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* antioxidantactivity(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 ratioof 55 mL/g were the optimal conditions for the extraction of carotenoids. Under these conditions, the carotenoid content of the extract was 124.61 ± 3.82µg/g.The microscopic analysis revealed the effectiveness of the ultrasound treatment that results in noticeable physical changes, like microscopic perforations and breakages.

Keywords: Cantaloupe,carotenoids, ultrasound assisted extraction, green extraction, antioxidant activity, mineral composition, waste.

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 andrepel 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, 2007a). 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 that occur. It is estimated that about 1.3 billion of food wastes are produced per year (Matharu, de Melo&Houghton,2016; Arshadi, Thomas, Lukasik, Brncic& da Costa Lopes, 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,AbertVian, &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, Zinoviadou, Galanakis, Orlien, Grimi, Vorobiev, & Barba, 2014;Kyriakopoulou, Papadaki, &Krokida, 2015).In this regard, ultrasound is considered as an emerging technology. Its applications in agro-food industry can lead to a reduction in treatment time and energy consumptionthrough theoptimization of the factors involved (Chemat, AbertVian, &Cravotto, 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, Papadaki, &Krokida, 2015;ŠicŽlabur, Voća, Dobričević, Brnčić, Dujmić, &RimacBrnčić, 2015;Zinoviadou, Galanakis, Brnčić, M., Grimi, Boussetta, Mota, Saraiva, Patras, Tiwari, & Barba, 2015).

Given the importance of carotenoids in agro-food health, other researchers such as Yolmeh, HabibiNajafi, &Farhoosh (2014),Dey, &Rathod(2013), Ofori-bcateng, & Lee (2013), Li, Fabiano-Tixier,Tomao, Cravotto, &Chemat(2013) and Lianfu, &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, theywere 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, 2007b).

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 & 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

161 acetonitrile (solvent A), methanol (solvent A) and ethyl acetate (solvent C). The linear gradient
162 elution was: 0 min, 85% A + 15% B; 5 min, 60% A + 20% B + 20% C; 7 min, 60% A + 20%
163 B + 20% C; 9 min, 85% A + 15% B; 12 min, 85% A + 15%B. The open labChem Station
164 software was used (Stinco, Benítez-González, Hernanz, Vicario, & Meléndez-Martínez, 2014).

165 **2.4. Analysis of powders by scanning electron microscopy**

166 Scanning electron microscopy was used in order to study the effect of the UAE on the
167 microstructure of the powder. Micrographs before and after the extraction processes were
168 obtained for morphological characterization. Three samples (non-extracted powder, powder
169 after UAE and a control, specifically apowder that was only mixed with the solvent at the same
170 time/temperature used during the UAE) were collected and dried until constant mass in an oven
171 at 40 °C before SEM analysis. The sample particles were fixed on a specific carbon film support
172 and coated with gold for 10 min in a SCANCOAT Six SEM sputter coater (Edwards, Crawley,
173 England). The shape and surface features were observed by using a secondary electron detector,
174 and the images were taken with a scanning electron microscope (JEOL JSM-6460LV, USA) at
175 25 kV.

176 ***2.5.*Assessment of the *in vitro* antioxidant activity by the DPPH assay**

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

183 $$\%DPPH\ inhibition = \frac{Abs\ control - Abs\ sample}{Abs\ control} \times 100 \quad (2)$$

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 tests was made to check the absence of interferences and for which the values of the recovery were between 85-115%.

2.6.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, Zeng, Xu, Zheng, Lin, Gan & Lo, 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^2_{adj}), 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.

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, Zoumpoulakis, Sinanoglou, Makris, Heropoulos & Calokerinos, 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, Zoumpoulakis, Sinanoglou, Makris, Heropoulos & Calokerinos, 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 & Orepoulou (2001), who reported that the acetone/hexane mixture was more efficient than the ethanol/hexane mixture in extracting carotenoids from tomato waste.

283 On the other hand, Lin & Chen (2003) demonstrated that the hexane/acetone mixture was more
284 efficient than hexane/ethanol and hexane/acetone/ethanol mixtures in the extraction of lutein
285 from tomato juice.

286 *3.3.2. Effect of solvent composition*

287 In order to visualize the response and experimental levels of each factor and to deduce
288 the optimal conditions, the regression coefficients were used and the fitted polynomial
289 equations were presented as surface plots (Figure 1).As it is shown in Figure 1a, the TCC yield
290 initially increased with increasing proportions of hexane in the hexane/acetone solvent mixture
291 and finally decreased at the highest concentration. The maximum TCC yield was obtained with
292 a mixture of hexane/acetone of80:20 (v/v). Strati & Orepoulou (2001) also demonstrated that
293 TCC yield from tomato waste increased with increasing of hexane percentage in the
294 hexane/ethyl acetate solvent mixture up to 50% (v/v) and then decreased for higher
295 concentrations. On the other hand, Poojary & Passamonti (2015) obtained a higher recovery of
296 lycopene with a high concentration of hexane in hexane/acetone system (75/25, v/v).

297 *3.3.3. Effect of extraction time*

298 Figure 1a shows the 3D surface plots of the effect of extraction time on extraction yield
299 for UAE. Indeed, an increase in extraction time caused a negative effect on the extraction yield
300 and the higher extraction yield was accomplished at the lower extraction time (10 min). This
301 phenomenon might be due to a possible oxidative degradation caused by the prolonged US
302 treatment (Tsiaka, Zoumpoulakis, Sinanoglou, Makris, Heropoulos, &Calokerinos, 2015).
303 Higher carotenoids content was also obtained at a shorter time (from 10 to 15 min) by Singh,
304 Barrow, Mathur, Tuli, & Puri (2015) when extracting them from the microalga *Chlorella*
305 *saccharophila*.

306 3.*3.4. Effect of amplitude*

307 Figure 1b shows the effect of amplitude on the response and its interaction with
308 extraction time. The result shows that when the amplitude is fixed at a minimum, increases in
309 time parameter result in significant increase in carotenoids yield; and when the time is set at
310 minimum, the increase in the amplitude parameter result in significant increases in the response.
311 The surface plots showed that the maximum TCC was achieved with the upper extreme
312 operational power used in this study, which was 100%. The increasing extraction of total
313 carotenoids with stronger ultrasonic intensity transmitted to the medium can be explained at
314 least in part by the greater number of cavitation micro-bubbles, which facilitate the disruption
315 of tissue cell walls and accelerate the diffusion of carotenoids into the medium (Ordonez-
316 Santos, Pinzon-Zarate, & Gonzalez-Salcedo, 2015; Tsiaka, Zoumpoulakis, Sinanoglou,
317 Makris, Heropoulos, & Calokerinos, 2015). Yolmeh, Habibi Najafi, & Farhoosh (2014) have
318 also reported that the increase in the extraction of carotenoids from peach palm fruit, annatto
319 seeds and red grapefruit is influenced by increasing ultrasonic intensity.
320 *3.3.5. Effect of solvent-to-solid ratio*
321 Figure1c shows the 3D surface plots of the ratio effect on extraction yield. It was observed
322 that the yield increased with the increase of the solvent-to-solid ratio. One of the main reasons
323 of this effect could be that higher solvent-to-solid ratio could cause greater concentration
324 differences between phases which accelerated mass transfer and facilitated the carotenoids
325 diffusion into the medium (Tsiaka, Zoumpoulakis, Sinanoglou, Makris, Heropoulos, &
326 Calokerinos, 2015). However, after the mass transfer process reached its maximum, further
327 increases of solvent-to-solid ratio prolonged the distance of diffusion from solvent to the matrix
328 and reduced the carotenoids extraction, thus indicating that there was no additional advantage
329 of increasing the solvent-to-solid ratio above 55 mL/g. This phenomenon was also observed by
330 Singh et al. (2015) who found that a higher ratio of solvent to raw material leads to the decrease
331 of the yield of ultrasonic extraction of β-carotene and zeaxanthin from *Chlorella saccharophila*.

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 of73 ± 1.39μg/mL represented the total carotenoids yield obtained during 2 hours 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, Vega-Vega, Rosas-Domínguez, Palafox-Carlos, Villa-Rodriguez, Wasim Siddiqui, Dávila-Aviña, & González-Aguilar, 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 Figure 2, shows that three main carotenoids (lutein, β-

carotene, and violaxanthin) are present in the cantaloupe peels analyzed. These compounds have also been reported byLaur,& 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% forviolaxanthin 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 toDietary 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, Jeon, Mohn, Griffiths, Johnson, & Erdan, 2017).

3.6. Assessment of structural changes by SEM

Figure 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, Zu, Fu, Liu , Chang & Gu, 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, Papadaki & Krokida, 2015). Indeed, ultrasound is considered to greatly affect the structure of the plant material by a sponge effect (Carcel Carrión, García Pérez, Benedito Fort, & Mulet Pons, 2012; Nowacka, & Wedzik, 2015). Concerning the facilitation of the diffusion process, ultrasound is thought to do it through the disruption of the solvent layer around the matrix, which is mainly formed by the solvents and cellulose from the cell wall (Mason, Chemat, & Vinatoru, 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 (Ordóñez-Santos, Pinzón-Zarate, & González-Salcedo, 2015;Teng, Chen, Huang, Wang, Lin, Liu, Lee, & Song, 2016).

3.7. Antioxidant Activity

Figure 4 shows the decrease of the DPPH radical as a function of the different concentrations of carotenoid extracts. Regarding the antiradical dose, the percentage of DPPH radical disappearance increases from 6.7 to 60.5% by increasing the concentration of carotenoids from 2 to 8.63µg/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 (13µg/ml) and lutein (35 µg/mL) found by Sindhu, Peethi, & Kuttan (2010).

High correlation has been reported between lutein and DPPH assay (Ingkasupart, Mandchai, Tae & Hwa, 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, Xue, Abbas, Feng, Zhang & Xia, 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 minerals 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 (24491.68 ± 710.26 mg/kg) and Ca (8260.17 ± 35.52 mg/kg). Also, there was no statistically difference between Mg (4904.11 ± 78.47 mg/kg), P (4811.69 ± 101.53 mg/kg) and Na (4470.89 ± 79.14 mg/kg) levels. Del Amor, Martinez & Cerda (1999) and Botía, Carvajal, Cerdá & Martínez (1998) founda 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, Carvajal, Cerdá & Martínez, 1998). Mineralsare 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, Souagui, Nabet, Ydjedd, Kefalas,

Madani, & Chibane, 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, Hilbers, & Poulsen, 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 minerals 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.

References

Arshadi, M., Thomas. M. A., Lukasik, R. M., Brncic, M., da Costa Lopes, A. M., Finell, M., &Yuste-Córdoba, F. J. (2016).Pre-treatment and extraction techniques for recovery of added value compounds from wastes throughout the agri-food chain.*Green Chemistry,* 18, 6160-6204. DOI: 10.1039/C6GC01389A.

455 Ayala-Zavala, J.F., Vega-Vega, V., Rosas-Domínguez, C.G., Palafox-Carlos, H., Villa-Rodriguez,
456 J.A., Wasim Siddiqui, Md., Dávila-Aviña, J.E., & González-Aguilar, G.A. (2011). Agro-
457 industrial potential of exotic fruit byproducts as a source of food additives. *Food Research*
458 *International*, 44, 7, 1866-1874. doi:10.1016/j.foodres.2011.02.021.

459 Barba, F. J., Galanakis, C. M., Esteve, M. J., Frigola, A., & Vorobiev, E. (2015). Potential use of
460 pulsed electric technologies and ultrasounds to improve the recovery of high-added value
461 compounds from blackberries. *Journal of Food Engineering*, 167, 38-
462 44.http://dx.doi.org/10.1016/j.jfoodeng.2015.02.001.

463 Beasley, D.E., Koltz, A. M., Lambert, J.E., Fierer, N., & Dunn, R. R, (2015). The Evolution of
464 stomach acidity and its relevance to the human microbiome. *PLOS ONE*,
465 DOI:10.1371/journal.pone.0134116.

466 Botía, P., Carvajal, M., Cerdá, A., & Martínez, V. (1998). Response of eight *Cucumis melo*
467 cultivars to salinity during germination and early vegetative growth. *Agronomie, EDP*
468 *sciences*, 18(8-9), 503-513. Id: hal-00885899.

469 Boudries, H., Souagui, S., Nabet, N., Ydjedd, S., Kefalas, P.,Madani, K., &Chibane, M. (2015).
470 Valorisation of Clementine peels for the recovery of minerals and antioxidants:
471 Evaluation and characterisation by LC-DAD-MS of solvent extracts. *International Food*
472 *Research Journal,* 22(3): 1218-1226.

473 Brand-Williams, W., Cuvelier, M.-E., & Berset, C. (1995). Use of a free radical method to
474 evaluate antioxidant activity. *LWT-Food science and Technology,* 28(1), 25-
475 30.https://doi.org/10.1016/S0023-6438(95)80008-5.

476 CarcelCarrión, J.A., García Pérez, J.V., Benedito Fort, J.J., &Mulet Pons, A. (2012). Food
477 Process Innovation Through New Technologies: Use of Ultrasound. *Journal of Food*
478 *Engineering*, 110(2):200-207. doi:101016/J.JFOODENG.2011.05.038.

479 Chemat, F., Vian, M. A., &Cravotto, G. (2012). Green Extraction of Natural Products: Concept
480 and Principles. *International Journal of Molecular Sciences*, 13, 8615-
481 8627.doi:10.3390/ijms13078615.

482 Clausen, M, V., Hilbers, F., & Poulsen, H, (2017). The structure and function of the Na,K-
483 ATPase isoforms in health and disease. *Frontiers in Physiology*, 8, 1-16. doi:
484 10.3389/fphys.2017.00371.

485 Del Amor, F., Martinez, V., & Cerda, A. (1999). Salinity duration and concentration affect fruit
486 yield and quality, and growth and mineral composition of melon plants grown in perlite.
487 *HortScience,* 34(7), 1234-1237.

488 Deng, Q., Zinoviadou, K. G., Galanakis, C. M., Orlien, V., Grimi, N., Vorobiev, E., & Barba,
489 F. J. (2014). The effects of conventional and nonconventional processing on
490 glucosinolates and its derived forms, isothiocyanates: Extraction, degradation, and
491 applications. *Food Engineering Reviews*, 7, 357-381.DOI 10.1007/s12393-014-9104-9.

492 Dey, S., & Rathod, V. (2013).Ultrasound assisted extraction of β-carotene from Spirulina
493 platensis. *Ultrasonics Sonochemistry*, 20, 1, 271-276.
494 https://doi.org/10.016/j.ultsonch.2012.05.010.

495 Eldahshan, O. A., &Singab, A. B. (2013).Carotenoids.*Journal of Pharmacognosy and*
496 *Phytochemistry,* 2 (1), 225-233.

El Mernissi, G., & Doucet, A. (1984). Quantitation of [3H] ouabain binding and turnover of Na-K-ATPase along the rabbit nephron. American. Journal of Physiology, 247, 158–167.

FDA. (1997). Food and Drug Administration, International conference on Harmonisation; Guidance on Impurities: Residual Solvents. *Federal Register*, December24, 62, 247.

Hiranvarachat, B., & Devahastin, S. (2014). Enhancement of microwave-assisted extraction via intermittent radiation: extraction of carotenoids from carrot waste. *Journal of Food Engineering*, 126, 17-26.https://doi.org/10.1016/j.jfoodeng.2013.10.024.

Ingkasupart, P., Mandchai, B., Tae SDNG, W &Hwa HDNG, J. (2015). Antioxidant activities and lutein content of 11 marigold cultivars (*Tagetes* spp.) grown in Thailand.*Food Science Technology Campinas*, 35(2), 380-385.D http://dx.doi.org/10.1590/1678-457X.6663.

Jiménez-Escrig, A., Jiménez-Jiménez, I., Sánchez-Moreno, C., & Saura-Calixto, F. (2000). Evaluation of free radical scavenging of dietary carotenoids by the stable radical 2, 2-diphenyl-1-picrylhydrazyl.*Journal of the Science of Food and Agriculture,*80 (11), 1686–1690.DOI: 10.1002/1097-0010(20000901)80:11<1686::AID-SFA694>3.0.CO;2-Y.

Johnson, E.J. (2014). Role of lutein and zeaxanthin in visual and cognitive function throughout the lifespan. *Nutrition Reviews*, 72, 605–612. doi:10.1111/nure.12133.

Knoblich, M., Anderson, B., & Latshaw, D. (2005). Analyses of tomato peel and seed byproducts and their use as a source of carotenoids. *Journal of the Science of Food and Agriculture*, 85, 7, 1166–1170. DOI: 10.1002/jsfa.2091.

Kong, Y., Zu, Y.G., Fu, Y.J., Liu, W., Chang, F.R., Li, J., Chen, Y.H, Zhang, S., & Gu, C.B. (2010). Optimization of microwave-assisted extraction of cajaninstilbene acid and

522 pinostrobin from pigeonpea leaves followed by RP-HPLC-DAD determination.*Journal*
523 *of Food Composition and Analysis*, 23(4), 382-
524 8.https://doi.org/10.1016/j.jfca.2009.12.009.

525 Kyriakopoulou K., Papadaki S., & Krokida M. (2015). Life cycle analysis of b-carotene
526 extraction techniques. *Journal of Food Engineering*, 167, 51-
527 58.http://dx.doi.org/10.1016/j.jfoodeng.2015.03.008.

528 Laur, L.M., & Tian, L. (2011). Provitamin A and vitamin C contents in selected California-
529 grown cantaloupe and honeydew melons and imported melons. *Journal of Food*
530 *Composition and Analysis*, 24 (2011) 194–201. doi:10.1016/j.jfca.2010.07.009.

531 Li, Y., Fabiano-Tixier, A.S., Tomao, V., Cravotto, G., &Chemat, F. (2013).Green ultrasound-
532 assisted extraction of carotenoids based on the bio-refinery concept using sunflower oil
533 as an alternative solvent. *UltrasonicsSonochemestry*, 20, 1, 12-18.
534 https://doi.org./10.1016/j.ultsonch.2012.07.005.

535 Lianfu, Z. &Zelong, L. (2008). Optimization and comparison of ultrasound/microwave assisted
536 extraction (UMAE) and ultrasonic assisted extraction (UAE) of lycopene from tomatoes.
537 *UltrasonicsSonochemestry*, 15, 5, 731-737.
538 https://doi.org./10.1016/j.ultsonch.2007.12.001.

539 Lin, C.H., & Chen, B.H. (2003). Determination of carotenoids in tomato juice by liquid
540 chromatography. *Journal of Chromatography A*, 1012(1), 103-
541 109.https://doi.org/10.1016/S0021-9673(03)01138-5.

542 Martins, N., Ferreira, I.C.F.R. (2017). Wastes and by-products: Upcoming sources of
543 carotenoids for biotechnological purposes and health-related applications. *Trends in Food*
544 *Science & Technology.*; 62:33-48. DOI: 10.1016/j.tifs.2017.01.014.

545 Martins, N., Roriz, C.L., Morales, P., Barros, L., Ferreira, I.C. (2016). Food colorants:
546 Challenges, opportunities and current desires of agro-industries to ensure consumer
547 expectations and regulatory practices. *Trends in Food Science & Technology*, 52, 1-
548 15.https://doi.org/10.1016/j.tifs.2016.03.009.

549 Matharu, A.S, de Melo, E.M.,&Houghton, J.A. (2016). Opportunity for high value-added
550 chemicals from food supply chain wastes, *Bioresource Technology*, 215,123-130.
551 https://doi.org/10.1016/j.biortech.2016.03.039.

552 Mason, T. J., Chemat, F., & Vinatoru, M. (2011). The extraction of natural products using
553 ultrasound or microwaves.*Current Organic Chemistry*, 2011, 15, 237-247.

554 Meléndez-Martínez, A.J., Britton, G., Vicario, I.M., & Heredia, F.J. (2007a). Relationship
555 between the colour and the chemical structure of carotenoid pigments. *Food Chemistry,*
556 101(3), 1145-1150. https://doi.org/10.1016/j.foodchem.2006.03.015.

557 Meléndez-Martínez, A.J., Vicario, I.M., & Heredia, F.J. (2007b). Carotenoids, color, and
558 ascorbic acid content of a novel frozen-marketed orange juice. *Journal of Agricultural*
559 *and Food Chemistry*, 55(4), 1347-1355.DOI: 10.1021/jf063025b.

560 Mermet, J.M. (1998). Revisitation of the matrix effects in inductively coupled plasma atomic
561 emission spectrometry: the key role of the spray chamber. *Journal of Analytical Atomic*
562 *Spectrometry*, 13, 419–422. ja13057197.

563 Mulkidjanian, A. Y., Bychkov, A. Y., Dibrova, D. V., Galperin, M. Y., &Koonin, E. V,
564 (2012). Origin of first cells at terrestrial, anoxic geothermal fields. *Proceedings of*
565 *the National Academy of Sciences of the United States of America (PNAS)*, 109, 821-830.

566 Navarro, J. M., Flores, P., Garrido, C., & Martinez, V. (2006). Changes in the contents of
567 antioxidant compounds in pepper fruits at different ripening stages, as affected by salinity.
568 *Food Chemistry*, 96, 1, 66–73. doi:10.1016/j.foodchem.2005.01.057.

569 Nowacka, M., &Wedzik, M, (2015).Effect of ultrasound treatment on microstructure, color and
570 carotenoid content in fresh and dried carrot tissue.*Applied Acoustics*,
571 http://dx.doi.org/10.1016/j.apacoust.2015.06.011.

572 Ofori-bcateng, C., & Lee, K.T. (2013). Response surface optimization of ultrasonic-assisted
573 extraction of carotenoids from oil palm (*Elaeisguineensis* Jacq.) fronds. *Food Science &*
574 *Nutrition*, 1, 3, 209–221. doi: 10.1002/fsn3.22.

575 Ordonez-Santos, L.E., Pinzon-Zarate, L.X., & Gonzalez-Salcedo, L.O. (2015). Optimization of
576 ultrasonic-assisted extraction of total carotenoids from peach palm fruit (*Bactris*
577 *gasipaes*) by-products with sunflower oil using response surface methodology.
578 *UltrasonicsSonochemistry,* 27, 560-566.https://doi.org/10.1016/j.ultsonch.2015.04.010.

579 Poojary, M.M., & Passamonti, P. (2015). Optimization of extraction of high purity all-trans-
580 lycopene from tomato pulp waste. *Journal of Food Chemestry*, 188, 84-
581 91.DOI:10.1016/j.foodchem.2015.04.133.

582 Ranard, K.M., Jeon, S., Mohn, E.S., Griffiths, J.C., Johnson, E.J., &Erdan Jr, J.W. (2017).
583 Dietary guidance for lutein: consideration for intake recommendations is scientifically
584 supported. European Journal of Nutrition 0, 0. doi:10.1007/s00394-017-1580-2.
585

586 Rinaldoni, A.N., Campderrós, M.E., Pérez Padilla, A., Perino, E., & Fernández, J.E. (2009).
587 Analytic determinations of minerals content by XRF, ICP and EEA in Ultrafiltered milk
588 and yoghurt. *Latin American Applied Research*, 39, 2, 113-118.
589
590 Rodriguez-Amaya, D.B., 2001. A guide to carotenoid analysis in foods, Life Sciences. ILSI
591 Press, Washington, D.C.

592 Saini R.M., Nile S.H., &Park S.W. (2015). Carotenoids from fruits and vegetables: Chemistry,
593 analysis, occurrence, bioavailability and biological activities. *Food Research*
594 *International*, 76, 735–750. http://dx.doi.org/10.1016/j.foodres.2015.07.047.

595 Scott, K.J. (2001). Detection and measurement of carotenoids by UV/VIS spectrophotometry.
596 *Current protocols in food analytical chemistry*,F:F2:F2.2.
597 DOI: 10.1002/0471142913.faf0202s00.

598 Sharma, K. D., Karki, S., Thakur, N. S., & Attri, S. (2010). Chemical composition, functional
599 properties and processing of carrot—a review. *Journal of Food Science and Technology*,
600 49, 1, 22–32. DOI 10.1007/s13197-011-0310-7.

601 Sindhu, E.R., Peethi, K.C., & Kuttan, R. (2010). Antioxydant activity of carotenoid lutein in
602 vitro and in vivo. *Indian journal of Experimental Biology*, 48, 8, 843-848.

603 Singh, D., Barrow, C.J., Mathur, A.S., Tuli, D.K., & Puri, M. (2015). Optimization of
604 zeaxanthin and β-carotene extraction from Chlorella saccharophila isolated from New
605 Zealand marine waters. *Biocatalysis and Agricultural Biotechnology,* 4(2), 166-
606 173.https://doi.org/10.1016/j.bcab.2015.02.001.

607 Sommerburg, O., Keunen, J. E. E., Bird, A.C & van Kuijk, F. J. G. M., (1998).Fruits and
608 vegetables that are sources for lutein and zeaxanthin: the macular pigment in human eyes.
609 *British Journal of Ophthalmology*, 82,907–910.http://dx.doi.org/10.1136/bjo.82.8.907.
610
611 Stinco, C.M., Benítez-González, A.M., Hernanz, D., Vicario, I.M., & Meléndez-Martínez, A.J.,
612 (2014). Development and validation of a rapid resolution liquid chromatography method
613 for the screening of dietary plant isoprenoids: Carotenoids, tocopherols and chlorophylls.
614 *Journal of Chromatography A*, 1370, 162-170.
615 https://doi.org/10.1016/j.chroma.2014.10.044.

616 Strati, I.F., & Oreopoulou, V. (2011). Process optimisation for recovery of carotenoids from
617 tomato waste. *Journal of Food Chemistry,* 129(3), 747-
618 752.https://doi.org/10.1016/j.foodchem.2011.05.015.

619 Šic Žlabur, J., Voća, S., Dobričević, N., Brnčić, M., Dujmić, F., & Rimac Brnčić, S. (2015).
620 Optimization of ultrasound assisted extraction of functional ingredients from *Stevia*
621 *rebaudiana* Bertoni leaves. *International Agrophysics*, 29, 2, 231-237.doi: 10.1515/intag-
622 2015-0017.

623 Tan, C., Xue, J., Abbas, S., Feng, B., Zhang, X., & Xia, S. (2014). Liposome as a delivery
624 system for carotenoids: comparative antioxidant activity of carotenoids as measured by
625 ferric reducing antioxidant power, DPPH assay and lipid peroxidation. *Journal of*
626 *agricultural and food chemistry*, 62(28), 6726-6735.DOI: 10.1021/jf405622f.

627 Teng, H., Chen, L., Huang, Q., Wang, J., Lin, Q., Liu, M., Lee W.Y., & Song H.
628 (2016).Ultrasonic-assisted extraction of raspberry seed oil and evaluation of its

physicochemical properties, fatty acid compositions and antioxidant activities.*Plos one*, 11(4): e0153457.doi:10.1371/journal. pone.0153457.

Tian, Y., Zeng, H., Xu, Z., Zheng, B., Lin, Y., Gan, C., & Lo, Y.M. (2012). Ultrasonic-assisted extraction and antioxidant activity of polysaccharides recovered from white button mushroom (*Agaricus bisporus*). *Carbohydrate Polymers,* 88(2), 522-529.https://doi.org/10.1016/j.carbpol.2011.12.042.

Tsiaka, T., Zoumpoulakis, P., Sinanoglou, V.J., Makris, C., Heropoulos, G.A., & Calokerinos, A.C. (2015). Response surface methodology toward the optimization of high-energy carotenoid extraction from Aristeus antennatus shrimp. *Analitica Chimica Acta,* 877, 100-110.https://doi.org/10.1016/j.aca.2015.03.051.

Wang, Y.C, Chuang, Y.C., & Hsu, H.W. (2008). The flavonoid, carotenoid and pectin content in peels of citrus cultivated in Taiwan. *Food Chemistry*, 106, 1, 277–284. doi:10.1016/j.foodchem.2007.05.086.

Yolmeh, M., Habibi Najafi, M.B., & Farhoosh, R. (2014). Optimisation of ultrasound-assisted extraction of natural pigment from annatto seeds by response surface methodology (RSM). *Journal of Food Chemistry*, 155, 319-324.https://doi.org/10.1016/j.foodchem.2014.01.059.

Zhang, Y.L., Yin, C.P., Kong, L.C., & Jiang, D.H. (2011). Extraction optimisation, purification and major antioxidant component of red pigments extracted from Camellia japonica. *Food Chemistry,* 129(2), 660-664. https://doi.org/10.1016/j.foodchem.2011.05.001.

649 Zinoviadou K.G., Galanakis, C.M, Brnčić, M., Grimi, N., Boussetta, N., Mota, M.J., Saraiva,
650 J., Patras, A., Tiwari, B.K., & Barba, F.J. (2015). Fruit juice sonication: Implications on
651 food safety, physicochemical and nutritional properties. *Food Research International*, 77,
652 4, 743-752.http://dx.doi.org/10.1016/j.foodres.2015.05.032.

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654

655 **Figure captions**

656 **Figure 1.** Response surface plots of UAEof carotenoids. Hexane/acetone was the extraction
657 solvent and hexane percentage with time (a), power with time (b) and hexane percentage with
658 ratio (c) were the interaction effects.

659 **Figure 2.** Scanning electron microscope images of cantaloupe waste powder before (A), after
660 extraction by ultrasound assisted extraction(B) and control (powder treated only withextraction
661 solvent) (C)

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

664 **Figure 4:** DPPH radical scavenging activity of UAE extract from waste cantaloupe

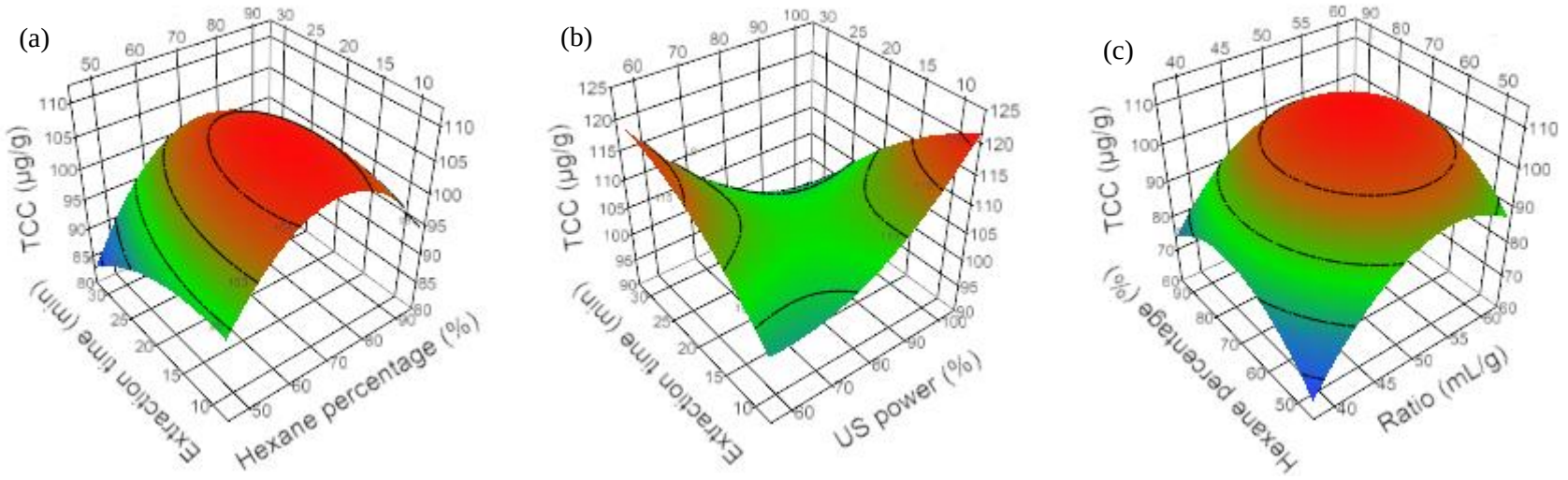
(a)
TCC (µg/g)
Extraction time (min)
Hexane percentage (%)
(b)
TCC (µg/g)
Extraction time (min)
US power (%)
(c)
TCC (µg/g)
Hexane percentage (%)
Ratio (mL/g)

Fig. 1

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	$110,18 \pm 0.76^{b}$	60	112.72 ± 0.4^{b}	50	128.08 ± 1.42^{a}
				40	$105,27 \pm 0.37^{c}$	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.*

Table 2. Total carotenoids and separated carotenoids from dried cantaloupe waste extracted ~~with different methods~~ 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	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.*

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	24491.68± 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.