https://doi.org/10.17221/26/2024-CJFS

Ultrasound-assisted ionic liquids extraction of carotenoids from Xinjiang apricots and evaluation of their antioxidant potential

Xiaohui Sun, Wanhui Guo, Na Jiang, Shuangyu Cao, Lei Ma, Shenghong Liu**

Key Laboratory of Agro-products Quality and Safety of Xinjiang, Institute of Quality Standards & Testing Technology for Agri-products, Xinjiang Academy of Agricultural Sciences, Urumqi, P.R. China.

**Corresponding authors: mal0416@163.com; shliu1028@163.com*

Citation: Sun X., Guo W., Jiang N., Cao S., Ma L., Liu S. (2025): Ultrasound-assisted ionic liquids extraction of carotenoids from Xinjiang apricots and evaluation of their antioxidant potential. Czech J. Food Sci., 43: 398–410.

Abstract: Xinjiang apricot is favoured by consumers because of its distinctive aroma, high nutritive value, and abundant functional active substances. Carotenoids of apricot are efficient antioxidants that can protect the human body from free radical attack. However, the extraction, quantification, and antioxidant activity of carotenoids from Xinjiang apricots have not been reported. In this work, ultrasound-assisted ionic liquid (ILs) extraction and optimisation of carotenoids from Xinjiang apricots and to evaluate their antioxidant potential. Based on Box–Behnken design (BBD), the best conditions were IL/ethanol ($R_{IL/E}$) ratio of 1 : 2, solid-liquid ratio ($R_{S/L}$) of 1 : 3, extraction time of 17 min and number of extractions of 3. The content of carotenoid extracted by ultrasonic-assisted [Bmim][BF_4] ILs was 32.98 ± 0.27 μg·g^{-1} that of traditional extraction method was 25.05 ± 0.35 μg·g^{-1}. Moreover, ultrasonic-assisted ILs extraction technology can shorten the extraction time, simplify the extraction steps and increase the extraction amount. Meanwhile, in order to recover and reuse ILs, ILs-ethanolic solution was frozen at temperatures lower than −80 °C, allowing the ILs precipitation and separation from the ethanol solution. Meantime, the antioxidant potential of five Xinjiang apricot varieties were evaluated by DPPH (2,2-diphenyl-1-picrylhydrazyl) and ABTS [2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)] assays *in vitro* and analysed by UV–vis spectroscopy and Raman spectroscopy. The results showed Shushanggan apricot has the highest carotenoid content and the strongest antioxidant activity. In conclusion, this research further proves the advantage of ultrasonic-assisted ILs in carotenoid extraction and the potential to obtain valuable carotenoids from the apricot industries.

Keywords: *Prunus armeniaca L.;* response surface design; ultrasonic-assisted ILs extraction; antioxidant activity

Apricot (*Prunus armeniaca* L.) is an edible drupe fruit described as 'medicine food homology' and is cultivated worldwide. It is worth noting that the apricot originated in China, with its centre of origin lying in central Asia including Xinjiang, China. Therefore, as an important origination centre, Xinjiang has almost all of the apricot cultivars in China, including Shushanggan, Liguang, Xiaobai, Saimaiti, Jianali, and Dajie. These apricot cultivars have become increasingly popular with consumers because of their distinct aroma, excellent taste, attractive appearance, and high nutritional value. More strikingly, Xinjiang apricots contain

Supported by an open fund project of Key Laboratory of Agro-products Quality and Safety of Xinjiang (xjnkywdzc-2023003-pt2) and Xinjiang dry fruit quality evaluation and plant active substances research team ability to improve Xinjiang key research and development projects (2022B30006).

https://doi.org/10.17221/26/2024-CJFS

abundant carotenoids, polyphenolic compounds, flavonoids, minerals, volatiles, organic acids, and vitamins (Farag et al. 2022). Among them are carotenoids, which are the most common natural pigments and antioxidants and include lycopene, α-carotene, β-carotene, lutein, and zeaxanthin (Nabi et al. 2020). Zhou et al. (2020) reported that the carotenoid content of 41 Xinjiang apricot cultivars varied from 2.536 to 98.179 $\mu g \cdot g^{-1}$. This result shows that Xinjiang apricot fruits have a rich content of carotenoids. However, the procedure of carotenoid extraction is tedious and time-consuming. At present, most commercial products of carotenoids are chemically synthesised, although little percentages are acquired from natural sources. Nevertheless, consumers prefer natural products over chemically synthesised compounds, which in some cases exhibit high toxicity, carcinogenicity, and teratogenicity (Cheng et al. 2020). This fact has increased interest of researchers in the extraction of carotenoids from natural sources such as *Lycium chinensis*, orange peel, and apricot. Solvent extraction is the most typical and widely used method, but it requires a long extraction time, uses significant amounts of solvents, and needs high temperature (Alara et al. 2018) However, the high temperature can cause rapid degradation of carotenoids and lead to isomerisation of *trans*-carotenoids to the *cis* form, thereby promoting the slight loss of colour and a reduction of provitamin activity (Aman et al. 2005). Therefore, the development of a sustainable and economical extraction method is an indispensable part of carotenoid recovery strategy. Ionic liquids are considered as green solvents for carotenoid extraction mainly because of their unique features such as high hydrolytic activity, low volatility, high solvation capacity, and excellent thermal and chemical stabilities. For example, the carotenoids from orange peel were extracted using a 1-butyl-3-methylimidazolium chloride IL via ultrasound-assisted extraction (Murador et al. 2019). de Souza Mesquita et al. (2021) utilised $[C_4mim][BF_4]$-ethanolic solution to achieve the maximum content of carotenoids from peach palm freeze-dried pulp. Zhao et al. (2024) adopted ionic liquids in synergy with ultrasound-enzyme-assisted extraction resveratrol from *Polygonum cuspidatum* (*P. cuspidatum*), the yield of resveratrol reached its maximum value, with a yield of 2.90 ± 0.15 $mg \cdot g^{-1}$. As indicated above, IL extraction is a simpler, more cost-effective, safer method than traditional organic-solvent extraction methods. In order to increase the extraction yield of carotenoids, IL extraction was used by combining microwave-assisted, ultrasound-assisted, supercritical fluid, and enzyme-assisted methods. In recent years, response surface methodology (RSM) has been successfully used in testing process parameters and their interactive effects. Among the RSM techniques, the central composite design (CCD) and Box–Behnken design (BBD) are the most commonly used. The advantage of BBD is that it obviates conducting experiments under extreme conditions because the model proposed by the combinations does not consider the study of the factors in their higher and lower limits simultaneously (Manzato et al. 2018). At present, BBD has been widely used in pharmaceuticals, bioprocessing, food engineering, agrochemicals, and other industries to extract biologically active compounds intended for human use, such as polysaccharides, phenolic compounds, anthocyanins and carotenoids (Sanahuja et al. 2024).

Thus, this study aims to establish an efficient and recyclable method for extracting carotenoids from Xinjiang apricots using ultrasonic-assisted ILs and evaluate its antioxidant capacity. Besides, in order to maximise the yield of carotenoids, the BBD response surface method was used to design 27-run experiments (4-factor, 3-level) to determine optimum levels for extraction parameters, including IL/ethanol ($R_{IL/E}$) ratio, solid-liquid ratio ($R_{S/L}$), time of extraction, and number of extractions. Meanwhile, a comparative study of antioxidant properties of five Xinjiang apricots by using DPPH (2,2-diphenyl-1-picrylhydrazyl) and ABTS [2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid)] *in vitro* assays. Raman spectroscopy was first used to display the reaction of ABTS with antioxidants, which can prevent colour interference with the reaction.

MATERIAL AND METHODS

Chemicals and standards

HPLC-grade methanol, ethanol, acetonitrile, petroleum ether, and methylene dichloride were purchased from Fisher Chemical (Shanghai, China). The standard all-*trans*-β-carotene (≥ 99.5% purity) was purchased from Anpel-Trace Standard Technical Services (Shanghai, China). The 1-Butyl-3-methylimidazolium tetrafluoroborate ([Bmim][BF4]), 1-hexyl-3-methylimidazolium chloride ([Hmim][Cl]), 1-Butyl-3-methylimidazolium chloride ([Bmim][Cl]), ascorbic acid (AA) and potassium hydroxide (KOH) were purchased from Aladdin (Shanghai, China). DPPH and ABTS were purchased from Macklin Reagent (Shanghai, China). All other reagents were of analytical grade.

Raw materials

The five Xinjiang apricot cultivars were Xiaobai, Mingxing, Shushanggan, Saimaiti, and Liguang. They

https://doi.org/10.17221/26/2024-CJFS

were respectively harvested from Luntai county, Aksu; Pishan county, Hotan; Khorgos, Ili kazahk autonomous prefecture; Yingjisha county, Kashgar; Turpan; Xinjiang, China. These apricots were harvested at commercial maturity stage around June to August every year. After harvest, the apricots were frozen in liquid nitrogen and stored at –80 °C in tightly closed packages until analysis. In the extraction experiment, the apricots were pureed in a blender.

Experimental Section

Ultrasound-assisted ionic liquids extraction of carotenoids and single-factor experimental design. In the preliminary tests, five different extraction solvents (acetone, ethanol, and ethanol-based ILs solutions ([Bmim][BF_4], [Hmim][Cl] and [Bmim][Cl] in ethanol at 1 : 2 ratio) were initially used to extract carotenoids. Details of the method are as follows. 1 g fresh apricot (Saimaiti) was put into contact with 3 mL of extraction solvent, the reaction lasted for 5 min under an ultrasonic power of 100%, a working frequency of 25 kHz, and a temperature of 25 °C (SK-36TC, Shanghai Kedao Ultrasonic Instrument Co., Ltd., China). Then superntant was centrifuged and transferred to another tube. The extraction step was then repeated three times and transferred to a petroleum ether solution. Afterward, the extracting solution was concentrated in a Termovap sample concentrator (Li Chen Technology Co., Ltd., China) at 37 °C.

Single-factor experimental design was used for the effect of type of IL, $R_{IL/E}$, $R_{S/L}$, ultrasound-assisted extraction time, and number of extractions on the yield of carotenoids.

BBD to optimise the carotenoid extraction process. BBD was used to optimise and evaluate the maximum percent yield of carotenoids from Saimaiti apricot, as shown in Table 1. Moreover, a 27-run experiment (4-factor, 3-level) was conducted to determine their optimum levels. The experimental designs of the code created by using the program Design Expert 13, as well as the actual levels of each factor, are shown in Table S1.

Traditional method of extracting carotenoids. According to GB 5009.83-2016, accurately weigh 5 g (precise to 0.001 g) of a uniformly mixed apricot sample, add 1 g of AA and 75 mL of ethanol, oscillate in a water bath at 60 °C for 30 min. Then, add 25 mL of KOH solution (1 g·mL^{-1}) and place it in a preheated constant-temperature oscillating water bath at 60 °C for saponification for 30 min. Remove and let it stand to cool to room temperature. Transfer the saponified solution into a 500 mL separatory funnel, add 100 mL of petroleum ether, oscillate at room temperature for 10 min before allowing it to stand for phase separation. Transfer the aqueous phase into another separatory funnel and repeat the above steps three times. Combine the organic phases and wash with water until near-neutral pH. Discard the aqueous phase, and dehydrate the organic phase by filtration through anhydrous sodium sulphate. Collect the filtrate in a 500 mL evaporation flask and concentrate under reduced pressure at 37 °C using a rotary evaporator until nearly dry, then accurately add 5.0 mL of dichloromethane to fully dissolve the extract. After filtration through a 0.45 μm membrane, discard the initial approximately 1 mL of filtrate and collect the remaining solution into an injection vial for further use.

HPLC analysis of carotenoid content. Extracted carotenoids were expressed as β-carotene (μg·g^{-1} of fresh weight). In order to measure precisely the amount of carotenoids, the precipitate was dissolved in 1 mL of dichloromethane and filtered through a 0.45 μm membrane. HPLC analysis was performed using an Agilent Technologies 1260 series liquid chromatograph equipped with an autosampler and

Table 1. Actual and coded levels of the variables studied in Box–Behnken design

Variables	Levels		
	low (–1)	middle (0)	high (+1)
Independent variables			
X_1: proportion [Bmim][BF_4]/ethanol ($R_{IL/E}$), (g·mL^{-1})	1 : 1	1 : 2	1 : 3
X_2: solid-liquid ratio ($R_{S/L}$), (g·mL^{-1})	1 : 1	1 : 2	1 : 3
X_3: time of extraction (min)	10	15	20
X_4: number of extraction	3	4	5
Dependent variables			
Y: carotenoid content (μg·g^{-1})	–	–	–

https://doi.org/10.17221/26/2024-CJFS

a UV detector for the chromatographic separation. Chromatographic conditions: XBridge C18 column (5 μm, 250 mm × 4.6 mm, Waters) and dichloromethane/acetonitrile/methanol (3 : 12 : 85, v/v) as the mobile phase, detection wavelength of 450 nm, column temperature of 40 °C, and injection volume of 20 μL at a flow rate of 1.0 mL·min^{-1}.

Antioxidant activity assay in vitro. Apricot ethanol extract: according to the extraction of carotenoids, the extract was dissolved in 10 mL of ethanol and stored in the dark.

DPPH assay. The assay was based on a previously reported method (Yang et al. 2024) with only minor modifications. In brief, 0.2 mmol·L^{-1} DPPH radical solution was precisely prepared and stored in the dark. Subsequently, 2.5 mL of DPPH and 2.5 mL of apricot ethanol extracts were mixed and allowed to react for 30 min in the dark. The absorbance and absorption spectra were obtained at 517 nm and a wavelength range of 200–800 nm by utilising a UV-viz spectrophotometer (T700; Persee, Beijing, China). The radical-scavenging activity (RSA) was calculated using

$$RSA\ (\%) = \left(1 - \frac{A_1 - A_0}{A_2}\right) \times 100 \quad (1)$$

Equation (1):
where: A_1 – the absorbance of the mixture (apricot ethanol extract was mixed with the DPPH radical solution); A_0 – the absorbance of the apricot ethanol extract; A_2 – the absorbance of the control group.

ABTS assay. ABTS+ radical cation solution (7.0 mmol·L^{-1} ABTS and 2.45 mmol·L^{-1} $K_2S_2O_8$ in ethanol/water, 50 : 50, v/v) was prepared and incubated in the dark at 25 °C overnight. The solution was then diluted with 0.1 mol·L^{-1} PBS (phosphate buffered saline) solution (pH = 7.4) to achieve an absorption of 1.0 ± 0.05 at 734 nm. Next, 4.5 mL of diluted ABTS+ radical cation solution was mixed with 1.5 mL of sample solution for 5 min, and the absorbance and absorption spectra were recorded at 734 nm and wavelength range of 200–800 nm by utilising a UV-viz spectrophotometer (Yang et al. 2024).

Raman spectroscopy has also been used to identify and characterise the redox behaviour of ABTS+ in the presence of antioxidants. Therefore, 600 μL of diluted ABTS+ radical cation and 200 μL of apricot ethanol extract were mixed for 0, 5, 15, and 30 min and analysed with a 785 nm laser excitation.

The chemical reaction is described by Equation (2):

$$\begin{aligned} &\text{ABTS} \xrightarrow{\text{oxidant}(K_2S_2O_8)} \text{ABTS}+ \\ &+\text{radical}\ (\text{dusty blue}) \xrightarrow{\text{attenuation}} \text{ABTS}+ \\ &+\text{radical}\ (734\ \text{nm, blue}-\text{green}) \xrightarrow{\text{antioxidant}} \\ &\text{decrease in the colour of ABTS}+\ \text{radical} \end{aligned} \quad (2)$$

Data statistics and analysis. Three replicates were set for all experiments in this paper, with the data as the average of the repeated trials. The response surface optimisation test data were analysed by quadratic regression and variance analysis using Design-Expert 13.0 software, and other test results were processed by Origin 2021 software.

RESULTS AND DISCUSSION

Single-factor experiments for extraction of carotenoids. To investigate the effect of extraction solvents on the extraction yield of carotenoids, acetone, ethanol, and three ILs ([BMIM][Cl], [BMIM][BF_4], and [HMIM][Cl]) were used to extract carotenoids of Saimaiti apricot. The $R_{S/L}$, extraction time, and number of extractions were fixed at 1 : 2, 5 min, and 3, respectively. Moreover, for the construction of calibration curves, standard solution of β-carotene was diluted to achieve the desired concentrations for the research. The concentrations ranged from 0.5 to 50.0 μg·mL^{-1} (R^2 = 0.999) following the equation $y = 274.85x - 66.913$, where: y – carotenoids content (μg·g^{-1}); x – sample concentration (μg·mL^{-1}). Moreover, three independent injections were performed to calculate the SD. β-carotene contents of Saimiaiti apricot from ultrasound-assisted extraction were 6.60 ± 0.037, 6.23 ± 0.33, 7.88 ± 0.68, 11.83 ± 0.51, and 11.07 ± 0.63 μg·g^{-1}, respectively (Figure 1A). The results show that ILs were significantly more efficient than acetone and ethanol, resulting in higher carotenoid yields probably because of the low surface tension and viscosity of ILs (Cheng et al. 2020). The reason is the greatest affinity between the carotenoids and ILs due to π–π, n–π, hydrophobic, and hydrogen-bond interactions (Bi et al. 2010). Low surface tension is essential for the solvent to better dissolve the plant matrix. Moreover, [BMIM][BF_4] is superior to [HMIM][Cl] because [HMIM][Cl] displays high viscosity with the simultaneous increase in alkyl chain length (Kateryna et al. 2020). However, the IL with high viscosity may be unfavourable to the destruction of cellulose cells of apricot, which leads to the decrease in carotene content. Thus, as the best extraction solvent, [BMIM][BF_4] was used in subsequent

https://doi.org/10.17221/26/2024-CJFS

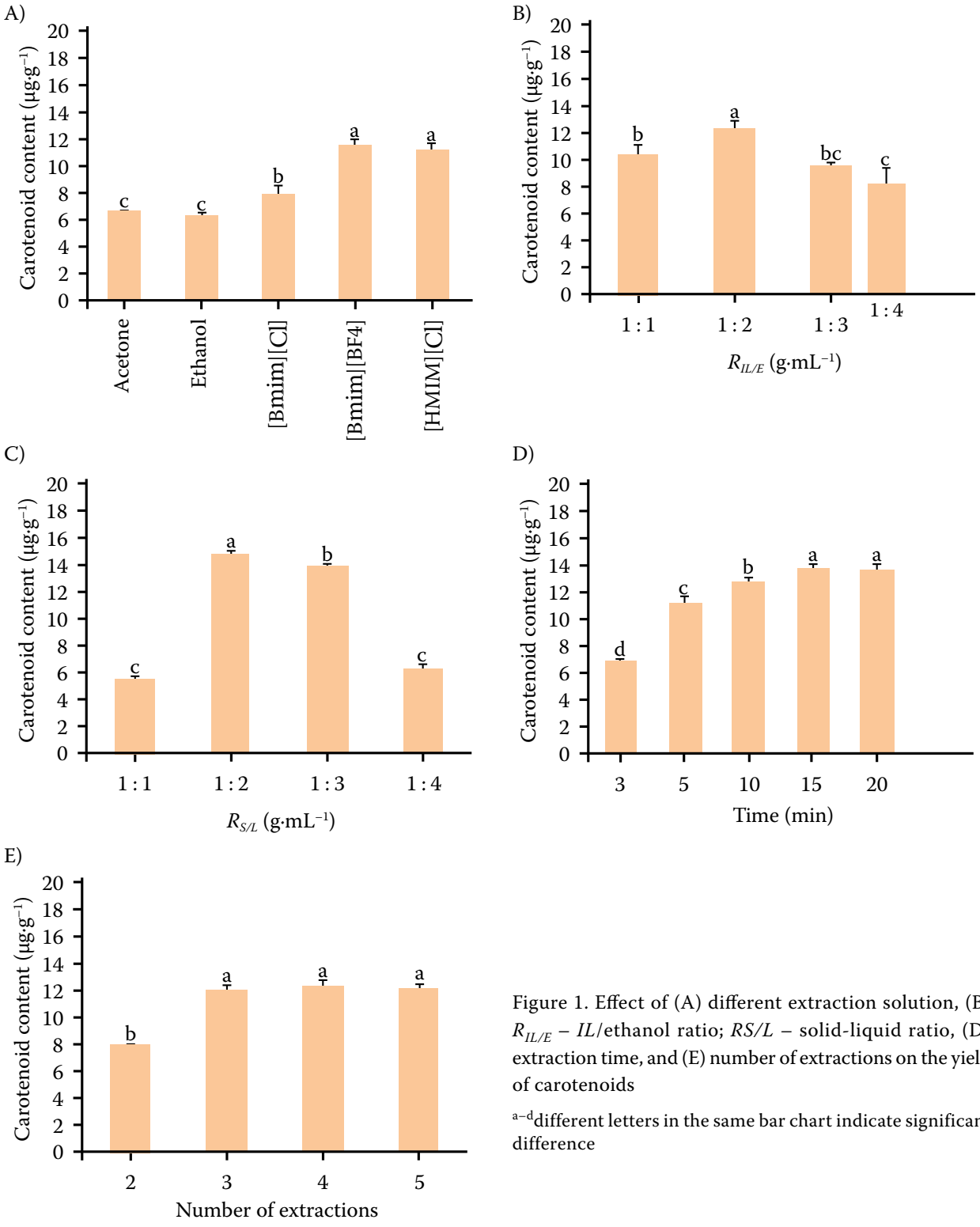

Figure 1. Effect of (A) different extraction solution, (B) $R_{IL/E}$ – *IL*/ethanol ratio; *RS/L* – solid-liquid ratio, (D) extraction time, and (E) number of extractions on the yield of carotenoids

[a–d]different letters in the same bar chart indicate significant difference

optimisation experiments. The proportion of [BMIM][BF_4] ILs and ethanol is another key factor that affects the extraction yield of carotenoids. In this experiment, the $R_{S/L}$, extraction time, and number of extractions were fixed at 1 : 3, 5 min, and 3 times, respectively. Carotenoid contents were 10.34 ± 0.77, 12.30 ± 0.65, 9.49 ± 0.28, and 8.60 ± 0.27 μg·g^{-1} when the $R_{IL/E}$ were 1 : 1, 1 : 2, 1 : 3, and 1 : 4, respectively (Figure 1B). The carotenoid content increased first and then decreased with the gradual increase in ethanol concentration.

https://doi.org/10.17221/26/2024-CJFS

Therefore, the proportion of [BMIM][BF_4] and ethanol was fixed at 1 : 2.

Different solid-liquid ratios were investigated in relation to carotenoid yield while the other variables, extraction time and number of extractions, were set at 5 min and 3 extraction cycles. The extraction ratio of carotenoids increased first and then decreased as the solid-liquid ratio rose from 1 : 1 to 1 : 4. The carotenoid yields are 5.63 ± 0.15, 14.875 ± 0.14, 13.99 ± 0.099, and 6.236 ± 0.42 μg·g^{-1} (Figure 1C). This result is due to the increase in contact surface area between apricot pulp and IL solution, which could enhance the solid/liquid concentration gradient as the driving force and thus increase the diffusion rate of the extraction (Yang et al. 2024). However, due to the possible saturation of carotenoids dissolved in the IL solution, the extraction ratio tended to decrease because the solid-liquid ratio continued to increase. Hence, as a significant factor, a solid-liquid ratio of 1 : 2 was chosen as the best ratio.

The extraction time and number of extractions were further studied (Figures 1C and 1D). The carotenoid content increased as the time of ultrasound-assisted extraction was extended from 5 to 20 min, the contents were 13.65 ± 0.33 and 13.59 ± 0.31 μg·g^{-1} for 15 and 20 min, respectively. Interestingly, the content decreased to 12.51 ± 0.024 μg·g^{-1} when the extraction time was 30 min (Figure 1D). Considering the time cost and economic value, we fixed the extraction time at 15 min. In addition, the number of extractions is a significant factor of carotenoid yield. The samples were extracted with IL/ethanol solution for different numbers of extractions (2, 3, 4, and 5; Figure 1E). The results show that the yield of carotenoids had an obvious increase with the number of extractions (3–5), which correspond to 11.980 ± 0.26, 12.209 ± 0.47, and 12.103 ± 0.28 μg·g^{-1}. The yield of carotenoids was the largest when the number of extractions was 4.

Optimisation of carotenoid extraction. RSM is an empirical modelling technique that is more advantageous than the traditional single-parameter optimisation, which can save time, space, and raw material. According to the single-factor experiments, a total of 27 runs in the BBD experiment were used to optimise the extraction conditions. The various combinations of experimental conditions (coded and uncoded) with their respective experimental responses, together with the actual values from the mathematical model, are presented in Table S1. The maximum yield of carotenoids (12.689 μg·g^{-1}) was obtained under the experimental conditions of $R_{IL/E}$ of 1 : 2, $R_{S/L}$ of 1 : 3, extraction time of 15 min, and number of extractions of 3.

Based on the Box–Behnken experimental design model, the empirical relationship between the experimental results and the input variables is a second-order polynomial equation with interaction terms. The

$$\begin{aligned} Y = {} & 12.12 + 0.1976 \times X_1 + 1.81 \times X_2 + \\ & + 0.7143 \times X_3 + 0.5267 \times X_4 - 0.2753 \times X_1 X_2 + \\ & + 0.1748 \times X_1 X_3 + 0.1472 \times X_1 X_4 - 0.1507 \times \\ & \times X_2 X_3 - 1.72 \times X_2 X_4 - 0.3703 \times X_3 X_4 - 1.02 \times X_1^{\,2} \\ & - 2.10 \times X_2^{\,2} - 0.9332 \times X_3^{\,2} - 0.5595 \times X_4^{\,2} \end{aligned} \quad (3)$$

final equation obtained in terms of coded factors is given in Equation (3):

The ANOVA analysis (Table 2) showed the linear, interactive, and quadratic relationships between the effects of independent variables on their dependent variables. The ANOVA concluded that a model F value of 15.39 and a P value less than 0.0001 implied that the model was significant. The P value of lack of fit is 0.1953 (> 0.05, non-significance), which shows that the regression model fits well and can better reflect the reliability of the test results. Moreover, the P values of linear coefficients (X_2, X_3, X_4), interaction term coefficients (X_2X_4), and quadratic term coefficients (X_1^2, X_2^2, X_3^2, and X_4^2) were lower than 0.05, indicating the significant effects on extraction yield of these quantities. However, the linear term (X_1) and interaction term coefficients (X_1X_2, X_1X_3, X_1X_4, X_2X_3, and X_3X_4) had insignificant effects.

Coefficient of determination (R^2) and adjusted R^2 were calculated to verify the adequacy and fitness of the model. Generally, R^2 values higher than 0.90 are considered to have a very high correlation (Prakash Maran and Manikandan 2012). The calculated R^2 values were 0.9473, which indicates that only 5.27% of the total variations could not be explained by the model. The predicted R^2 of 0.7040 is in reasonable agreement with the adjusted R^2 of 0.8857; the difference is less than 0.2, which is required for the fit model. Furthermore, the statistical significance of the correlation was verified by comparing the calculated correlation coefficient (R = 0.973, derived from $\sqrt{R^2}$) with the critical R value. At a significance level of α = 0.05 and 13 degrees of freedom (df = $n - p$ = 27 − 14 = 13, where: n – the number of data values; p – the number of equation parameters), the critical R value was determined to be 0.514 based on the reference statistical tables (Štěpánek 1975). The fact that the calculated R value (0.973) substantially exceeded this critical value (0.514) provides strong evidence for the statistical significance of the model correlations.

https://doi.org/10.17221/26/2024-CJFS

Table 2. ANOVA of the regression model for the prediction of carotenoid extraction

Source	Sum of squares	df	Mean square	*F*-value	*P*-value
Model	86.7300	14	6.1900	15.3900	< 0.0001*
X_1	0.4685	1	0.4685	1.1600	0.3019
X_2	39.1500	1	39.1500	97.2800	< 0.0001*
X_3	6.1200	1	6.1200	15.2100	0.0021*
X_4	3.3300	1	3.3300	8.2700	0.0139*
$X_1 X_2$	0.3031	1	0.3031	0.7530	0.4026
$X_1 X_3$	0.1222	1	0.1222	0.3035	0.5918
$X_1 X_4$	0.0867	1	0.0867	0.2155	0.6508
$X_2 X_3$	0.0909	1	0.0909	0.2259	0.6431
$X_2 X_4$	11.8000	1	11.8000	29.3200	0.0002*
$X_3 X_4$	0.5483	1	0.5483	1.3600	0.2658
X_1^2	5.5900	1	5.5900	13.8900	0.0029*
X_2^2	23.5000	1	23.5000	58.4000	< 0.0001*
X_3^2	4.6400	1	4.6400	11.5400	0.0053*
X_4^2	1.6700	1	1.6700	4.1500	0.0643
Residual	4.8300	12	0.4025	–	–
Lack of fit	4.6200	10	0.4624	4.5000	0.1953
Pure error	0.2054	2	0.1027	–	–
Cor total	91.5600	26	–	–	–
SD	0.6344	–	–	–	–
Mean	10.0700	–	–	–	–
CV (%)	6.3000	–	–	–	–
R^2	0.9473	–	–	–	–
Adjusted R^2	0.8857	–	–	–	–
Predicted R^2	0.7040	–	–	–	–
Adeq precision	14.9043	–	–	–	–

*significance level at 0.05

A high coefficient of variation (CV) points out that there is extreme variation in the mean value and inadequacy for development of a sufficient model (Koocheki et al. 2009). In this experiment, the CV was 6.30%, suggesting that the predicted and experimental values were similar. Adeq precision greater than 4 is normally desirable, in this model, the ratio of 14.904 indicates an adequate signal, suggesting that this model can be used to navigate the design space. These results further demonstrate that the BBD model can be used to analyse the extraction conditions for carotenoids from the apricots. The main effect sequence of the influence factors is known according to the parameter estimates: $R_{S/L}$ > extraction time > number of extraction > $R_{IL/E}$ (Table 2). Besides, to perform adequacy testing on the BBD model, Figure S1A shows that the Cook's distance of the simplified polynomial model is within 1.0. Figure S2B shows that the extraction rates of carotenoids all follow a normal distribution, and there is no deviation from the variance. Figure S2C shows that the externally studentised residuals at each run number are all within the ± 3 range. The actual values of carotenoid extraction content obtained from the experiment are basically consistent with the predicted values (Figure S2D). The results confirm that the BBD model is applicable for estimating carotenoid yields from Xinjiang apricots across different extraction process parameters. All in all, the adequacy test can further verify the feasibility of using ultrasonic-assisted ionic liquid extraction to extract carotenoids from Xinjiang apricots.

3D surface plots and 2D contour plots illustrate the key collaborative effects generated for each pair of factors. The content of extracted carotenoid was investigated when two varieties were kept in the experimental

https://doi.org/10.17221/26/2024-CJFS

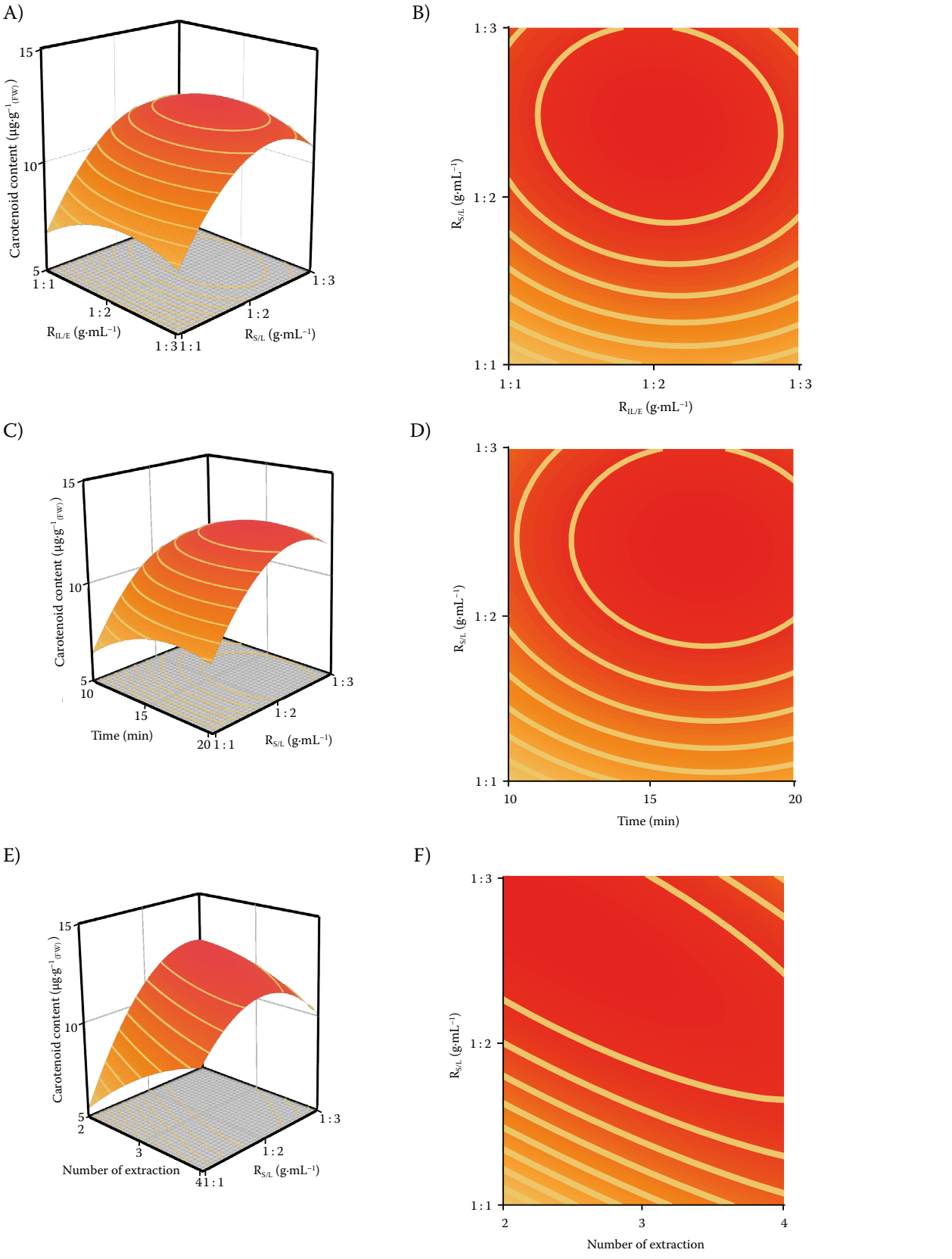

Figure 2. Three-dimensional response surface plots and two-dimensional contour plots for the reciprocal effect of $R_{S/L}$ with $R_{IL/E}$ (A, B), time (C, D) and number on carotenoid content (E, F)

https://doi.org/10.17221/26/2024-CJFS

range and other varieties were fixed at zero (Figure 2 and Figure S2). Figure 2 displays the interactive effect of $R_{S/L}$ with $R_{IL/E}$, time, and number the extractions on the carotenoid content. The carotenoid content increased first and then decreased with increasing $R_{IL/E}$ and $R_{S/L}$ when the time and number were 15 and 3, respectively, and the contour plot was elliptical, indicating that the mutual interactions between $R_{S/L}$ and $R_{IL/E}$ were significant (Figure 2A and 2B). The interaction effect of time and $R_{S/L}$ showed a similar trend (Figure 2C and 2D). The 3D surface and contour plot are steeper and more elliptical, indicating that the mutual interactions between $R_{S/L}$ and number of extractions was highly significant (Figures 2E and 2F). Furthermore, the 3D surface plot and 2D contour plot of $R_{IL/E}$ with time, number of extractions, and time with number are shown in Figure S2. The significant influence of time and number on the carotenoid content are seen in Figures S2E and S2F. However, Figures S2A–S2D displays a slight interaction effect of $R_{IL/E}$ on time and number; $R_{IL/E}$ had lower effect on the model that the *F* value of $R_{IL/E}$ was lower (Table 2). All in all, these results were in good agreement with the above-mentioned single-factor test and BBD model showing that factors affecting carotenoid yield in order of influence are $R_{S/L}$ > extraction time > number of extractions > $R_{IL/E}$.

Verification of optimised conditions and predictive model. Applying the methodology of desired function, we obtained the optimum level of various parameters. They indicate an $R_{IL/E}$ of 1 : 2, solid-liquid ratio of 1 : 3, ultrasound-assisted extraction time of 17 min, and number of extractions of 3 (Figure S3). Under these optimal conditions, the predicted carotenoid content is 12.72 μg·g^{-1} with a desirability value of 1.0. A desirability ramp developed for the optimised conditions indicates the optimised conditions obtained in this study. Triplicate experiments were carried out under the optimised conditions, and mean values of experimental results were compared with the predicted values. Figure 3 shows that under the optimum conditions, the carotenoid content of Mingxing, Xiaobai, Saimaiti, Shushanggan, and Liguang apricots were 3.55 ± 0.012, 6.68 ± 0.065, 12.84 ± 0.31, 32.98 ± 0.27, and 20.08 ± 0.21 μg·g^{-1}, respectively. The mean values of the carotenoid content obtained were compared with the predicted values and were used as indicators of the suitability of the developed quadratic models. The percentage deviation of the experimental and theoretical results was found to be + 0.12%. The results obtained through confirmation experiments indicate the suitability of the developed quadratic models, it may

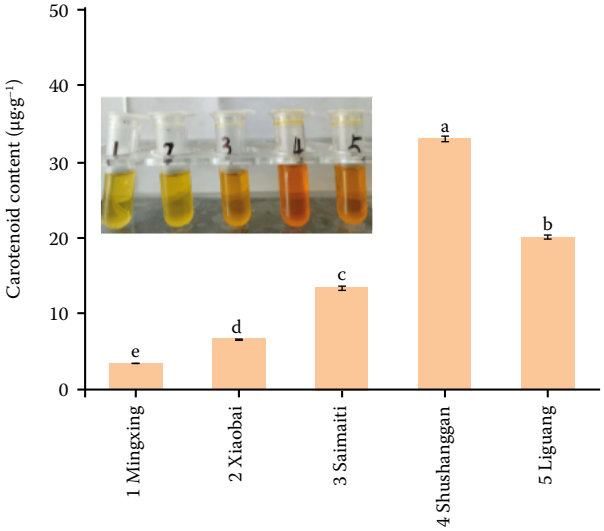

Figure 3. Carotenoid content of Xinjiang apricots under optimal extraction conditions

be noted that these optimal values are valid within the specified range of process parameters.

Thus, in order to obtain maximum concentration of carotenoids from apricots, the best conditions are [BMIM][BF_4] liquid/ethanol ratio of 1 : 2, a solid/liquid ratio of 1 : 3, and three extraction repetitions of 17 min each under ultrasound assistance. Moreover, the high carotenoid content may indicate greater antioxidant activity.

Compared with the traditional extraction method. Ultrasonic-assisted ionic liquid extraction demonstrates superior performance compared to traditional extraction methods (Table 3). The technique yields 8 660.0 ± 0.29 μg·g^{-1} of lycopene from dried guava (*Psidium guajava* L.), representing a 2.36-fold increase over ultrasonic-assisted water extraction (Wang et al. 2024). Similarly, for zeaxanthin extraction from freeze-dried *Lycium barbarum* L., the ultrasonic-assisted ILs method yields 2 964.5 ± 7.53 μg·g^{-1}, exceeding the 2 408.6 ± 10.23 μg·g^{-1} obtained through traditional organic solvent extraction (Shen et al. 2022). For carotenoids from Xinjiang 'Shushanggan' apricot (fresh sample), the ultrasonic-assisted ILs extraction achieves a yield of 32.98 ± 0.27 μg·g^{-1}, outperforming the 25.05 ± 0.35 μg·g^{-1} yield obtained using the traditional extraction method (GB 5009.83-2016). Moreover, the method offers substantial time savings, completing carotenoid extraction from Xinjiang apricot in 51 min versus 150 min for traditional approaches. For zeaxanthin isolation, ultrasonic-assisted ILs extraction reduces processing time from

https://doi.org/10.17221/26/2024-CJFS

Table 3. Comparing ultrasonic-assisted ionic liquid extraction method to traditional extraction methods

Sample	Extraction substance	Extraction method	Extraction yield ($\mu g \cdot g^{-1}$)	Extraction time	Reference
Chlorella sorokiniana (dried sample)	carotenoids	ultrasound-assisted ionic-liquid extraction	1 290.0	extraction: 35 min saponification: 16 h	Bianchini et al. (2024)
Orange peel (freeze-dried sample)	carotenoids	ultrasound-assisted ionic-liquid extraction	32.08 ± 2.05	extraction: 35 min saponification: 16 h	Murador et al. (2019)
Guava (*Psidium guajava* L., dried sample)	lycopene	ultrasound-assisted water extraction	3 670.0 ± 0.21	35 min	Wang et al. (2024)
Guava (*Psidium guajava L.*, dried sample)	lycopene	ultrasound-assisted ionic-liquid extraction	8 660.0 ± 0.29	35 min	Wang et al. (2024)
Lycium barbarum L. (freeze-dried sample)	zeaxanthin	traditional organic solvents	2 408.6 ± 10.23	extraction: 40 min saponification: 6 h	Shen et al. (2022)
Lycium barbarum L. (freeze-dried sample)	zeaxanthin	ultrasound-assisted ionic-liquid extraction	2 964.5 ± 7.53	40 min	Shen et al. (2022)
Xinjiang ‚Shushanggan' apricot (fresh sample)	carotenoids	traditional extraction method (GB 5009.83-2016)	25.05 ± 0.35	pre-treatment: 30 min saponification: 1 h liquid-liquid extraction: 1 h	this work
Xinjiang ‚Shushanggan' apricot (fresh sample)	carotenoids	ultrasound-assisted ionic-liquid extraction	32.98 ± 0.27	51 min	this work

6.7 h to 40 min by eliminating extended saponification requirements (Shen et al. 2022).

Notably, ultrasonic-assisted ILs extraction still exists limitations including prolonged saponification durations (16 h) for certain matrices like *Chlorella sorokiniana* (Bianchini et al. 2024) and orange peel (Murador et al. 2019). Future studies should focus on (*i*) optimising saponification duration to streamline the extraction workflow, and (*ii*) elucidating the mechanistic role of ionic liquids in extraction enhancement, thereby establishing a theoretical framework for broader applications in bioactive compound recovery.

Antioxidant activity. Antioxidants play a crucial role in human health because they can inhibit or delay undesired oxidation reactions and thus prevent oxidative stress related to diseases such as high blood pressure, neurodegenerative disorders, and cancer (Rumof et al. 2023). At present, no single method can completely describe the natural reactions occurring *in vivo*. In contrast, antioxidant assay *in vitro* is more simple and less affected and is therefore very suitable for rapid screening of samples with antioxidant activity. Thus, the DPPH assay, which is based on the hydrogen atom transfer (HAT) reaction mechanism, is the most common for investigating antioxidant capacity (Wołosiak et al. 2022).

The carotenoid ethanol extract displays a strong absorption band in the region of 400 to 500 nm, and the absorption intensity gradually weakens from Shushanggan to Mingxing ethanol extract (Figure 4A). These results are consistent with Figure 3. Meanwhile, DPPH radical solution is a stable free radical that possesses two distinct bands at around 325 nm and 517 nm due to π–π^* transitions and an unpaired electron (Ngueumaleu et al. 2023). Figure 4B displays the absorbance at 517 nm after 30 min of reaction of carotenoids with DPPH. The violet colour of DPPH radical disappears gradually as a result of radical reduction by HAT from carotenoid antioxidants to form DPPH-H (Gulcin and Alwasel 2023). Moreover, the removal rates of DPPH radical by Mingxing, Xiaobai, Saimaiti, Shushanggan, and Liguang apricot ethanol extracts were 10.2, 13.9, 27.0, 64.8, and 42.1%, respectively. The result further proves that Shushanggan apricot possesses higher carotenoid content and antioxidant capacity, consistent with Figure 3. However, the DPPH assay was limited by the intrinsic absorption of the sample at the wavelength of detection. Carotenoids have a certain absorption at 517 nm (Figure 4A). Therefore, ABTS assay is less prone to interference from coloured samples (such as carotenoids) due to the higher wavelength used.

https://doi.org/10.17221/26/2024-CJFS

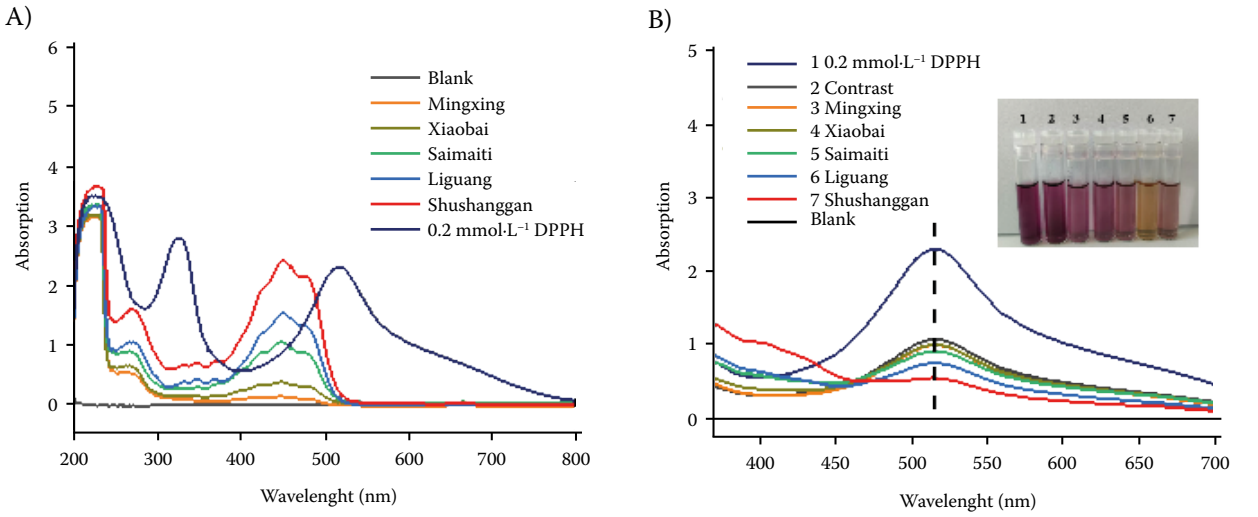

Figure 4. (A) UV-vis spectrum of 0.2 mmol·L^{-1} DPPH (2,2-diphenyl-1-picrylhydrazyl) and carotenoid extracts from Xinjiang apricots and (B) antioxidant activity of apricot ethanol extract against DPPH radical

ABTS is a compound that is extensively employed to evaluate the free-radical-trapping capacity of antioxidant agents based on the mechanism of single-electron transfer. First, the colourless ABTS is oxidised to the green radical cation (ABTS+ radical) using oxidant ($K_2S_2O_8$). The second step then detects the presence of the antioxidant via the decolourisation of the green ABTS+ radical to the colourless ABTS as it is reduced by the antioxidant (Schaich et al. 2015). In Figure 5A, the absorbance of ABTS+ radical decreases gradually upon addition of the carotenoid ethanol extract. UV-vis absorption intensities listed according to the extract are Xiaobai > Mingxing > Saimaiti > Liguang > Shushanggan, which correspond to ABTS+ radical removal rates of 18.1, 19.2, 20.0, 36.3, and 53.0%, respectively. However, the antioxidant capacity of Xiaobai, Mingxing, and Saimaiti is not easily distinguished by the UV-vis spectrum. Therefore, the Raman spectrum was used for the first time to describe the reaction of ABTS and carotenoid (Figures 5B and S4). The Raman spectrum peak

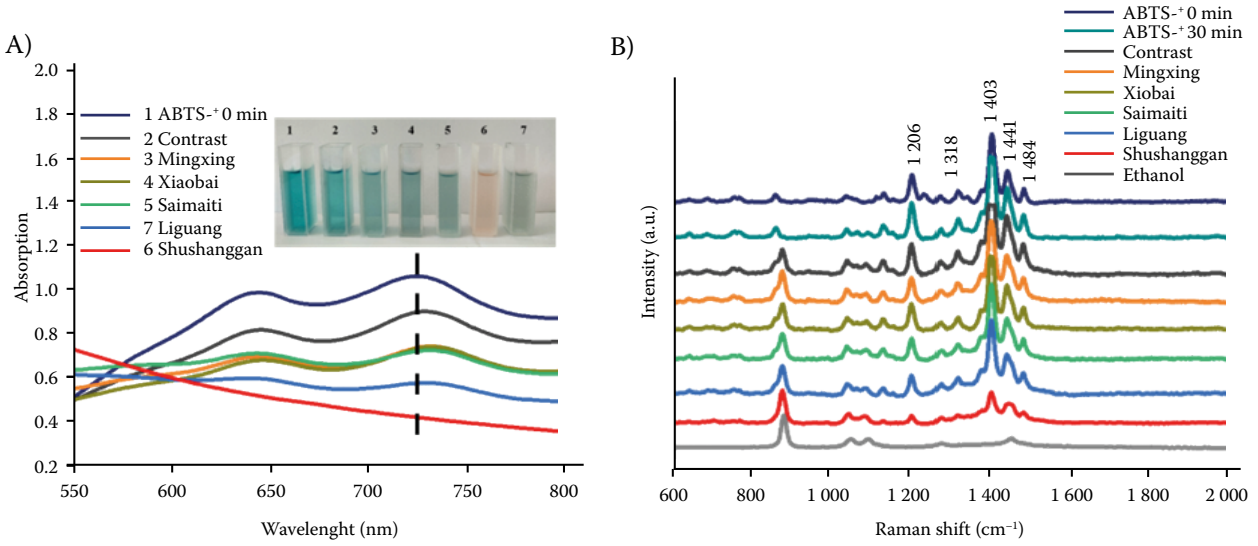

Figure 5. UV-vis spectroscopy (A) and Raman spectroscopy (B) of ABTS+ radical and extracts of the Xinjiang apricots after 30 min of reaction

ABTS – 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)

https://doi.org/10.17221/26/2024-CJFS

at 1206, 1318, 1403, 1439 and 1485 cm^{-1} are assigned to ABTS+ radical (Sloan-Dennison et al. 2017). As the carotenoid ethanol extract of Xinjiang apricot was added, the intensity of ABTS+ radical decreased because green ABTS+ radical is reduced to colourless. The intensity of the 1 403 cm^{-1} and 1 206 cm^{-1} bands gradually decreased, that is, Mingxing > Xiaobai > Saimaiti > Liguang > Shushanggan. In contrast, the intensity at 879 cm^{-1} is high and sharp, which may be caused by the antioxidants reacting completely with the ABTS, leaving only the ethanol solution. These results are consistent with the carotenoid content of apricot (Figure 3). Moreover, the Raman spectra of Shushanggan after reaction with ABTS+ radical after 0, 5, 10, 15, and 30 min are shown in Figure S4. The intensity of the ABTS peak gradually decreased because of extension of reaction time. This result strongly suggests that antioxidants play a crucial role in oxidation reactions because they are able to inhibit or delay undesired oxidation reactions.

CONCLUSION

In this research, an efficient and simple method that ultrasound-assisted ionic-liquid extraction of carotenoids from Xinjiang apricot was studied. RSM was used to optimise the extraction process in order to obtain the best extraction conditions and carotenoid content. The statistical analysis based on BBD showed that a [Bmim][BF_4] liquid/ethanol ratio of 1 : 2, a solid/liquid ratio of 1 : 3, an ultrasound-assisted extraction time of 17 min, and number of extractions of 3 are optimal conditions. Under the optimal conditions, the carotenoid contents of Mingxing, Xiaobai, Saimaiti, Shushanggan, and Liguang were 3.55 ± 0.012, 6.68 ± 0.065, 12.84 ± 0.31, 32.98 ± 0.27, and 20.08 ± 0.21 $\mu g \cdot g^{-1}$, respectively. The experimental values were in agreement with those predicted, thus indicating adequacy of the developed models. Moreover, DPPH and ABTS methods were used to evaluate the antioxidant activities of the five Xinjiang apricots. The results showed that the antioxidant activities were as follows: Shushanggan > Liguang > Saimaiti > Xiaobai > Mingxing. These results provide a theoretical basis for screening Xinjiang apricot varieties with high carotenoid content and excellent antioxidant activity, as well as promote the healthy and sustainable development of apricots and the apricot industry in Xinjiang.

REFERENCES

Alara O.R., Abdurahman N.H., Ukaegbu C.I. (2018): Soxhlet extraction of phenolic compounds from *Vernonia cinerea* leaves and its antioxidant activity. Journal of Applied Research on Medicinal and Aromatic Plants, 11: 12–17.

Aman R., Schieber A., Carle R. (2005): Effects of heating and illumination on *Trans–cis* isomerization and degradation of β-carotene and lutein in isolated spinach chloroplasts. Journal of Agricultural and Food Chemistry, 53: 9512–9518.

Bi W., Tian M., Zhou J., Row K.H. (2010): Task-specific ionic liquid-assisted extraction and separation of astaxanthin from shrimp waste. Journal of Chromatography B, 878: 2243–2248.

Bianchini C.B., Ramos-Souza C., Schappo F.B., Farina M., De Rosso V.V., Nunes I.L. (2024): Ionic liquid and ultrasound as a fast and innovative combination for improved extraction of *Chlorella sorokiniana*-derived carotenoids. Algal Research, 82: 103650.

Cheng S.H., Khoo H.E., Kong K.W., Prasad K.N., Galanakis C.M. (2020): Extraction of carotenoids and applications. In: Galanakis C.M. (ed.): Carotenoids: Properties, Processing and Applications. Cambridge, Academic Press: 259–288.

de Souza Mesquita L.M., Murador D.C, Neves B.V., Braga A.R.C., Pisani L.P., de Rosso V.V. (2021): Bioaccessibility and cellular uptake of carotenoids extracted from *Bactris gasipaes* fruit: Differences between conventional and ionic liquid-mediated extraction. Molecules, 26: 3989.

Farag M.A., Ramadan N.S., Shorbagi M., Farag N., Gad H.A. (2022): Profiling of primary metabolites and volatiles in apricot (*Prunus armeniaca* L.) seed kernels and fruits in the context of its different cultivars and soil type as analyzed using chemometric tools. Foods, 11: 1339.

Gulcin İ., Alwasel S.H. (2023): DPPH radical scavenging assay. Processes, 11: 2248.

Kateryna Y., Konieczna K., Marcinkowski Ł., Kloskowski A. (2020): Ionic liquids in the microextraction techniques: The influence of ILs structure and properties. TrAC Trends in Analytical Chemistry, 130: 115994.

Koocheki A., Taherian A.R., Razavi M.A.S., Bostan A. (2009): Response surface methodology for optimization of extraction yield, viscosity, hue and emulsion stability of mucilage extracted from *Lepidium perfoliatum* seeds. Food Hydrocolloids, 23: 2369–2379.

Manzato L., Takeno M. L., Pessoa-Junior W. A. G., Mariuba L. A. M., & Simonsen J. (2018): Optimization of Cellulose Extraction from Jute Fiber by Box-behnken Design. Fibers Polymers, 19, 289-296

Murador D.C., Salafia F., Zoccali M., Martins P.L.G., Ferreira A.G., Dugo P., Mondello L., de Rosso V.V., Giuffrida D. (2019): Green extraction approaches for carotenoids and esters: Characterization of native composition from orange peel. Antioxidants, 8: 613.

https://doi.org/10.17221/26/2024-CJFS

Nabi F., Arain M.A., Rajput N., Alagawany M., Soomro J., Umer M., Soomro F., Wang Z., Ye R., Liu J. (2020): Health benefits of carotenoids and potential application in poultry industry: A review. Journal of Animal Physiology and Animal Nutrition, 104: 1809–1818.

Ngueumaleu Y., Deutchoua A.D.D., Hanga S.S.P., Liendji R.W., Dedzo G.K., Ngamenia E. (2023): Probing the reactivity of 2,2-diphenyl-1-picrylhydrazyl (DPPH) with metal cations and acids in acetonitrile by electrochemistry and UV-Vis spectroscopy. Physical Chemistry Chemical Physics, 25: 5282–5290.

Prakash Maran J., Manikandan S. (2012): Response surface modeling and optimization of process parameters for aqueous extraction of pigments from prickly pear (*Opuntia ficus-indica*) fruit. Dyes and Pigments, 95: 465–472.

Sanahuja A.B., García A.V., Baenas N., Ferrando B.O., Periago M.J., Alonso N.C., Sánchez R., Todolí J.L. (2025): Valorization of pineapple core waste for sequential extraction of phenolic compounds and carotenoids: Optimization through ultrasound-assisted method and box-behnken design. Food and Bioprocess Technology, 18: 2618–2631.

Schaich K.M., Tian X., Xie J. (2015): Hurdles and pitfalls in measuring antioxidant efficacy: A critical evaluation of ABTS, DPPH, and ORAC assays. Journal of Functional Foods, 18: 782–796.

Shen Q., Zhu T., Wu C., Xu Y., Li C. (2022): Ultrasonic-assisted extraction of zeaxanthin from *Lycium barbarum* L. with composite solvent containing ionic liquid: Experimental and theoretical research. Journal of Molecular Liquids, 347: 118265.

Sloan-Dennison S., Shand N.C., Grahama D., Faulds K. (2017): Resonance Raman detection of antioxidants using an iron oxide nanoparticle catalysed decolourisation assay, Analyst, 142: 4715–4720.

Štepánek V. (1975): (Mathematical statistics in chemistry). Prague, Státní nakladatelství technické literatury: 313–314. (in Czech)

Wang J., Zhao H., Xue X., Han Y., Wang X., Sheng Z. (2024): Application of ionic liquid ultrasound-assisted extraction (IL-UAE) of lycopene from guava (*Psidium guajava* L.) by response surface methodology and artificial neural network-genetic algorithm. Ultrasonics Sonochemistry, 106: 106877.

Wołosiak R., Drużyńska B., Derewiaka D., Piecyk M., Majewska E., Ciecierska M., Worobiej E., Pakosz P. (2022): Verification of the conditions for determination of antioxidant activity by ABTS and DPPH assays – A practical approach. Molecules, 27: 50.

Yang S., Li X., Zhang H. (2024): Ultrasound-assisted extraction and antioxidant activity of polysaccharides from Tenebrio molitor. Scientific Reports, 14: 28526.

Zhao H., Wang J., Han Y., Wang X., Sheng Z. (2024): Optimization of process conditions for ionic liquid-based ultrasound-enzyme-assisted extraction of resveratrol from *Polygonum Cuspidatum*. Ultrasonics Sonochemistry, 108: 106973.

Zhou W., Niu Y., Ding X., Zhao S., Li Y., Fan G., Zhang S., Liao K. (2020): Analysis of carotenoid content and diversity in apricots (*Prunus armeniaca* L.) grown in China. Food Chemistry, 330: 127223.

Received: February 11, 2025
Accepted: August 8, 2025
Published online: December 18, 2025