot samples were fresh and the pressure was constant (50 bar), while in the present study, the carrot samples were freeze-dried and the pressure varied in dependence on the temperature. The amount of *TCar* in the carrot extracts ranged from 8.42–29.01 mg 100 g^{-1}, which corresponds with the reports of Mustafa *et al.*[4] for the content of α- and β-carotene in carrot by-products (10.3–27 mg 100 g^{-1}). The highest *TPh* amount was obtained using the highest temperature (120 °C), with 3 cycles and a moderate duration of the process (40 min, experiment 12), whereas the lowest *TPh* was obtained when the extraction was performed longer (60 min), in 2 cycles and at a relatively low temperature (40 °C, experiment 2). Liu *et al.* reported that elevated temperature improves diffusion rates and solubility in extraction solvents.[10] According to

Herrero *et al.* ASE with high temperature is an effective way to increase the recovery of bioactive compounds.[11] Longer extraction times, higher extraction temperature, and more extraction cycles also resulted in higher *SA* and *RP* of the extracts. Both *SA* and *RP* reached the highest values (2615.99 and 1324.80 μmol TE 100 g^{-1}, respectively) when the extraction was performed using the parameters: 120 °C, 60 min and 2 cycles (experiment 4).

TABLE I. Experimental design, total carotenoid (*TCar*) and polyphenol (*TPh*) contents, radical scavenging activity (*SA*) and reducing power (*RP*) of carrot extracts; the results are presented as mean values of three replicates ± *SD*

Run	*t* / min (X_1)	*t* / °C (X_2)	*n*[a] (X_3)	*TCar*[b]	*TPh*[c]	*SA*[d]	*RP*[e]
1	20 (–1)	40 (–1)	2 (0)	8.98±0.01	94.21±0.98	404.30±1.59	278.05±0.48
2	60 (+1)	40 (–1)	2 (0)	11.07±0.02	78.51±0.13	473.96±10.38	274.82±0.30
3	20 (–1)	120 (+1)	2 (0)	24.55± 0.01	322.71±0.69	1135.87±3.01	997.85±6.53
4	60 (+1)	120 (+1)	2 (0)	24.20±0.01	470.01±3.03	2615.99±23.41	1324.80±5.27
5	20 (–1)	80 (0)	1 (–1)	22.01±0.01	127.79±0.37	619.30±6.90	569.97±1.91
6	60 (+1)	80 (0)	1 (–1)	23.72±0.01	157.11±0.43	532.43±1.29	730.67±8.40
7	20 (–1)	80 (0)	3 (+1)	24.24±0.01	154.88±0.07	658.47±1.12	483.99±2.28
8	60 (+1)	80 (0)	3 (+1)	30.57±0.01	232.15±0.37	949.60±0.02	649.73±0.85
9	40 (0)	40 (–1)	1 (–1)	8.42±0.01	87.26±0.50	733.54±1.07	192.66±1.40
10	40 (0)	120 (+1)	1 (–1)	24.14±0.03	454.65±0.60	2356.49±1.41	921.57±0.81
11	40 (0)	40 (–1)	3 (+1)	12.57±0.002	107.79±0.82	657.94±3.26	129.56±1.00
12	40 (0)	120 (+1)	3 (+1)	29.01±0.02	472.74±0.84	2071.03±10.79	1040.04±6.07
13	40 (0)	80 (0)	2 (0)	26.02±0.02	144.14±0.19	824.66±2.28	469.38±1.86
14	40 (0)	80 (0)	2 (0)	26.95±0.01	143.26±0.01	997.41±2.11	429.32±0.73
15	40 (0)	80 (0)	2 (0)	27.50±0.01	208.80±0.21	837.78±1.72	514.45±2.13

[a]Number of cycles; [b]mg *β*-carotene/100 g; [c]mg GAE/100 g; [d]μmol TE/100 g; [e]μmol TE/100 g

The experimental values of all quality indicators obtained in the optimization experiments (Table I) were analyzed by single and multi-response optimization and the results are reported in Table II and Figs. S-1 and S-2.

TABLE II. Single and multi response optimization of the extraction parameters

Optimization	Variable code			Variable value			Optimal response			
	X_1	X_2	X_3	X_1	X_2	X_3	*TCar*[a]	*TPh*[b]	*SA*[c]	*RP*[d]
Single response (*TCar*)	0	0.21	0.95	40	88.4	3	30.80	–	–	–
Single response (*TPh*)	1	1	–1	60	120	1	–	472.78	–	–
Single response (*SA*)	1	1	1	60	120	3	–	–	2572.28	–
Single response (*RP*)	1	1	0.59	60	120	3	–	–	–	1325.67
Multi response	1	1	1	60	120	3	28.84	530.81	2572.29	1336.26

[a]mg *β*-carotene/100 g; [b]mg GAE/100 g; [c]μmol TE/100 g; [d]μmol TE/100 g

It was found that single-cycle extraction applying a high temperature (120 °C) for a longer time (60 min) ensures the maximum extraction of carotenoids.

The optimal conditions maximizing *TPh* were lower temperature and duration, in several cycles. Considering sample 4 that showed the highest *SA* and *RP*, it could be concluded that the higher temperature (120 °C) and longer extraction time (60 min) are the main contributors to these responses.

The simultaneous optimization of multiple responses is the main concern for industrial applications, especially in view of energy cost reduction. The optimal extraction conditions for all observed responses were 120 °C for 60 min with three extraction cycles.

CONCLUSIONS

Response surface methodology (RSM) and Box–Behnken design were developed to determine the optimum process parameters of carrot ASE extraction. The optimal conditions to obtain the highest extraction yield of carotenoids and polyphenols in carrot extracts, as well as maximum antioxidant activity were: 120 °C, 60 min in 3 extraction cycles. Under the optimal conditions, the experimental values were in agreement with the predicted values.

SUPPLEMENTARY MATERIAL

Analysis of variance (ANOVA) of the modelled responses, as well as single- and multi--response optimization of the influence of extraction parameters on the total carotenoid contents (*TCar*), total polyphenol contents (*TPh*), scavenging activity (*SA*) and reducing power (*RP*) of carrot extracts are available electronically at the pages of journal website: http:// //www.shd.org.rs/JSCS/, or from the corresponding author on request.

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ИЗВОД

ОПТИМИЗАЦИЈА ЕКСТРАКЦИЈЕ БИОАКТИВНИХ ЈЕДИЊЕЊА ИЗ ШАРГАРЕПЕ РАСТВАРАЧИМА ПОД ПРИТИСКОМ МЕТОДОМ ОДЗИВНИХ ПОВРШИНА

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Шаргарепа се сматра богатим извором биоактивних антиоксидативних једињења, и липофилних (каротеноиди) и хидрофилних (полифеноли). У овом раду извршена је оптимизација екстракције биоактивних компонената из шаргарепе (*Daucus carota* L.) растварачима под притиском, употребом методе одзивних површина (RSM). Експерименти су планирани употребом Box–Behnken дизајна у циљу одређивања оптималне комбинације екстракционе температуре, времена и броја циклуса екстракције. Садржај укупних каротеноида (*TCar*), укупних полифенола (*TPh*), способност хватања радикала (*SA*) и редукциона способност (*RP*) добијених екстраката коришћени су као одзиви за оптимизацију. Узимајући у обзир ове одзиве добијени су следећи оптимални услови екстракције: 120 °C, 60 min и три екстракциона циклуса. Под овим експерименталним условима предвиђене вредности *TCar* (28,84 mg *β*-каротена на 100 g), *TPh* (530,81 mg

GAE на 100 g), *SA* (2572,29 μmol TE на 100 g) и *RP* (1336,26 μmol TE на 100 g) су добијене са високим фактором пожељности (0,975) и без статистички значајне разлике ($p < 0,05$) у поређењу са експериментално добијеним вредностима.

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