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Extraction of carotenoids from cantaloupe waste and determination of its mineral composition

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ABSTRACT

The carotenoid and mineral levels as well as the *in vitro* antioxidant capacity, using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay, of waste from cantaloupe was assessed. Then the matrix was subjected to ultrasound-assisted extraction (UAE) and response surface methodology (RSM) was used for the optimization of the extraction of carotenoids. The effect of the extraction procedure on the microstructure of the powder was assessed by scanning electron microscopy (SEM) analysis. The major carotenoids identified were lutein (63.24 ± 0.73 μg βCE/g dw) and β-carotene (56.43 ± 0.11 μg βCE/g dw). Several mineral elements (K, Na, P, Mg, Ca, Fe, Cu, Mn and Zn) were identified, potassium being the major one.The extract exhibited *in vitro* antioxidant activity (IC_{50} = 7.33 ± 0.22 μg/mL). The RSM results showed that an amplitude of 100%, extraction time of 10 min, hexane percentage of 80% in hexane/acetone solvent, and solvent-to-solid ratio of 55 mL/g were the optimal conditions for the extraction of carotenoids. Under these conditions, the carotenoid content of the extract was 124.61 ± 3.82 μg/g.The microscopic analysis revealed the effectiveness of the ultrasound treatment that results in noticeable physical changes, like microscopic perforations and breakages.

1. Introduction

Colorants are extensively used in food industry as they are much related to the sensory quality and therefore to food choice and preference, hence the production of colorants continues to increase (Martins, Roriz, Morales, Barros, & Ferreira, 2016). Synthetic colorants are perceived as potentially harmful for many consumers, therefore, there is a constant trend to try to replace them with natural pigments (Zhang, Yin, Kong, & Jiang, 2011).

Plant or natural pigments are important in signalling, they attract pollinators and seed dispersal agents and repel herbivores (Eldahshan & Singab, 2013). They are also important for humans, because colour is one of the attributes of appearance related to food acceptability (Meléndez-Martínez, Britton, Vicario, & Heredia, 2007). In addition to their role in providing colour, natural pigments such as carotenoids can be involved in a wide variety of health-promoting biological functions (Saini, Nile, & Park, 2015).

Fruits and vegetables are rich in carotenoids and are the most important contributors to these compounds in the typical human diet. During the treatments applied on these foods, large amounts of waste were occured. It is estimated that about 1.3 billion of food wastes are produced per year (Arshadi et al., 2016; Matharu, de Melo, & Houghton, 2016), which poses important problems for the industry and the environment. Fruits and vegetables waste might be rich sources of bioactive compounds and can be used to obtain products with high added-value for the agro-food, cosmetic or pharmaceutical industry.

Currently there is a trend to extract such compounds not only from foods but also from by-products and wastes by means of green extraction. This consists in the extraction procedures design that use environmental friendly solvents and renewable products, reduce the consumption of energy and have a suitable extract in terms of safety and other quality parameters as result (Chemat, Vian, & Cravotto, 2012).

Recently, different novel and emerging technologies for green extraction such as High Hydrostatic Pressures (HHP), Ultrasound (US), Pulsed electric fields (PEF) and Microwaves (MW) are being increasingly used (Barba, Galanakis, Esteve, Frigola, & Vorobiev, 2015; Deng et al., 2014; Kyriakopoulou, Papadaki, & Krokida, 2015). In this regard,

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ultrasound is considered as an emerging technology. Its applications in agro-food industry can lead to a reduction in treatment time and energy consumption through the optimization of the factors involved (Chemat et al., 2012).

The ultrasound work in a liquid medium is based on the generation of micro-bubbles filled with gas, a phenomenon called cavitation. The produced cavitation helps disrupt the cell wall, increasing its permeability and allowing the solvent to penetrate in the plant material. Therefore, the release of the compounds of interest is accelerated. Ultrasound is considered a non-thermal technology, since it leads to instantaneous increases in local temperature without raising the temperature of the treated liquid substantially, which reduces the risk of food degradation. Besides, the time of treatment using ultrasonic approach can be shortened (Kyriakopoulou et al., 2015; Šic Žlabur et al., 2015; Zinoviadou et al., 2015).

Given the importance of carotenoids in agro-food health, other researchers such as Yolmeh, Habibi Najafi, and Farhoosh (2014), Dey and Rathod (2013), Ofori-bcateng and Lee (2013), Li, Fabiano-Tixier, Tomao, Cravotto, and Chemat (2013) and Lianfu and Zelong (2008) have used the ultrasound method for their extraction, although the variables evaluated (like the matrix, the nature of the carotenoids, the type of solvent, among others) were different.

Consumption of cantaloupe and its processing to produce juices and jams generate large quantities of waste which can be valorised through the extraction of its health-promoting compounds. Thus, the objective of the present study was the extraction of carotenoids from cantaloupe peels.The ultrasound technique (UAE) used for this extraction was optimized by the response surface methodology (RSM). This optimization will allow us to reduce in treatment time and energy, to have a better yield in carotenoids and to use a less solvent. A rapid resolution liquid chromatography method (RRLC-DAD) was applied to determine the individual carotenoids in the extract. The mineral elements content of the cantaloupe waste and its *in vitro* antioxidant activity were also studied as well as the sonication effect on the matrix structure using *scanning electronmicroscopy*.

2. Materials and methods

2.1. Plant material and reagents

Cantaloupe fruits (*Cucumis melo* L.) were harvested at the same period, from different regions of Bejaia (northeast of Algeria), and at optimal ripening stage, they were obtained from a local market at Bejaia city during the summer of 2014. The samples were prepared as follows: after washing with distilled water, the rinds were removed manually from the rest of the fruit by means of a rind peeler and subsequently sliced into small cylinders (with 2 mm of diameter and 2–3 mm of thickness). They were dried in an oven set at 40 °C (Binder E28, Germany) until constant weight. The dried samples were ground with an electric grinder (IKA A11, Retsch, Germany) to granulometry lower than 250 µm. The powder so obtained was stored in airtight bags. The water activity (a_w) was determined by Hygro Palm AW1 (EminTech, Lund, Sweden) and was 0.33 ± 0.01 at 27 °C. Hexane, ethanol, acetone, DPPH, β-carotene and β-cryptoxanthin were purchased from Sigma-Aldrich. Methanol, acetonitrile and ethyl acetate, were of analytical grade and were purchased from Merck (Darmstadt, Germany). Water was purified in a NANO pure® DIamond™ system. Violaxanthin, α-carotene, and lutein were obtained by standard procedures from appropriate sources as described elsewhere (Rodriguez-Amaya, 2001; Meléndez-Martínez, Vicario, & Heredia, 2007).

2.2. Extraction of carotenoids

2.2.1. Ultrasound assisted-extraction

The frequency of the apparatus (SONICS Vibra cell, VCX 130 PB No. 630-0422, Newtown, Connecticut, USA) was fixed at 20 kHz. One gram of the powder was added to 30 mL of extraction solvent. The solution was subjected to the action of acoustic waves with different solvent mixtures, hexane contents in the solution, extraction times, amplitudes and solvent-powder ratios, as explained below. The temperature was continuously monitored using a T-type thermocouple (± 0.2 °C) connected to a data logger, and kept at 21 ± 2 °C by an external cold water bath. So, by the elimination of any temperature effect, it can be accepted that the observed effect was related only to the application of ultrasound. After the treatments, 40 mL of distilled water were added to the extracts and the mixtures were then filtered and left to settle. After decantation, the coloured phase containing carotenoids was recovered and evaporated to dryness. The crude extracts obtained were stored in a freezer (Samsung RL60GQERS1/XEF, France) at −20 °C under a nitrogen atmosphere until their analyses.

2.2.2. Response surface methodology

The methodology followed to optimize the conditions was basically that described by Hiranvarachat and Devahastin (2014).Three food-compatible solvents were considered, namely acetone, ethanol, and hexane and then three mixtures were prepared (hexane/acetone, hexane/ethanol, and hexane/acetone/ethanol). According to the US Department of Health and Human Services, Food and Drug Administration (FDA), ethanol and acetone may be regarded as less toxic and with lower risk to human health as compared to other solvents. Daily exposures of 50 mg per day were recommended. Hexane belongs to a class of solvent to be limited, with a lower recommended daily exposure (2.9 mg) (FDA, 1997).

In order to minimize the number of experiments, a preliminary study whose parameters were studied separately in single-factor experiments was conducted. The extraction conditions were optimized, using the Central Composite Design (CCD), with respect to four variables, *i.e.* hexane percentage in the hexane/acetone solvent mixture (X_1), extraction time (X_2), amplitude (X_3), and solvent-to-solid ratio (X_4). The levels of the four factors were selected based on preliminary experiments and the response variable was the total carotenoids yield (Y). The CCD required in total 30 combinations of factors, including six tests at the center point level. The data obtained were modelled with the following second-order polynomial equation (Eq. (1)):

$$Y = B_0 + \sum_{i=1}^{k} B_i X_i + \sum_{i=1}^{k} B_{ii} X_i^2 + \sum_{ij}^{k} B_{ij} X_i X_j + E \quad (1)$$

where Y represents the response function, which represents, in this study, the total carotenoids content (TCC) yield; B_0 is a constant coefficient, B_i, B_{ii} and B_{ij} are the coefficients of the linear, quadratic and interactive terms, respectively; and X_i and X_j represent the coded independent variables.

2.3. Determination of total carotenoids content

The total carotenoid contents (TCC) of the extracts were determined by spectrophotometry at 450 nm using a UV–Vis spectrophotometer (UV-mini 1240, Shimadzu, Japan). Total carotenoids concentration was calculated according to Scott (2001), and was expressed as micrograms of β-carotene equivalent per gram of dry weight (βCE µg/g of dw). Individual carotenoids were quantified by using a RRLC-DAD methodology which was carried out on an Agilent 1260 system (Agilent, Palo Alto, CA) fitted with a diode-array detector (DAD). This was set at 285 nm for the detection of phytoene, at 350 nm for that of phytofluene, and at 450 nm for coloured carotenoids and chlorophylls. A C_{18} Poroshell 120 column (2.7 µm, 5 cm × 4.6 mm) kept at 28 °C was used as stationary phase. The injection volume was in the range 1–10 µL. The mobile phase was pumped at a flow rate of 1 mL/min and consisted of acetonitrile (solvent A), methanol (solvent A) and ethyl acetate (solvent C). The linear gradient elution was: 0 min, 85% A + 15% B; 5 min, 60% A + 20% B + 20% C; 7 min, 60% A + 20% B + 20% C; 9 min, 85%

A + 15% B; 12 min, 85% A + 15% B. The open labChem Station software was used (Stinco, Benítez-González, Hernanz, Vicario, & Meléndez-Martínez, 2014).

2.4. Analysis of powders by scanning electron microscopy

Scanning electron microscopy was used in order to study the effect of the UAE on the microstructure of the powder. Micrographs before and after the extraction processes were obtained for morphological characterization. Three samples (non-extracted powder, powder after UAE and a control, specifically a powder that was only mixed with the solvent at the same time/temperature used during the UAE) were collected and dried until constant mass in an oven at 40 °C before SEM analysis. The sample particles were fixed on a specific carbon film support and coated with gold for 10 min in a SCANCOAT Six SEM sputter coater (Edwards, Crawley, England). The shape and surface features were observed by using a secondary electron detector, and the images were taken with a scanning electron microscope (JEOL JSM-6460LV, USA) at 25 kV.

2.5. Assessment of the in vitro antioxidant activity by the DPPH assay

The *in vitro* antioxidant activity using 1,1-diphenyl-2-picrylhydrazyl (DPPH) as a free radical was measured according to the method of Brand-Williams, Cuvelier, and Berset (1995). Different concentrations were prepared (2–8.63 μg/mL) and tested to determine the amount of TCC that reduces 50% of the initial DPPH concentration (IC_{50}). The results were expressed as the percentage of inhibition of DPPH radical (% DPPH inhibition) calculated according to the following equation:

$$\%DPPH\ inhibition = \frac{Abs\ control - Abs\ sample}{Abs\ control} \times 100 \quad (2)$$

where *Abs control* is the absorbance of the DPPH radical + extraction solvent and *Abs sample* is the absorbance of DPPH radical + sample extract. For comparison, the β-carotene and Trolox radical standards were also tested at different concentrations from (0.01 to 0.4 mg/mL) and (50 to 250 μg/mL), respectively.

2.6. Mineral elements analysis

The mineral elements composition of cantaloupe peels were determined using a Horiba Jobin-Yvon Inductively Coupled Plasma-Optical Emission Spectrometry (ICP-OES) model LAST 2 instrument (Longjumeau, France), which is among the most appropriate instrumental techniques for determination of minerals. About 0.4 g of the lyophilized material received in the Microanalysis Service was weighed, with an accuracy of 0.1 mg on a precision balance (Sartorius AG Gottingen, CP124S). Then the sample has been digested by using a Digiprep Jr. digester of 24 positions (SCP Science, Baie-D'Urfe, Canada).The reagent volumes used for these digestions were 6 mL of concentrated HNO_3 (Plasma Pure quality, SCP SCIENCE) and 2 mL of concentrated H_2O_2 (Suprapure quality, Merck). Once the treatments were completed, the samples were made up to mark with ultrapure quality water in 25 mL. The temperature program was: Step 1, 60 min, 115 °C; Step 2, 120 min, 115 °C. The wavelengths used for the measurement of each element were: 317.933 nm for Ca (ionic), 324.754 nm for Cu (atomic), 259.940 nm for Fe (ionic), 766.490 nm for K (atomic), 279.553 nm for Mg (ionic), 257.610 nm for Mn (ionic), 589.592 nm for Na (atomic), 178.229 nm for P (atomic), and 213.856 nm for Zn (atomic).The detection limits used for the quantification, were as follow: Ca and Na: 0.005 mg/kg; Fe, Mg, Mn and Zn: 0.001 mg/kg; Cu and P: 0.002 mg/kg; and K: 0.012 mg/kg.

To ensure the accuracy of the results, all the calibration lines had an RSD less than or equal to 0.99%, and they were built with a minimum of 5 points at the corresponding wavelength of emission of each element. After calibrating, a standard was analyzed between ten samples and at the beginning and at the end of each sequence to ensure that its values were between +/− 10% of the theoretical value. Also, all the values of the measurements were within the calibration range. In parallel to the preparation of the sample, a blank without sample was also digested, only with the reagents used in the digestion, to verify the absence of signal in each analyte due to the preparation procedure. Finally, in each sample matrix a recovery test was made to check the absence of interferences and for which the values of the recovery were between 85 and 115%.

2.7. Statistical analysis

All experiments were performed in triplicate and the presented results are means ± standard deviation. Analysis of variance (ANOVA) with Tukey's *post hoc* test was used to evaluate the influence of each factor on the TCC yield in the single-factor experiment for the UAE at 95% confidence level. Data obtained from CCD was statistically analyzed using ANOVA for the response variable in order to test the model significance and suitability. $p < 0.05$ and $p < 0.01$ were taken as significant and highly significant level, respectively. To construct the CCD and to analyze all the results, the JMP software (Version 10.0, SAS, USA) was used.

3. Results and discussion

3.1. Optimization of the carotenoid extraction

In this study, four independent variables at three levels were selected for the optimization of carotenoids extraction using the CCD approach. The complete experimental planning for CCD parameters in coded values consisted of 30 experimental combinations in random order with six replicates at the center point, in order to avoid possible artificial systematic effects (Tian et al., 2012). The repetitions at the center point are necessary for the estimation of pure error associated with them. ANOVA was employed to estimate the statistical significance of the factors and their interactions.

3.2. Regression models for carotenoids extraction yield

The experimental data were fitted to the polynomial regression and the predicted model addressed to the data could be expressed, in terms of coded values, neglecting the non-significant terms ($p > 0.05$), in Eq. (3).

$$Y(TCC) = 107.34 - 2.23X_2 + 7.02X_4 + 7.90X_1X_3 - 7.42X_2X_3 - 4.97X_2X_4 - 8.19X_1^2 - 10.23X_4^2 \quad (3)$$

This equation describes an empirical relationship between the response and the tested variables for UAE. The regression analysis showed that some of the linear factors and the interactions among the factors had an effect on carotenoids extraction. The positive and negative coefficients of the factors show how the response changes with regard to these variables. Based on the regression coefficients, it can be seen in Eq. (3) that linear term of hexane percentage in hexane/acetone solvent mixture (X_1) and the ultrasound (US) amplitude (X_3) did not influence the extraction yield but its interaction did. The solvent-to-solid ratio (X_4) had significant influence and its quadratic term had the largest negative effects on the extraction yield. It can be concluded that, at a confidence level of 95%, the proposed models showed accuracy and good fit, as the value of the coefficient of determination (R^2) was higher than 0.95 which indicates an important agreement between the observed and the predicted model values. The adjusted coefficient of determination (R_{adj}^2), which verified the adequacy of the model had a value higher than 0.90. In addition, the significance of the model was confirmed by a *p*-value < 0.05. Furthermore, no significance of lacks of fit $p > 0.05$ ($p = 0.37$) also strengthened the reliability of the model.

Table 1
Results of single-factors experiments of ultrasound assisted extraction.

Solvent		Hexane/acetone fraction		Sonication time		Amplitude radiation		Solvent-to-solid ratio	
Type	TCC yield ($\mu g_{\beta CE}/g_{dw}$)	(%, v/v)	TCC yield ($\mu g_{\beta CE}/g_{dw}$)	(min)	TCC yield ($\mu g_{\beta CE}/g_{dw}$)	(%)	TCC yield ($\mu g_{\beta CE}/g_{dw}$)	(mL/g)	TCC yield ($\mu g_{\beta CE}/g_{dw}$)
H/E (50/50, v/v)	78.46 ± 1.50^{c}	50/50	101.44 ± 4.84^{b}	10	111.40 ± 0.25^{ab}	20	89.09 ± 3.49^{d}	30	126.41 ± 0.89^{ab}
H/A (50/50, v/v)	107.74 ± 2.42^{a}	70/30	111.22 ± 1.59^{a}	20	112.46 ± 1.21^{a}	40	105.64 ± 0.26^{c}	40	127.78 ± 1.09^{a}
H/A/E (50/25/25, v/v)	87.10 ± 0.56^{b}	90/10	104.61 ± 3.44^{ab}	30	110,18 ± 0.76^{b}	60	112.72 ± 0.4^{b}	50	128.08 ± 1.42^{a}
				40	105,27 ± 0.37^{c}	80	125.92 ± 0.4^{a}	60	124.31 ± 0.54^{b}
						100	116.92 ± 0.4^{b}		

H/A: hexane/acetone; H/E: hexane/ethanol; H/A/E: hexane/acetone/ethanol.
*Results are reported as means ± S.D. Same letters in the same column refer to means not statistically different according to ANOVA and Tukey's test. TCC, total carotenoids yield referred to dry weight (dw) of *Cucumis melo* peels; βCE, β-carotene equivalents.

3.3. Effects of extraction conditions on the extraction yield

3.3.1. Effect of solvent

The combination of polar solvents with typical non-polar (hexane) solvents for lipid-soluble compounds extraction seems to enhance the solubilization of the non-polar carotenoids (β-carotene), whereas individual polar solvents (ethanol and acetone) are thought to enhance the solubilization of the polar ones like lutein (Strati & Oreopoulou, 2011). Table 1 shows the results of the single-factor experiments carried out for preliminary optimization of UAE. At the beginning of this study, the effect of the solvent mixture type was investigated in order to obtain higher extraction yields. Hexane/acetone (1/1, v/v), hexane/ethanol (1/1, v/v) and hexane/acetone/ethanol (2/1/1, v/v/v) mixtures were investigated. Table 1 shows that for UAE method, the hexane/acetone mixture gave higher extraction yield followed by hexane/acetone/ethanol and hexane/ethanol mixture.

In general, for UAE, the recovery of the extracted compounds is mainly attributed to the acoustic cavitation phenomenon, which is thought to be enhanced when solvents with low vapor pressures or solvents with low viscosity are used (Tsiaka et al., 2015).

Table 1 show that the solvent mixtures containing acetone gave slightly higher extraction yield. Since acetone has a higher vapor pressure, it was expected that the extraction yield with hexane/acetone would have been lower compared to that of the hexane/acetone/ethanol and hexane/ethanol mixture solvents. A probable reason for the higher yield in case of hexane/acetone could be the polarity and the lower viscosity of acetone. Indeed, the polarity of the solvents lead to the increase of the permeability of the cell wall, and their lower viscosity help create an acoustic cavitation (Tsiaka et al., 2015). For UAE, the non-polar solvent hexane was chosen as a component of the mixture of solvents, which can help prevent degradation of heat-sensitive components and solubilize non-polar compounds (Hiranvarachat & Devahastin, 2014). The mixture of hexane/acetone, with the physicochemical properties mentioned above, provides optimum extraction yields and was so selected as the solvent for the RSM trials. Similar solvents systems were used by Strati and Orepoulou (2011), who reported that the acetone/hexane mixture was more efficient than the ethanol/hexane mixture in extracting carotenoids from tomato waste. On the other hand, Lin and Chen (2003) demonstrated that the hexane/acetone mixture was more efficient than hexane/ethanol and hexane/acetone/ethanol mixtures in the extraction of lutein from tomato juice.

3.3.2. Effect of solvent composition

In order to visualize the response and experimental levels of each factor and to deduce the optimal conditions, the regression coefficients were used and the fitted polynomial equations were presented as surface plots (Fig. 1). As it is shown in Fig. 1a, the TCC yield initially increased with increasing proportions of hexane in the hexane/acetone solvent mixture and finally decreased at the highest concentration. The maximum TCC yield was obtained with a mixture of hexane/acetone of 80:20 (*v*/v). Strati and Orepoulou (2011) also demonstrated that TCC yield from tomato waste increased with increasing of hexane percentage in the hexane/ethyl acetate solvent mixture up to 50% (v/v) and then decreased for higher concentrations. On the other hand, Poojary and Passamonti (2015) obtained a higher recovery of lycopene with a high concentration of hexane in hexane/acetone system (75/25, v/v).

3.3.3. Effect of extraction time

Fig. 1a shows the 3D surface plots of the effect of extraction time on extraction yield for UAE. Indeed, an increase in extraction time caused a negative effect on the extraction yield and the higher extraction yield was accomplished at the lower extraction time (10 min). This phenomenon might be due to a possible oxidative degradation caused by the prolonged US treatment (Tsiaka et al., 2015). Higher carotenoids content was also obtained at a shorter time (from 10 to 15 min) by Singh, Barrow, Mathur, Tuli, and Puri (2015) when extracting them from the microalga *Chlorella saccharophila*.

3.3.4. Effect of amplitude

Fig. 1b shows the effect of amplitude on the response and its interaction with extraction time. The result shows that when the amplitude is fixed at a minimum, increases in time parameter result in significant increase in carotenoids yield; and when the time is set at minimum, the increase in the amplitude parameter result in significant increases in the response. The surface plots showed that the maximum TCC was achieved with the upper extreme operational power used in this study, which was 100%. The increasing extraction of total carotenoids with stronger ultrasonic intensity transmitted to the medium can be explained at least in part by the greater number of cavitation micro-bubbles, which facilitate the disruption of tissue cell walls and accelerate the diffusion of carotenoids into the medium (Ordonez-Santos, Pinzon-Zarate, & Gonzalez-Salcedo, 2015; Tsiaka et al., 2015). Yolmeh et al. (2014) have also reported that the increase in the extraction of carotenoids from peach palm fruit, annatto seeds and red grapefruit is influenced by increasing ultrasonic intensity.

3.3.5. Effect of solvent-to-solid ratio

Fig. 1c shows the 3D surface plots of the ratio effect on extraction yield. It was observed that the yield increased with the increase of the solvent-to-solid ratio. One of the main reasons of this effect could be that higher solvent-to-solid ratio could cause greater concentration differences between phases which accelerated mass transfer and facilitated the carotenoids diffusion into the medium (Tsiaka et al., 2015). However, after the mass transfer process reached its maximum, further increases of solvent-to-solid ratio prolonged the distance of diffusion from solvent to the matrix and reduced the carotenoids extraction, thus indicating that there was no additional advantage of increasing the solvent-to-solid ratio above 55 mL/g. This phenomenon was also observed by Singh et al. (2015) who found that a higher ratio of solvent to raw material leads to the decrease of the yield of ultrasonic extraction of β-carotene and zeaxanthin from *Chlorella saccharophila*.

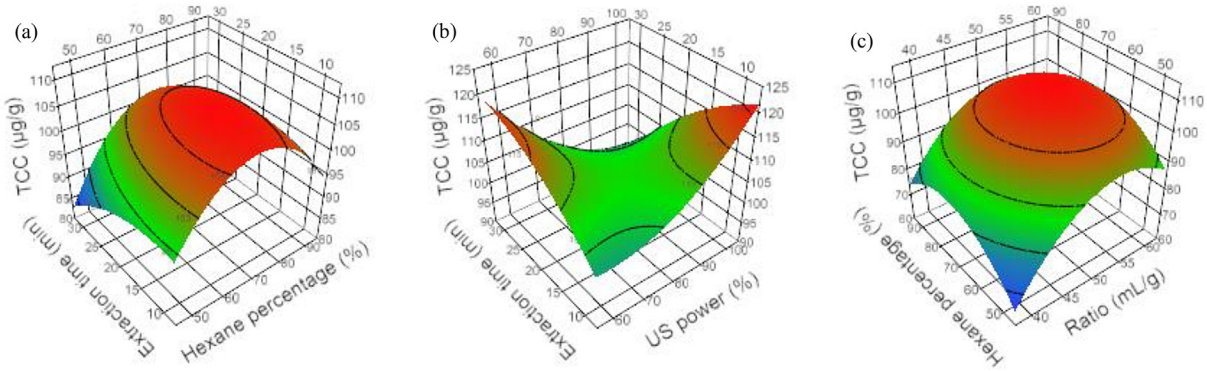

Fig. 1. Response surface plots of UAE of carotenoids. Hexane/acetone was the extraction solvent and hexane percentage with time (a), power with time (b) and hexane percentage with ratio (c) were the interaction effects.

3.4. Model validation and efficiency of the UAE optimisation

Under the operating conditions, the predicted carotenoids yield was about 121.3 µg/g, while the experimental yield obtained in the extraction procedure was 124.61 ± 3.82 µg/g. No significant difference was observed between the theoretical and experimental responses. The results obtained by RSM optimization verified that the models of UAE process are valid and adequate for carotenoids extraction. The concentration of 73 ± 1.39 µg/mL represented the total carotenoids yield obtained during 2 h of conventional extraction carried out with the aid of a stirring plate, with equivalent conditions used for UAE (in terms of solvent, solvent concentration, temperature and ratio) and a consumption energy of 4.536×10^6 J. Ultrasound with a reduced time (10 min), led to a higher yield (124.61 ± 3.82 µg/g) and a lower consumption energy (2.999×10^6 J). Some carotenoid-rich food products are pepper (with reported values of 988 $\mu g/g_{dw}$) (Navarro, Flores, Garrido, & Martinez, 2006), tomato peel (with reported values of 793.2 $\mu g/g_{dw}$) (Knoblich, Anderson, & Latshaw, 2005) and carrot (with reported values of 239 $\mu g/g_{dw}$) (Sharma, Karki, Thakur, & Attri, 2010), although of course, it is to be taken into account that the carotenoid levels depend of many several factors. Cantaloupe waste with a concentration of 124.61 ± 3.82 $\mu g/g_{dw}$ occupies also an important place compared to other sources used in the industry such as avocado peel (15.2 $\mu g/g_{dw}$), guava (138 $\mu g/g_{dw}$) (Ayala-Zavala et al., 2011) and lemon peel (110 $\mu g/g_{dw}$) (Wang, Chuang, & Hsu, 2008). In addition to the important carotenoids yield of cantaloupe waste, optimizing the extraction of these compounds by ultrasound makes the technique more cost-effective by saving time and energy.

3.5. RRLC-DAD analysis

Table 2 shows the concentration of total and individual carotenoids identified. The RRLC profile and chromatograms in Fig. 2, shows that three main carotenoids (lutein, β-carotene, and violaxanthin) are present in the cantaloupe peels analyzed. These compounds have also been reported by Laur and Tian (2011) in cantaloupe fruit tissue. Quantitatively, dried cantaloupe peels were characterized by a higher content of lutein (63.24 ± 0.73 µg/g), followed by β-carotene (56.43 ± 0.11 µg/g), with traces of violaxanthin. These carotenoids were previously reported in cantaloupe fruit with a proportion of 87% for β-carotene, 1% for lutein, and 9% for violaxanthin and neoxanthin (Sommerburg, Keunen, Bird, & van Kuijk, 1998).β-carotene and lutein are among the natural colorants authorized to be used in the food industry (Martins & Ferreira, 2017). They are also thought to contribute to some health benefits. More specifically, lutein is attracting much attention for its possible role in eye and brain health together with zeaxanthin (Johnson, 2014). Interestingly, it appears that in years to come there will be values similar to Dietary Reference Intakes (DRIs) for non-essential health-promoting bioactive compounds, lutein being one of this class of molecules for which such recommendations could be established sooner (Ranard et al., 2017).

3.6. Assessment of structural changes by SEM

Fig. 3 shows the micrographs of non-extracted powder, extracted powder by the UAE and a powder that was only mixed with the solvent at the same time/temperature used during the UAE as a control. Unlike the untreated powder that is intact, it is clearly observed that UAE caused noticeable changes in the integrity of the matrix that facilitated the release of cellular components. This could be attributed to a disruption of the wall cells *via* cavitation phenomenon (Kong et al., 2010). Indeed, excluding the use of ultrasound, the solvent used solubilizes also certain amount of matrix compounds, which is indicate by the observed alteration of the microstructure of the matrix. However, this alteration is not very pronounced compared to that caused by ultrasound which causes marked perforations on the structure, changes that are not readily observed when the solvent is used alone. The cavitation phenomenon produces enough energy to favor collisions among plant cell constituents (Mason, Chemat, & Vinatoru, 2011). The ultrasound treatment can generate pores and micro-fractures, which facilitate the diffusion of solvents inside the cell and the release of solutes outside the structures that contain them (Kyriakopoulou et al., 2015). Indeed, ultrasound is considered to greatly affect the structure of the plant material by a sponge effect (CarcelCarrión, García Pérez, Benedito Fort, & Mulet Pons, 2012; Nowacka & Wedzik, 2015). Concerning the facilitation of the diffusion process, ultrasound is thought to do it through

Table 2
Total carotenoids and separated carotenoids from dried cantaloupe waste extracted using the ultrasound-assisted extraction.

Extraction methods	Carotenoids yield ($\mu g_{\beta CE}/g_{dw}$)			RRLC identified carotenoids ($\mu g_{\beta CE}/g_{dw}$)			
	Predicted values (RSM)	UV–Vis	RRLC-DAD	lutein	β-carotene	violaxanthin	β-cryptoxanthin
UAE	121.30 ± 7.00[a]	124.61 ± 3.82[a]	119.67 ± 0.71[a]	63.24 ± 0.73[a]	56.43 ± 0.11[a]	traces	nd

UAE: Ultrasound assisted extraction; nd: not detected.
*Results are reported as means ± S.D. Values with different letters (a < b < c) differ significantly ($p < 0.05$) according to ANOVA and tukey's test. βCE, β-carotene equivalents.

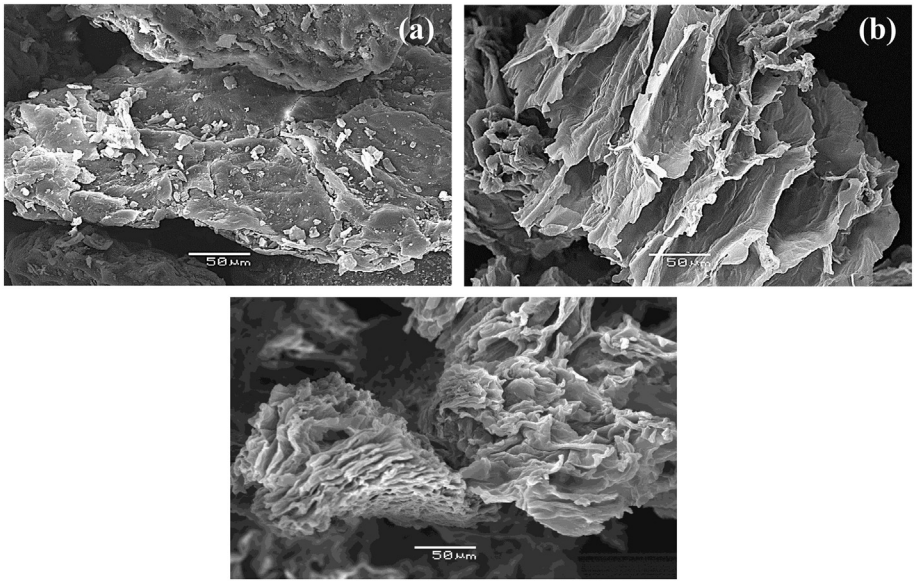

Fig. 2. Scanning electron microscope images of cantaloupe waste powder before (A), after extraction by ultrasound assisted extraction(B) and control (powder treated only with extraction solvent) (C).

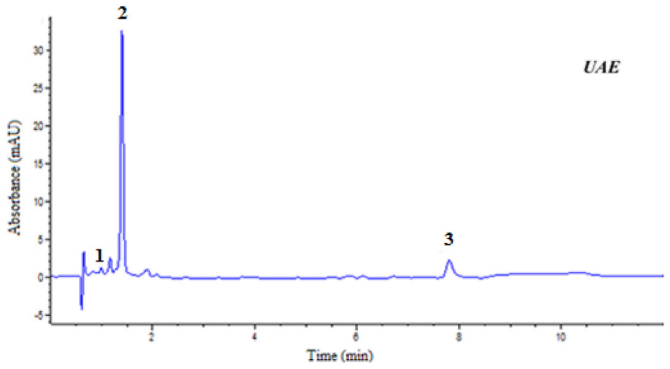

Fig. 3. Identification of selected individual carotenoids at 450 nm using a RRLC-DAD system. Carotenoids identified were violaxanthin (1), lutein (2) and β-carotene (3).

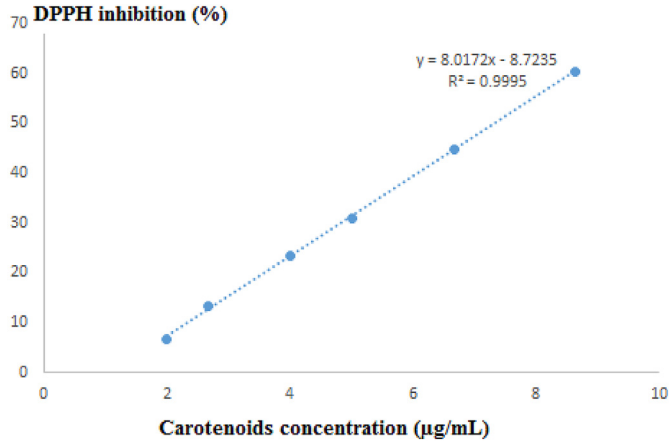

Fig. 4. DPPH radical scavenging activity of UAE extract from waste cantaloupe.

the disruption of the solvent layer around the matrix, which is mainly formed by the solvents and cellulose from the cell wall (Mason et al., 2011).The cavitation produced leads to the formation of bubbles on the cell surface that release vapor leading to the bursting of the cell walls (Ordonez-Santos et al., 2015; Teng et al., 2016).

3.7. Antioxidant activity

Fig. 4 shows the decrease of the DPPH radical as a function of the different concentrations of carotenoid extracts. Regarding the anti-radical dose, the percentage of DPPH radical disappearance increases from 6.7 to 60.5% by increasing the concentration of carotenoids from 2 to 8.63 µg/mL. The concentration needed to reduce the DPPH radical by 50% (IC_{50}) was 7.33 ± 0.22 µg/mL. This concentration is low and it is lower than that of β-carotene and Trolox standards (350 ± 1.00 and 102.34 ± 5.79 µg/mL, respectively) indicating the effective elimination of DPPH by carotenoids extracted from cantaloupe waste and their strong activity against this radical. In addition, the IC_{50} value of carotenoids extract is lower than that of Trolox (13 µg/mL) and lutein (35 µg/mL) found by Sindhu, Peethi, and Kuttan (2010).

High correlation has been reported between lutein and DPPH assay (Ingkasupart, Mandchai, Tae SDNG, & Hwa HDNG, 2015). The scavenging ability of carotenoids is thought to be mainly affected by their structural features, like the number and arrangement of conjugated double bonds and the presence of certain chemical groups (Jiménez-Escrig, Jiménez-Jiménez, Sánchez-Moreno, & Saura-Calixto, 2000; Martins & Ferreira, 2017; Tan et al., 2014). Due to their *in vitro* antioxidant capacity, it appears interesting to assess the utility of cantaloupe peel extracts as ingredients of cosmetic products (total screen) intended for the protection of the skin against external aggressions triggered by oxidative species or as antioxidants to preserve and extend the shelf life of cosmetic (Martins & Ferreira, 2017). Of course, such extracts would also be interesting for the food industry not only to protect products from oxidation, but also to impart colour, fortify them with the provitamin A carotenoid β-carotene or for the development of health-promoting products.

3.8. Mineral composition

The mineral composition of dried cantaloupe waste is shown in Table 3. The mineral levels of the fruit *Cucumis melo* are influenced by several parameters namely, salt composition of the cultivated soils, the collection period and the ripening phase (Del Amor, Martinez, & Cerda, 1999). The results indicated that cantaloupe waste contained a higher concentration of K (24,491.68 ± 710.26 mg/kg) and Ca (8260.17 ± 35.52 mg/kg). Also, there was no statistically difference

Table 3
Mineral composition of cantaloupe waste.

Minerals	Concentration (mg/kg)
Ca	8260.16 ± 35.51[b]
Cu	4.56 ± 0.09[d]
Fe	26.28 ± 0.43[d]
K	24,491.68 ± 710.25[a]
Mg	4904.11 ± 78.46[c]
Mn	7.50 ± 0.10[d]
Na	4470.88 ± 79.13[c]
P	4811.69 ± 101.52[c]
Zn	17.15 ± 0.51[d]

Mineral concentration was expressed as mg of mineral salt per Kg of dried cantaloupe waste. Values with different letters (a < b < c < d) differ significantly (p < 005) according to ANOVA and tukey's test.

between Mg (4904.11 ± 78.47 mg/kg), P (4811.69 ± 101.53 mg/kg) and Na (4470.89 ± 79.14 mg/kg) levels. Amor et al. (1999) and Botía, Carvajal, Cerdá, and Martínez (1998) found a similar mineral composition in other *Cucumis melo* cultivars. In addition to K, Ca, Mg, P, and Na, low levels of Fe (26.28 ± 0.4 mg/kg), Zn (17.16 ± 0.52 mg/kg), Mn (7.51 ± 0.11 mg/kg) and Cu (4.56 ± 0.4 mg/kg) were found (Botía et al., 1998). Minerals are involved in different key biological actions and are essential nutrients that our organism can not synthesize, so they must be acquired from foods or other products. Some minerals are cofactors of enzymes involved in the antioxidant reactions of the endogenous system such as superoxide dismutase which involves Mn, Cu and Zn; catalase using Fe and glutathione peroxidase using Se (Boudries et al., 2015). The major mineral of cantaloupe peel analyzed was potassium. This element is one of the three electrolytes that circulate in the blood vessels along with sodium and chlorine and the most important ion of the cell cytoplasm (Mulkidjanian, Bychkov, Dibrova, Galperin, & Koonin, 2012). Potassium is the principal compound of membrane transporters, namely sodium/potassium and hydrogen potassium pumps (Clausen, Hilbers, & Poulsen, 2017; El Mernissi & Doucet, 1984). The first one plays a very important role in the nerve conduction and absorption of nutrients such as glucose (Clausen et al., 2017) and the second one is involved in the digestive tract in the stomach, it is responsible for the gastric acidity essential to the digestion of food and the protection of the stomach and intestine from pathogenic bacteria (Beasley, Koltz, Lambert, Fierer, & Dunn, 2015).

4. Conclusion

The UAE method was effective for carotenoids extraction from cantaloupe waste allowing for higher recovery yield. The RRLC analysis revealed the predominance of lutein and β-carotene in the extracts obtained, which exhibited *in vitro* antioxidant capacity as assessed by the DPPH method. The waste also proved to be a good source of several mineral elements. In summary, it can be concluded that waste from cantaloupe fruit can be used to obtain a series of health-promoting compounds that have multiple uses in the food, pharmaceutical and cosmetic industries.

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