https://doi.org/10.26651/allelo.j/2023-60-1-1452 0971-4693/94 Euro 20.00
Allelopathy Journal 60 (1): 45-56 (September, 2023) *International Allelopathy Foundation* 2023
Table: 3, Figs: 3

Optimization of ultrasound-assisted aqueous 2-phase extraction system of carotenoid-allelochemicals from sweet potato roots by response surface methodology

Yi Kuang, Xiating Shen, Diego A. Sampietro[1,]* and Shengxiang Yang*
College of Chemical and Materials Engineering,
Zhejiang A & F University, Lin'an, Zhejiang Province, 311300, China;
E-mail: shengxiangyang2000@163.com, dasampietro@hotmail.com

(Received in revised form: June 30, 2023)

ABSTRACT

Orange sweet potato (*Ipomoea batata* L., Convolvulaceae) roots are an attractive source of β-carotene and other carotenoids. These compounds (allelochemicals) are used in the pharmaceutical, medicinal and food industries. In this study, the extraction of carotenoid-allelochemicals from roots of orange sweet potato cultivar 'pushu 32' was optimized by response surface methodology (RSM) in aqueous two-phase systems (ATPS) based on polymer/salt mixtures. Preliminary tests yielded higher contents of carotenoids in ATPS, based on sodium sulfate-PEG than on ammonium sulfate-PEG. Hence the effects of PEG-4000 concentration, sodium sulfate concentration, liquid-to-solid ratio and ultrasonic time were further investigated on carotenoids yield in single factor experiments. Four factors were used in a 3-level Box-Behnken design (BBD) of response surface model (RSM) to find the best conditions for carotenoid yield. Optimum carotenoids yield was obtained with 35.96 % (w/w) PEG-4000; 15.40 % (w/w) sodium sulfate; an ultrasonic time applied for 31.84 min and a liquid-to-solid ratio of 25.96:1 (v/w). Under these conditions, carotenoids yield was 0.594 mg/g which was very close to the predicted value (0.597 mg/g). The new proposed extraction method is promising for higher recovery of carotenoids from roots of orange sweet potatoes.

Keywords: Allelochemicals, aqueous two-phase system, β-carotene, carotenoids, *Ipomoea batata* L., response surface methodology, sweet potato roots.

INTRODUCTION

Sweet potato (*Ipomoea batata* L., Convolvulaceae) is a staple food crop. China has about 70 % of the total world area, hence, plays great role in ensuring food security (29). Its roots are rich in secondary metabolites such as carotenoids and anthocyanins (3,6,8). The carotenoids are abundant in yellow and orange-fleshed sweet potatoes, its variety Pushu 32 (Fig. 1) is rich in carotenoid and β-carotene (13). While purple roots are rich in anthocyanin (16). In plants, β-carotene and other carotenoids act as light-energy receptors/dissipators in photosynthesis as well as antioxidant agents and allelochemicals (25). β-carotene is used in pharmacy, medicine and the food industry as a natural colourant and antioxidant (11). Diets rich in β-carotene reduces the risk of heart diseases and some cancers (1). The β-carotene is also a precursor of vitamin A, which prevents several health disorders viz., the xerophthalmia and night blindness (12).

Biotechnologically advanced extraction methods of secondary metabolites from sweet potato are developed to extract anthocyanins and polyphenols but not carotenoids

*Correspondence author, [1]LABIFITO, Universidad Nacional de Tucumán, Ayacucho 471 (4000), San Miguel de Tucumán, Argentina.

(3,8,17,32). The traditional methods for carotenoids extraction from plant organs used organic solvents, supercritical fluids, microwaves, ultrasound and adsorption by coarse pored resins (4,5,14-16,21,23). These methods have many drawbacks (a) partial recovery of carotenoids from the extraction source, (b) large consumption of solvents and (c) degradation losses (15). Aqueous two-phase systems might improve carotenoid extraction from sweet potato roots. They are a promising tool to separate the natural products from plant sources (24,28), offer simple separation steps and absence of toxic residues in organic solvents (24). Aqueous two phase systems are widely used to purify various enzymes, nucleic acids, growth hormones and secondary plant compounds (18). They often contain polyethylene glycol (PEG), which is a non-toxic and non-volatile polymer, more environmentally friendly than organic solvents used in conventional extraction systems (2).

Figure 1. Orange-fleshed sweet potato roots variety Pushu 32.

Response surface methodology (RSM) is a multivariate regression analysis, it predicts the effects of several factors or independent variables provided at several levels on the response of a dependent or output variable (26). RSM also allows to know how factors interact each other in the response observed of an output variable and means an important advantage respect to data provided by the orthogonal method (22). It is widely used in extraction processes designed for plant metabolites (anthocyanins, phenolic compounds and oils) (14).

This research aimed to optimize carotenoid ultrasound-assisted aqueous two phase system extraction from sweet potato roots. Key extraction parameters (PEG-4000 concentration, sodium sulfate concentration, ultrasonic time and liquid-to-solid ratio) were optimized using the RSM methodology.

MATERIALS AND METHODS

Materials and Chemicals

This research was done in the College of Chemical and Materials Engineering, Zhejiang A&F University from September 2020 to September 2021. Roots of the orange sweet potato (*Ipomoea batatas* L.) cultivar pushu 32 were provided by the sweet potato research base of our University in September 2020. The roots were cut into small pieces and freeze dried at -40 °C for 48 h. The dry samples were pulverized, passed through a sieve of 40-60 mesh and stored at 4 °C for further use. Polyethylene glycol 4000 (PEG 4000) and sodium sulfate were obtained from Zhejiang Lanxi Chemical Reagent Factory (China). β-carotene was purchased in Sigma (China). All chemicals were of analytical grade.

Preparation of phase diagram

Phase diagrams were prepared for two ATPS by titration method using an aqueous salt solution (20 %, w/w sodium sulfate or 40 %, ammonium sulfate) and 48 % PEG 4000 (9). The first diagram was prepared by dropwise addition of sodium sulfate solution to PEG-4000 solution until the mixture becomes turbid, indicating the appearance of a biphasic system. Then, water drops were added until the mixture turned clear (monophasic system), thereafter the sodium sulfate solution was added dropwise till reach turbidity again. These additions were done under constant vortexing. The masses of sulfate solution and water required to reach each turbid point were determined in a balance and used to calculate the percentage of sodium sulfate and PEG 4000 as described elsewhere (2,7). The same procedure was used to prepare the binodal curve involving ammonium sulfate. The partition coefficient (K) and phase ratio (R) were calculated as under:

$$R = \frac{V_t}{V_b} K_c = \frac{C_t}{C_b}$$

Where, C_t and C_b : Concentrations of carotenoids in the top and bottom phase, respectively. V_t and V_b : Volumes of top and bottom phase.

The relative carotenoid recovery in the top phase (Y) was expressed in percentage and was determined as under:

$$Y = \frac{RK}{1+RK} \times 100\% \quad (3)$$

2.3. Single factor experiments

Preliminary experiments showed that salting out occurred when the salt concentration was above 20 % and precipitation occurred when PEG-4000 concentration was over 40 wt %. Accordingly, we select PEG-4000 concentrations of 25, 30, 35 and 40 % and sodium sulfate concentrations of 14, 16, 18 and 20 % to maximize the carotenoids extraction efficiency. Other two factors were the ultrasonic time at 20, 30, 40 and 50 min, and the liquid-to-solid ratio at 15:1, 20:1, 25:1 and 30:1. Ultrasonic extraction was done in ATPS at an operating power of 300W. Each extraction was done on a sweet potato sample of 0.5 g. After that, the mixture was filtered and centrifuged at 3000 rpm for 10 min. Concentration of carotenoids was estimated in the top phase at 450 nm using a microplate spectrophotometer (Epoch2, Bio Tek) from a 6-point standard calibration curve. Each time 3-replicates were used. The carotenoids content (ET) was calculated as under:

ET (mg/g) = CVD/W

Where, C : Concentration of carotenoids determined from the standard curve as mg β-carotene equivalents (mg/ml), V : Volume (ml), D : Dilution factor performed in the top phase before entering in the curve, and W : Sample weight (g).

Statistical analysis

RSM was used to determine the optimum condition for extraction of carotenoids based on the results recorded in the single factor experiment. Four independent factors viz., (A). PEG-4000 concentration, (B). Sodium sulfate concentration, (C). Liquid-to-solid ratio and (D). Ultrasonic time were studied, at three levels using a Box-Behnken design. Levels included in the design for each factor were set according to the results of single factor experiments. The statistical design and the data analysis were done using the Design Expert 8.0.6 (Stat-Ease, USA). The expt data were fitted by the second-order polynomial model:

$$Y = \beta_0 + \sum \beta_i X_i + \sum \beta_{ii} X_i^2 + \sum \beta_{ij} X_i X_j$$

Where, Y : Predicted response; β_0, β_i, β_{ii} and β_{ij} are regression coefficients for intercept, linear, quadratic and interaction effects, respectively; and X_i and X_j are the factors (20).

RESULTS AND DISCUSSION

Phase diagrams and selection of the ATPS

Phase diagrams were built with PEG-4000 and either sodium sulfate or ammonium sulfate to find which of their combinations will provide biphasic systems. Each binodal curve separates monophasic systems beneath the curve from biphasic systems located above (Figure 2). Concerning the sulfate salts tested, the binodal curves showed that ammonium sulfate had only a slightly higher phase-forming ability than sodium sulfate. Hence, the partition effectiveness of salts tested depended on the sulfate anion, irrespective of salt-forming cation. Investigations in other salt-PEG systems suggest the same (7,9,24). However preliminary experiments, where root slices were extracted with salt-PEG mixtures at salt conc of 16 or 20 % (w/w) combined with PEG conc of 25 % to 40 % (w/w), yielded higher carotenoids with sodium sulfate (0.354±0.012 mg/g) than with ammonium sulfate (0.217±0.012 mg/g). Thus we selected sodium sulfate/PEG-4000 ATPS for further studies.

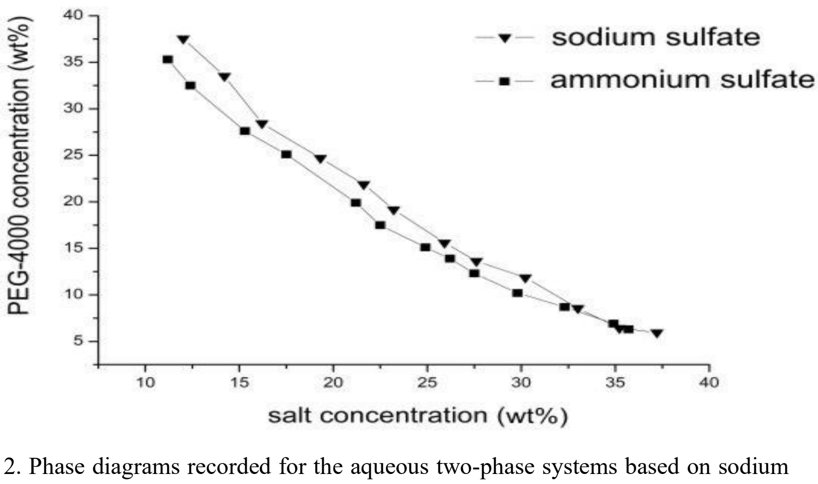

Figure 2. Phase diagrams recorded for the aqueous two-phase systems based on sodium sulphate-PEG 4000 and ammonium sulphate-PEG 4000.

Effects of single factors on carotenoids yield

Several carotenoids are absorbed in the wavelength range of 425 to 475 nm. Hence, the middle value of 450 nm was selected to measure their presence in the PEG-rich layers of the salt-PEG systems. This value agrees with maximum absorbance of β-carotene which is the main constituent of carotenoids fraction present in roots of sweet potato cv. Pushu 32. Figure 3 showed how carotenoids recovery varied with the change in PEG-4000 concentration (20-40 %), liquid-to-solid ratio (15:1-30:1), sodium sulfate concentration (14-20 %) and ultrasonic time (20-50 min). Factors not represented in each graph, were assigned at fixed levels at 30 % and 18 % concentrations for PEG-4000 and sodium sulfate, respectively, a liquid-to-solid ratio of 25:1 and an ultrasonic time of 30 min.

(A). PEG-4000 : Its addition favoured carotenoids solubility in the hydrophobic top phase till reached yield of 0.476 mg/g at 35 % (Figure 3). This advantage disappeared at 40 % PEG, where the high viscocity slowed down carotenoids diffusion from the bottom salt-rich phase to the PEG-rich layer (17,30). In case of liquid-to solid ratio, the contact area of the root samples increased with growing liquid volumes leading to the highest yield of 0.468 mg/g at ratio of 25:1 (v/w) (Figure 3). The fall in carotenoids content at higher liquid volumes could be due to oxidation or another degradative process.

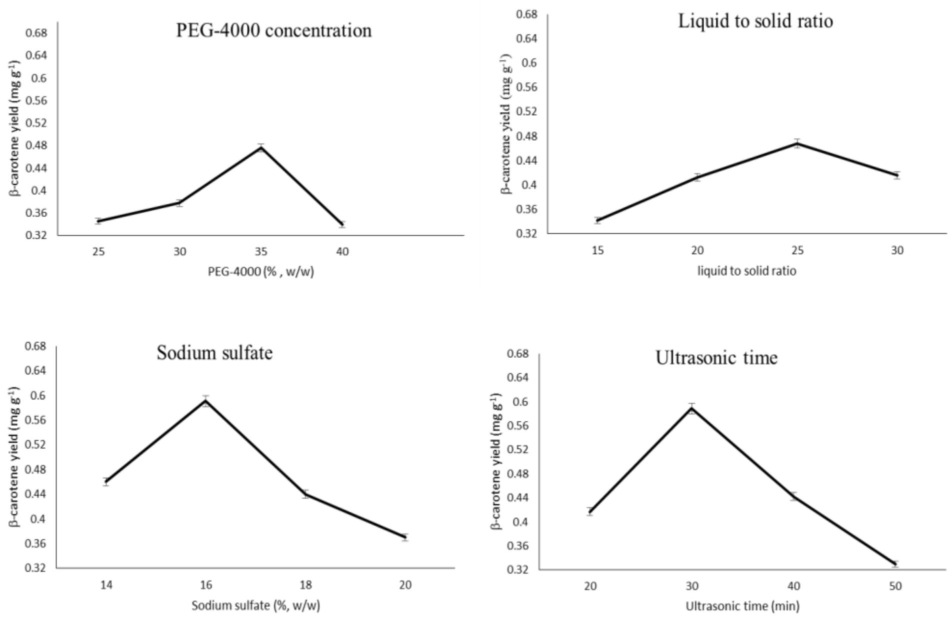

Figure 3. Carotenoids yields obtained in single factor experiments. Factors tested were PEG-4000 concentration, liquid-to-solid ratio, sodium sulfate concentration and ultrasonic time. Factors not represented in each graph were kept constant, with values of 30 % PEG-4000, 18 % sodium sulfate, liquid-to-solid ratio of 25:1, and ultrasonic time of 30 min. Carotenoid yields are expressed as mg β-carotene equivalents.

(B). **Sodium sulfate**: It was the single factor achieving the highest carotenoids retrieval (Figure 3). Carotenoids contents varied from 0.441 mg/g to 0.591 mg/g, when salt concentrations was changed from 14 to 16 %, respectively. The large amount of water absorbed by sodium sulfate at 16 % probably maximized PEG richness in the upper phase helps in carotenoids solubility (30). However, concentrations equal and higher than 18 % hinders carotenoids transfer to the top phase (31).

(C). Liquid to Solid Ratio: The contact area of the root samples increased with growing liquid volumes leading to maximum yield of 0.468 mg/g at ratio of 25:1 (v/w) (Figure 3). The decrease in β-carotene content at higher liquid volumes could be due to oxidation or some other degradative process.

(D). Ultrasonic time: It required 30 min to provide the best carotenoids retrieval (0.589 mg/g). The time was shorter to destroy cell compartmentalization in root samples, while took longer time to oxidize the carotenoids molecules (10,27).

The results of above single factor experiment led us to set the centerpoints and ranges for each BBD factor as under: **(A).** 35 % PEG (30-40 %), **(B).** Liquid-to-solid ratio of 25:1 (20:1-30:1, v/w), **(C).** 16 % Sodium sulfate (14-18 %) and **(D).** Ultrasonic time of 30 min (20-40 min).

Optimization of carotenoids extraction by BBD and predicted regression equation model

The carotenoids yields obtained are also indicated. The BBD consisted of 29-experiments, with 25-randomized runs (values of 1-25) and 4-replicates (values of 26-29) at the central point, to estimate the experimental error (Tables 1 and 2).

Table 1. Factors levels used in the Box-Behnken design.

#	**Factor**	**Levels**
A	PEG-4000	30, 35, 40 % w/w
B	Liquid-to-solid ratio	20:1, 25:1, 30:1
C	Sodium sulphate	14, 16, 18 %
D	Ultrasonic time	20, 30, 40 min

The regression model is as under:

$$Y = -6.40550 + 0.17218A + 0.25747B + 0.24777C + 0.11643D - 1.350 \times 10^{-3}AB + 3.80 \times 10^{-4}AC + 4.0 \times 10^{-5}AD + 4.0 \times 10^{-4}BC - 8.0\times10^{-4}CD - 2.284 \times 10^{-3}A^2 - 6.775 \times 10^{-3}B^2 - 8.185 \times 10^{-4}C^2 - 2.114 \times 10^{-3}D^2$$

Where, Y: Predicted carotenoids yield (mg/g), A : PEG concentration, B : Sodium sulfate concentration, C : Ultrasonic time and D : Liquid to solid ratio, respectively.

Table 2. Box-Behnken experimental design and the carotenoids yields.

Run	A: PEG 4000 (%, w/w)	B: Sodium sulfate (%, w/w)	C: Ultrasonic time (min)	D: Liquid to solid ratio (v/w)	Actual values of carotenoids yield (mg/g)
1	40 (+1)	18	30	25:1	0.432
2	30	16	30	20:1	0.394
3	30	16	40	25:1	0.454
4	35	14	40	25:1	0.502
5	35	16	20	30:1	0.444
6	35	16	40	20:1	0.406
7	40	14	30	25:1	0.546
8	35	14	30	30:1	0.570
9	40	16	30	20:1	0.446
10	30	16	20	25:1	0.408
11	30	16	30	30:1	0.476
12	35	18	30	30:1	0.526
13	35	16	20	20:1	0.382
14	35	16	40	30:1	0.528
15	40	16	20	25:1	0.412
16	35	18	30	20:1	0.468
17	40	16	40	25:1	0.534
18	30	14	30	25:1	0.522
19	40	16	30	30:1	0.532
20	35	18	40	25:1	0.458
21	30	18	30	25:1	0.462
22	35	14	30	20:1	0.480
23	35	16	30	25:1	0.578
24	35	14	20	25:1	0.484
25	35	18	20	25:1	0.408
26	35	16	30	25:1	0.578
27	35	16	30	25:1	0.596
28	35	16	30	25:1	0.566
29	35	16	30	25:1	0.578

The ANOVA (Table 3) indicated that A, B, C, D, AB, AC, A^2, C^2 and D^2 had significant participation in the quadratic regression model. The F-values revealed that significance of individual factors followed the order: Salt concentration > Ultrasonic time > PEG-4000 concentration > Liquid-solid ratio. PEG concentration significantly interacted with sodium sulfate and ultrasonic time to determine the carotenoids yields. The F-values indicated that the ionic strength provided by sodium sulfate was the main factor in carotenoids recovery. The same was concluded from the variables of the regression model, where B (sodium sulfate concentration) doubled the magnitude of A (PEG-4000 concentration) and C (ultrasound time), and was 6-folds higher than D, A x B or A x C.

Values of the adjusted R2 (89.76 %) and the predicted R2 (79.81 %) were very near to the determined R2 coefficient (94.86 %) demonstrating a strong match between the predicted and empirical carotenoids yields (9). Good model fitting was also reflected in a short standard deviation of 0.023 and a small variation coefficient of 4.62 (19).

Table 3. Analysis of variance (ANOVA) for response surface quadratic model

Source	Sum of Squares	Df	Mean Square	F-Value	p-value	Remarks
Model	0.11	14	7.79E-03	15.29	<0.0001	Significant
Linear Model						
A - PEG-4000 conc	9.86E-03	1	9.86E-03	19.37	0.0006	Significant
B - Sodium sulfate conc	0.021	1	0.021	40.92	<0.0001	Significant
C - Ultr. extraction time	0.01	1	0.01	20.05	0.0005	Significant
D - Liquid-solid ratio	2.88E-03	1	2.88E-03	5.66	0.0321	Significant
Two way interaction Model						
AB - PEG-4000 conc x sodium sulfate conc	0.030	1	7.29E-03	7.10	0.0351	Significant
AC - PEG-4000 conc x ultr. extraction time	0.021	1	1.44E-03	6.43	0.0398	Significant
AD - PEG-4000 conc x liquid-solid ratio	4.00E-06	1	4.00E-06	7.86E-03	0.9306	NS
BC -Sodium sulfate conc x ultr. extraction time	2.56E-04	1	2.56E-04	0.5	0.4899	NS
BD - Sodium sulfate conc x liquid-solid ratio	2.56E-04	1	2.56E-04	0.5	0.4899	NS
CD - Extraction time x liquid-solid ratio	9.00E-04	1	9.00E-04	1.77	0.2049	NS
Square Model						
A^2 - PEG-4000 conc x PEG-4000 conc	0.018	1	0.018	35.59	<0.0001	Significant
B^2 - Sodium sulfate conc x sodium sulfate conc	0.043	1	0.043	85.35	<0.0001	Significant
C^2 - Ultr. extraction time x ultr. extraction time	0.021	1	0.021	41.54	<0.0001	Significant
D^2 - Liquid-solid ratio x liquid-solid ratio	4.76E-03	1	4.76E-03	9.36	0.0085	Significant
Residual	7.13E-03	14	5.09E-04			
Lack of fit	6.67E-03	10	6.67E-03	5.79	0.0526	NS
Pure Error	4.61E-04	4	1.15E-04			
Cor Total	0.12	28				

Analysis of the response surface plots

Response surface plots for pair factors PEG-4000 x sodium sulfate (A x B) and PEG-4000 x ultrasonic time (A x C) are shown in Figure 4 together with their contour plots. Figure 3 predicts carotenoids yields ≥ 0.58 mg/g in the concentrations range of 34-38 % PEG-4000 and 13.61-15.92 % sodium sulfate. PEG concentrations needed to reach carotenoids yields

≥ 0.58 mg/g were reduced to the interval 34.67-36.90 %, when co-tested with ultrasonic time in the range 28.49-34.11 min. The regression equation predicted the maximum carotenoids yield as 0.597 mg/g under the following conditions: 35.96 % PEG, 15.40 % sodium sulfate, ultrasonic time of 31.84 min and liquid-solid ratio of 25.96:1.

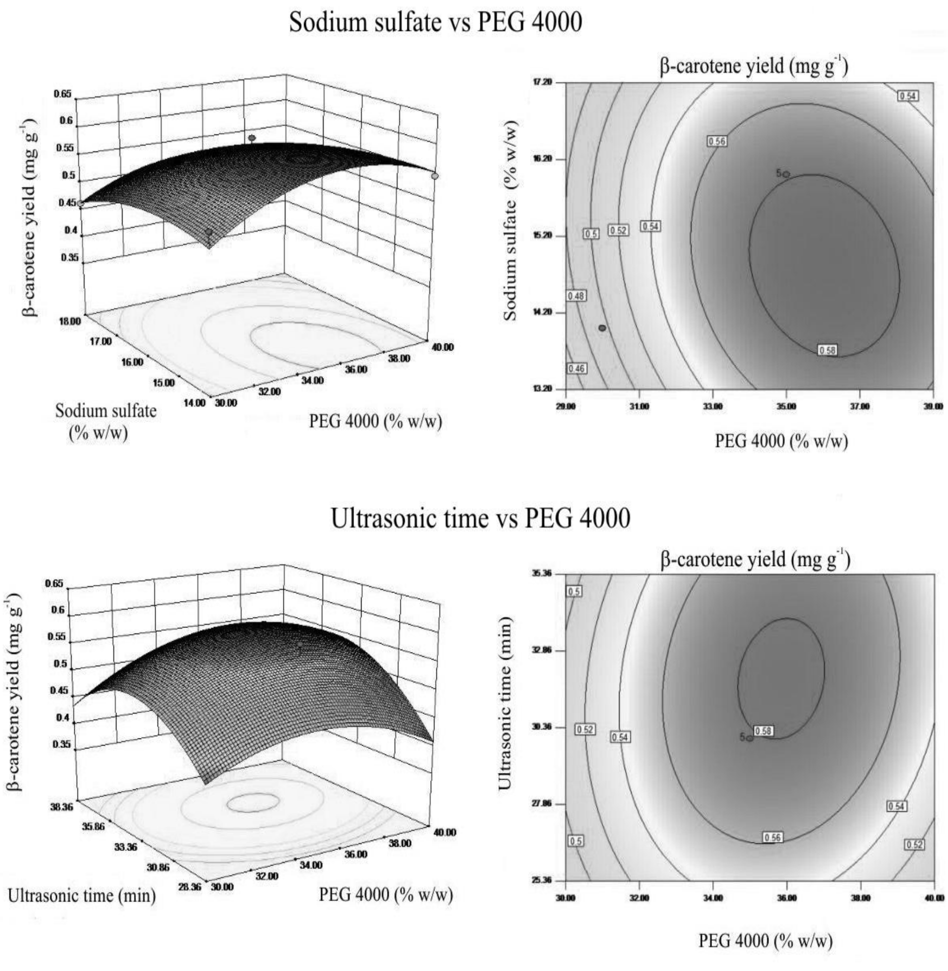

Figure 4. Surface and contour plots showing the interactive impact of factor pairs on β-carotene yields: PEG-4000 concentration vs sodium sulphate concentration and PEG-4000 concentration vs ultrasonic time. Liquid-to-solid ratio was 25:1 in both graphs, while ultrasound time of 30 min was in PEG-4000 concentration vs sodium sulphate concentration, and 16 % sodium sulphate in PEG-4000 concentration vs ultrasonic time. Carotenoid yields are expressed as mg β-carotene equivalents.

Verification of the model

The optimal extraction conditions defined in the regression equation previously shown needed to be empirically confirmed. They were slightly fixed to ensure their reproducibility. Hence, factors were set at 36 % of PEG 4000, 15 % sodium sulfate, an ultrasonic time of 31 min and a liquid-solid ratio of 26:1. These conditions provided a carotenoids yield of 0.594 mg/g, similar to the value predicted by the regression equation (0.597 mg/g). The carotenoids recovery was high, if we consider that carotenoids contents reported for roots of Pushu 32 are about 0.600 mg/g (13).

CONCLUSIONS

Four factors were optimized for carotenoids extraction from sweet potato roots in an aqueous two phase system through RMS. Sodium sulfate concentration was the main factor affecting the carotenoids recovery in both single factor experiments and the regression model obtained by the BBD design. A set of empirical conditions, 36 % (w/w) PEG 4000, 1 5% (w/w) sodium sulfate, ultrasonic time of 31 min and liquid-solid ratio of 26:1, established from the optimization process achieved the highest carotenoids yield. The optimized method is a potent tool to extract carotenoids from sweet potato roots and may be used on other plant materials.

ACKNOWLEDGEMENTS

This work was supported by the National Natural Science Foundation of China (No. 32271527), National Science & Technology Fundamental Resources Investigation Program of China (2018FY100800) and PICT 2019 3228 (National Agency for Science and Technology Promotion, Tucumán, Argentina).

DECLARATION

We declare that all authors of this manuscript have made substantial contributions. We did not exclude any author who substantially contributed to this manuscript. We have followed the ethical norms established by our institution.

CONFLICT OF INTEREST

The authors announce that they have no conflict of interest.

ETHICAL APPROVAL

The authors declare that the study was carried out following scientific ethics and conduct. However, this study did not involve any use of animals, hence no ethical approval has been obtained from the concerned committee.

REFERENCES

1. Akinosho, H.O. and Wicker, L. (2015). Stability of β-carotene loaded emulsions vary by viscosity of hydroxypropyl methylcellulose dispersions. *LWT - Food Science and Technology* **63**: 582-589.

2. Azevedo, A.M., Rosam, P.A.J., Ferreira, I.F. and Aires-Barros, M.R. (2009). Chromatography-free recovery of biopharmaceuticals through aqueous two-phase processing. *Trends in Biotechnology* **27**: 240-247.
3. Cai, Z., Qu, Z., Lan, Y., Zhao, S., Ma, X., Wan, Q., Jing, P. and Li, P. (2016). Conventional, ultrasound-assisted and accelerated-solvent extractions of anthocyanins from purple sweet potatoes. *Food Chemistry* **197**: 266-272.
4. Chumnanpaisont, N., Niamnuy, C. and Devahastin S. (2014). Mathematical model for continuous and intermittent microwave-assisted extraction of bioactive compound from plant material: Extraction of β-carotene from carrot peels. *Journal of Chemistry and Engineering Science* **116**: 442-451.
5. Davarnejad, R., Kassim, K.M., Zainal, A. and Sata, S.A. (2008). Supercritical fluid extraction of β-carotene from crude palm oil using CO_2. *Journal of Food Engineering* **89**: 472-478.
6. Drapal, M., Rossel, G., Heider, B. and Fraser, P. (2019). Metabolic diversity in sweet potato (*Ipomoea batatas*, Lam.) leaves and storage roots. *Horticulture Research* **6**: 2.
7. Fakhari, M.A., Rahimpour, F. and Taran, M. (2017). Response surface methodology optimization of partitioning of xylanase form *Aspergillus Niger* by metal affinity polymer-salt aqueous two-phase systems. *Journal of Chromatography B* **1063**: 1-10.
8. Filho, E.G., Sousa, V., Rodrigues, S., de Brito, E. and Fernandez, F. (2020). Green ultrasound-assisted extraction of chlorogenic acids from sweet potato peels and sonochemical hydrolysis of caffeoylquinic acids derivatives. *Ultrasonics Sonochemistry* **63**: 104911.
9. Glyk, A., Solle, D., Scheper, T. and Beutel, S. (2015). Optimization of PEG-salt aqueous two phase systems by design of experiments. *Chemometrics and Intelligent Laboratory Systems* **149**: 12-21.
10. González-Centeno, M.R., Kai, K., Sabarez, H., Simal, S., Rosselló, C. and Femenia, A. (2014). Effects of acoustic frequency and power density on the aqueous ultrasonic-assisted extraction of grape pomace (*Vitis vinifera* L.)-a response surface approach. *Ultrasonics Sonochemistry* **21**: 2176-2184.
11. Hou, Z., Zhangm, M., Lium, B., Yanm, Q., Yuanm, F., Xum, D. and Gao, Y. (2012). Effects of chitosan molecular weight on the stability and rheological properties of β-carotene emulsions stabilized by soybean soluble polysaccharides. *Journal of Food Hydrocolloids* **26**: 205-211.
12. Islam, S.N., Nusrat, T., Begum, P. and Ahsan, M. (2016). Carotenoids and β-carotene in orange fleshed sweet potato: A possible solution to vitamin A deficiency. *Journal of Food Chemistry* **199**: 628-631.
13. Jia, R., Zhang, R., Gangurde, S.S., Tang, C., Jiang, B., Li, G. and Wang, Z. (2022). Integrated analysis of carotenoid metabolites and transcriptome identifies key genes controlling carotenoid compositions and content in sweet potato tuberous roots (*Ipomoea batatas L.*). *Frontiers in Plant Science* **13**: 993682.
14. Jiang, H.L., Yang, J.L. and Shi, Y.P. (2017). Optimization of ultrasonic cell grinder extraction of anthocyanins from blueberry using response surface methodology. *Ultrasonics Sonochemistry* **34**: 325-331.
15. Kyriakopoulou, K., Papadaki, S. and Krokida, M. (2015). Life cycle analysis of β-carotene extraction techniques. *Journal of Food Engineering* **167**: 51-58.
16. Lien, C., Chan, C., Huang, C., Lai, Y. and Liao, W.C. (2021). Studies of carotene extraction from sweet potato variety CYY95-26, *Ipomoea batatas* L. *International Journal of Food Engineering* **8**: 1-14.
17. Lien, C., Chan, C., Lai, Y., Huang, C. and Liao, W.C. (2012). Ultrasound assisted anthocyanin extraction of purple sweet potato variety TNG73, *Ipomoea batatas*, L. *Separation Science and Technology* **47**: 1241-1247.
18. Lin, L., Yang, W., Wei, X., Wang, Y., Zhang, L., Zhang, Y., Zhang, Z., Zhao, Y. and Zhao, M. (2019). Enhancement of solasodine extracted from fruits of *Solanum nigrum* L. by microwave-assisted aqueous two-phase extraction and analysis by high-performance liquid chromatography. *Molecules* **24**: 2294.
19. Liu, J., Yan, M., Zhang, Y.K. and Du, K.F. (2011). Study of glutamate-modified cellulose beads for Cr(III) adsorption by response surface methodology. *Industrial Engineering and Chemistry Research* **20**: 10784-10791.
20. Martínez-Patiño, J.C., Gullón, B., Romero, I., Ruiz, E., Brnčić, M., Šic Žlabur, J. and Castro, E. (2019). Optimization of ultrasound-assisted extraction of biomass from olive trees using response surface methodology. *Ultrasonics Sonochemistry* **51**: 487-495.
21. Mojaat, M., Foucault, A., Pruvost, J. and Legrand, J. (2008). Optimal selection of organic solvents for biocompatible extraction of β-carotene from *Dunaliella salina*. *Journal of Biotechnology* **133**: 433-441.
22. Niizawa, I., Espinaco, B.Y., Zorrilla, S. and Sihufe, G.A. (2019). Natural astaxanthin encapsulation: Use of response surface methodology for the design of alginate beads. *International Journal of Biological Macromolecules* **121**: 601-608.

23. Purohit, A.J. and Gogate, P.R. (2015). β-Carotene from waste carrot residue: Effects of operating parameters and type of ultrasonic irradiation. *Separation Science and Technology* **50**: 1507-1517.
24. Phong, W.N., Show, P.L., Chow, Y.H. and Ling, T.C. (2018). Recovery of biotechnological products using aqueous two phase systems. *Journal of Bioscience and Bioengineering* **126**: 273-281.
25. Sasamoto, H., Suzuki, S., Mardani-Korrani, H., Sasamoto, Y. and Fujii, Y. (2021). Allelopathic activities of three carotenoids, neoxanthin, crocin and β-carotene, assayed using protoplast co-culture method with digital image analysis. *Plant Biotechnology* (Tokyo) **38**: 101-107.
26. Singla, M. and Sit, N. (2023). Theoretical aspects and applications of aqueous two-phase systems. *Chemical Bio Engineering Reviews* **10:** 65-80.
27. Song, J.F., Li, D.J., Pang, H.L. and Liu, C.Q. (2015). Effects of ultrasonic waves on the stability of all-*trans* lutein and its degradation kinetics. *Ultrasonics Sonochemistry* **27**: 602-608.
28. Walter, H., Krob, E.J. and Brooks, D.E. (1796). Membrane surface properties other than charge involved in cell separation by partition in polymer, aqueous two-phase systems. *Journal of Biochemistry* **15**: 2959-2964.
29. Wang, S., Nie, S. and Zhu, F. (2016). Chemical constituents and health effects of sweet potato. *Journal of Food Research International* **89**: 90-116.
30. Wang, C., Zhang, Z., Wang, Q., Wang, J. and Shang, L. (2023). Aqueous two-phase emulsions toward biologically relevant applications. *Trends in Chemistry* **5**: 61-75.
31. Yu, L., Zhang, H., Yang, L. and Tian, K. (2019). Optimization of purification conditions for papain in a polyethylene glycol-phosphate aqueous two-phase system using quaternary ammonium ionic liquids as adjuvants by BBD-RSM. *Protein Expression and Purification* **156**: 8-16.
32. Zhu, Z., Jiang, T., He, J., Barba, F.J., Cravotto, G. and Koubaa, M. (2016). Ultrasound-assisted extraction, centrifugation and ultrafiltration: multistage process for polyphenol recovery from purple sweet potatoes. *Molecules* **21**:1584.

PUBLISHER NOTE