Improving the extraction of carotenoids from tomato waste through the application of ultrasound under pressure

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Abstract

The influence of the application of moderate pressure on the ultrasound-assisted extraction (manosonication-assisted extraction) of carotenoids from dry tomato pomace using a mixture of hexane/ethanol as a solvent was investigated. In comparison with conventional solvent extraction, the carotenoid-extraction yield (CEY) increased with the application of ultrasound, and it increased even more so when static pressure was increased.

The maximum CEY for the control extraction (6.95 mg/100 g DW) was considerably lower than that for the extraction conducted with ultrasound with a vibration amplitude of 76 μm without an increase static pressure (11.32 mg/100 g DW). Furthermore, the application of ultrasound at a static pressure of 100 kPa and 76 μm increased the extraction yield by 149% compared to the control. Response surface methodology (RSM) was used to calculate the optimization of the extraction temperature (25 to 45 ºC) and the proportion of hexane in the solvent (25%, 50%, 75%) for the manosonication-assisted (94 μm of amplitude, 50 kPa, 6 min) extraction of carotenoids. In the range of experimental conditions investigated, the optimal solvent hexane/ethanol mixture for the extraction of carotenoids from tomato pomace was different for the conventional extraction (75/25) than for the extraction assisted by ultrasound under pressure (58/42). The increment of CEY, the possibility of decreasing the percentage of hexane, and the fact that the treatment did not cause the degradation of carotenoid extracts are key benefits of the manosonication-assisted extraction of carotenoids from tomato pomace.

Keywords: Extraction, Manosonication, Ultrasound, tomato pomace, carotenoids

1. Introduction

During the processing of fruits and vegetables, a large amount of by-products are generated, representing a major disposal problem for the food-processing industry in terms of costs and potential negative impacts on the environment [1]. However, in some cases, these by-products represent a source of valuable compounds because of their technological and nutritional properties [2,3]. Extraction is one of the crucial steps for preparing these compounds for use in the food, pharmaceutical, or cosmetic industries. Conventional extraction of these compounds is generally performed by the maceration of dried by-products using water or organic solvents, depending on whether the component of interest is water-soluble or lypophilic and water-insoluble. Generally, this technique is very time consuming and requires the use of a large volume of solvents [4]. The ability of several methods such as enzyme-aided extraction, supercritical CO_2, pressurized liquid extraction, microwaves, pulsed electric fields, or ultrasound has been evaluated to optimize the extraction of bioactive compounds from by-products by improving the extraction yield, diminishing the extraction time, and/or reducing the use of organic solvents [5].

Ultrasound is a non-thermal technology that has been shown to be particularly effective for improving the extraction of heat-labile compounds [6,7]. The improvement of extraction through high-power ultrasound is attributed to acoustic cavitation, which consists of the formation, growth, and collapse of microbubbles inside a liquid submitted to high-frequency sound waves (≥20 kHz) [8]. This collapse is accompanied by localized extreme pressures and temperatures that cause the formation of shock waves and high-velocity liquid jets. These mechanical effects of ultrasound may facilitate the release of desired compounds from their matrices by disrupting cellular tissues and by providing the greater penetration of the solvent into the cellular materials

[9]. Several studies have investigated the effect of ultrasound on the extraction of bioactive compounds such as polyphenols or carotenoids from different plant by-products. The application of ultrasound throughout the entire maceration process enhanced the extraction yield of polyphenols from orange peels by around 40% and of carotenoids from freeze-dried tomatoes by up to 100% [10-13] .

It is known that the application of moderate external pressure (up to 300 kPa) during sonication (manosonication) increases the intensity of the collapse of the bubbles [14,15]. It has been demonstrated that manosonication drastically increases the inactivation effect of ultrasound on microorganisms and enzymes [16-18]. However, the effect of manosonication on the extraction of bioactive components from plant by-products has not yet been investigated.

Industrial processing of tomatoes generates a considerable amount of waste (10-40% of total tomatoes processed) consisting of peel, seeds, and part of the pulp, which is known as tomato pomace [19]. This tomato by-product is a rich source of carotenoids, mainly in the form of lycopene and ß-carotene, that are authorized as natural colorants for enhancing the color of processed foods [20]. Moreover, carotenoids have been claimed to provide health benefits such as modulation of the immune system, reduction of the risk of cancer and cardiovascular diseases, and pro-vitamin A activity [21-23].

The extraction of carotenoids from tomato pomace is generally performed by the maceration of dried pomace using organic solvents such as hexane and ethanol because carotenoids are lypophilic, water-insoluble compounds [24,25]. The process generally requires large amounts of solvents per mass of final products. These solvents define a major part of the environmental performance of the extraction process, and they also impact cost and safety issues [26]. Studies performed by Capello et al. [26] have shown

that hexane has a higher environmental impact than ethanol. Therefore, reducing the amount of this solvent without affecting the efficiency of carotenoid extraction is desirable.

The primary aim of this study was to investigate the influence of the application of moderate pressure on the ultrasound-assisted extraction of carotenoids from dry tomato pomace. The second objective was to optimize the extraction conditions under moderate temperatures to obtain the highest carotenoid extraction yield (CEY) with a reduced hexane concentration.

2. Material and Methods

2.1. Plant Material

Red tomatoes (commercial variety: Canario) were purchased from a local supermarket. The tomatoes were passed though a laboratory peeler-pulper to obtain the tomato pomace composed of skin, seeds, and part of the pulp. The tomato pomace was dried in an oven with an air circulation of 25 ºC, and the dried pomace was grounded in a laboratory mill. The dry sample was stored in the dark at 4 ºC until needed.

2.2. Chemicals

Hexane and ethanol, analytical grade, were purchased from VWR International (Fontenay-sous-Bois, France). All solvents for HPLC analysis (acetonitrile, hexane, and methanol) were of HPLC gradient grade and were obtained from Fisher Scientific (Fair Lawn, NJ). All-trans lycopene was purchased from the Sigma Chemical Co. (Sigma-Aldrich Co., St. Louis, MO).

2.3. Reference Extraction Process

In order to identify the advantages of applying manosonication to the extraction of carotenoids, the conventional maceration of dried tomato pomace with a solvent (hexane-ethanol) was used in the control experiments. The first series of experiments

was conducted with a mixture of equal volumes (50:50) of both solvents. The extraction of carotenoids was performed in a 250 mL vessel placed in a temperature-controlled (±1 ºC) water bath with agitation. Dry tomato waste (3 g) was placed in an extraction vessel containing 100 mL of solvent at the extraction temperature. To investigate the effect of extraction time, 1 mL of the extract was removed at different time intervals.

2.4. Ultrasound and Manosonication-Assisted Extraction

Ultrasound and manosonication treatments were carried out in the equipment previously described, which permits the application of ultrasound treatments of different amplitudes and at different hydrostatic pressures [24,25]. A treatment chamber of 100 mL pressurized with nitrogen was used for the extraction experiments. The tip of a sonication horn (13 mm in diameter) connected to a Digital Sonifier® ultrasonic generator (Branson Ultrasonics Corp., Danbury, CT) that emits sound vibration at a frequency of 20 kHz and different amplitudes (34-145 μm) was located in the bottom of the chamber. A cooling coil located in the treatment chamber was used to dissipate the heat generated by ultrasound and to maintain a constant temperature (±2 ºC) by circulating a cooled water-ethylene glycol mixture. The temperature was monitored by a thermocouple located in the treatment chamber. An entry to the treatment chamber sealed with a rubber septum was used to sample the extraction medium during the extraction time using a syringe.

Dried tomato pomace (3 g) was placed in the treatment chamber with 100 mL of solvent (hexane/ethanol), and samples of 1 mL were taken at different time intervals. The first series of experiments was conducted with a mixture of equal volumes (50:50) of both solvents.

The power input (W) of the treatment medium was determined using the calorimetric method previously described [27].

2.5. Carotenoid Quantification

The extracts obtained at different extraction times were centrifuged at 5400 × g for 6 min to separate the supernatant. One-tenth of a milliliter of water was added to 1 mL of the supernatant in order to separate the supernatant into distinct polar and non-polar layers. The absorbance of the non-polar layer (hexane layer) containing carotenoids was measured at 472 nm on a spectrophotometer (Jenway 6505 UV/VIS, Jenway, Felsted, UK). CEY was determined using the molar extinction coefficient of lycopene in hexane at 472 nm (E1 % 1 cm 3450) [28], and it was expressed as mg of carotenoids/100 g dry weight (DW) of tomato pomace.

2.6. Kinetics of Carotenoid Extraction

The experimental data corresponding to the evolution of the carotenoid extraction yield over time were fitted to the following equation, which is commonly used to describe the solid-liquid extraction of different intracellular compounds [29,30].

$$Y_t = Y_{max} (1-e^{-kt}) \quad (1)$$

where Y_t is the carotenoid extraction yield at time *t* (min), Y_{max} is the carotenoid extraction yield at equilibrium, and k (min^{-1}) is a rate constant.

2.7. HPLC Analysis of Carotenoids

Prior to HPLC analysis, the extracts were concentrated in a miVac concentrator (GeneVac Ltd., City, UK) for 15 min at 30 ºC with vacuum evaporation of 10 mL of the hexane layer and re-dissolution in 2 mL of hexane.

HPLC/DAD analyses were performed on a Varian ProStar high performance liquid chromatograph (Varian Inc., Walnut Creek, CA) equipped with a ProStar 240 ternary pump, a ProStar 410 autosampler, and a ProStar 335 photodiode array detector. The system was controlled with a Star chromatography workstation v.6.41 (Varian). A reversed-phase column Microsorb-MV 100-5 C18 (25 x 0.46 cm; 5 μm particle size)

with a precolumn (5 x 0.46 cm; 5 µm particle size) of the same material was used. The temperatures of the column and precolumn were maintained at 30 ºC.

A linear gradient consisting of acetonitrile (A), hexane (B), and methanol (C) was used as follows: from 70% A, 7% B, and 23% C to 70% A, 4% B, and 26% in 10 min. The flow rate through the column was 1.5 mL/min, the sample injection was 10 µL, and the absorbance detection wavelength was 472 nm. Prior to injection, all samples were filtered through a 0.2 µm sterile syringe filter of cellulose acetate (VWR, West Chester, PA).

Lycopene and ß-carotene were identified by comparing their retention times and visible absorption spectra with those of their standards.

2.8. Experimental Design

Response surface methodology (RSM) was used to determine the optimal manosonication extraction of carotenoids from dried tomato pomace with respect to the hexane percentage in a hexane-ethanol solvent mixture and the extraction temperature. Preliminary kinetic experiments indicated that extraction equilibrium was reached after approximately 6 min, and 50 kPa of pressure and 94 µm of amplitude were the optimal ultrasonication treatment conditions. Therefore, this extraction time and these ultrasonication conditions were selected for subsequent experiments. A central composite design (CCD) was constructed to investigate the effects of hexane/ethanol solvents (25-75% of hexane) and extraction temperatures (25-45 ºC) on ultrasound-under-pressure CEY. The data obtained were modeled with the following second-order polynomial equation:

$$Y = \beta_0 + \sum_{i=1}^{k} \beta_i X_i + \sum_{i=1}^{k} \beta_{ii} X_i^{\,2} + \sum_{i>j}^{k} \beta_{ij} X_i X_j \qquad (2)$$

where Y is the response variable to be modeled, X_i and X_j are independent factors, β_0 is the intercept, β_i is the linear coefficient, β_{ii} is the quadratic coefficient, β_{ij} is the cross-

product coefficient, and k is the total number of independent factors. A backward regression procedure was used to determine the parameters of the models. This procedure systematically removed the effects that were not significantly associated ($p > 0.05$) with the response until a model with only a significant effect was obtained.

The CCD and the corresponding analysis of the data were carried out using the software package Design-Expert 6.0.6 (Stat-Ease Inc., Minneapolis, MN).

2.9. Statistical Analysis

Experiments were performed in duplicate, and the presented results are means ± standard deviation. A one-way analysis of variance (ANOVA) using the Tukey test was performed to evaluate the significance of differences between mean values. Differences were considered significant at $p < 0.05$. GraphPad PRISM (GraphPad Software, San Diego, CA) was used to perform the statistical analysis and nonlinear regression analysis of the data obtained from the experiments conducted to assess the effects of external hydrostatic pressure and vibration amplitude on CEY over time.

3. Results and Discussion

3.1. Effect of Hydrostatic Pressure and Vibration Amplitude on Carotenoid Extraction Yield

Figure 1 shows the influence of hydrostatic pressure (1A) and of the vibration amplitude of an ultrasonic horn (1B) on the ultrasound-assisted extraction yield of carotenoids from dry tomato pomace over time. The extraction curve obtained when the extraction was conducted without the application of ultrasound is also shown in Figure 1A in order to illustrate the effect of ultrasound application. It was observed that, in all cases, CEY depended on extraction time, with extraction curves showing a high initial rate of extraction that decreased with time until it reached almost an equilibrium condition. Conversely, CEY increased with the application of ultrasound, and it was

higher when external pressure was increased from atmospheric pressure to 100 kPa or when a constant pressure (50 kPa) amplitude was increased from 58 to 94 μm.

The enhancement of the extraction yield of carotenoids from dry tomatoes through the application of ultrasound has already been observed by Eh and Teoh [13]. However, the effect of the pressurization of the extraction medium or of the ultrasound amplitude had not been previously investigated. The enhancement of the ultrasound effect on microbial inactivation or sonochemical reaction by increasing static pressure or vibration amplitude has been observed by other authors [17, 31]. This effect has been attributed to the influence of these two parameters on the dynamic of bubble growth and collapse of the bubbles. The two ways to increase the physicochemical effects of ultrasound are to increase the number of cavitating bubbles or to increase the power of bubble implosion. Increasing external pressure hinders the cavitation phenomenon. However, if the ultrasonic power applied is sufficient to cause cavitation, the intensity of the collapse of the bubbles increases [14]. In contrast, at higher vibration amplitudes, the effective size of the zone of the liquid undergoing cavitation and the range of bubble size undergoing cavitation also increase [32].

The mathematical model described by Equation 1 shows a good fit to the experimental data. The equation parameters and the corresponding correlation coefficients describing the influence of static pressure and amplitude on the extraction yield of carotenoids are listed in Table 1. Correlation coefficients higher than 0.90 indicated that the simplified extraction model used could be applied satisfactorily to estimate the extraction rate and maximum CEY.

A comparison of the coefficients shown in Table 1 illustrates that the rate of extraction indicated by the constant k was not influenced by increasing the static pressure or vibration amplitude. No statistically significant ($p < 0.05$) differences were

observed between the *k* constant of the extraction curves obtained under the different experimental conditions investigated. Rather, the concentration at equilibrium (Y_{max}) drastically increased with the application of ultrasound or with an increase in static pressure or vibration amplitude. The maximum CEY for the control extraction (6.95 mg/100 g DW) was considerably lower than that for the extraction conducted with ultrasound with a vibration amplitude of 76 µm without an increase in static pressure (11.32 mg/100 g DW). Conversely, the application of ultrasound at a static pressure of 100 kPa and 76 µm increased the extraction yield by 149% as compared with the control extraction and by 53% as compared with the ultrasound extraction without an increase in static pressure (0 kPa) at the same vibration amplitude. An increase in the static pressure from 50 to 100 kPa did not significantly increase the extractability of carotenoids at equilibrium ($p < 0.05$) at 76 µm of amplitude. The effect of amplitude on improving the extraction yield at a constant pressure of 50 kPa was not as effective as the increase in hydrostatic pressure. An enhancement in extraction of around 25% was observed by increasing the amplitude from 76 to 94 µm, but an increase in amplitude from 58 to 76 µm did not significantly increase the extractability ($p < 0.05$).

These studies indicated that the maximum CEY was obtained through the application of a vibration amplitude of 94 µm and an external pressure of 50 kPa. In contrast, extraction equilibrium was reached after approximately 6 min of extraction (Figure 1). A similar extraction time was reported for the optimal extraction of lycopene from tomato paste using ultrasound/microwave-assisted extraction [33].

3.2. Relationship between Ultrasonic Power Delivery to the Treatment Medium and Carotenoid Extraction

It is well know that the power delivery to a liquid medium by ultrasound depends on the external hydrostatic pressure and vibration amplitude. Data reported by

different authors indicate that the power delivery increases with these two treatment parameters [34, 35]. The effect on CEY of ultrasonic power transmitted to the medium in the experiments in Figure 1 is shown in Figure 2. Ultrasonic power had a positive effect on CEY. A similar effect of ultrasonic power on the yield of phenolic compounds from citrus peels was observed by Ma et al. [11]. In the experimental conditions investigated in our study, the maximum extraction yield of carotenoids was linearly related to the ultrasonic power delivered to the treatment medium independent of the external pressure or amplitude applied. These results are also in agreement with those reported by Mañas et al. [36] that demonstrated a linear relationship between the inactivation of *Listeria monocytogenes* by ultrasound under pressure and the ultrasonic power delivery to the treatment medium.

3.3. Optimization of Extraction Temperature and % Hexane in the Solvent for Manosonication-Assisted Extraction of Carotenoids.

The conditions of ultrasound amplitude (94 μm), static pressure (50 kPa), and extraction time (6 min) that permitted us to obtain the maximum CEY were used for subsequent experiments in which the influence of the extraction temperature and hexane percentage in the solvent mixture on manosonication-assisted extraction of carotenoids was investigated.

CEYs resulting from the experimental conditions investigated for the control extraction (without ultrasound) and the manosonication-assisted extraction are shown in Table 2. Due to the thermal susceptibility of carotenoids, the maximum extraction temperature used in this study was 45 ºC.

CEYs varied from 3.54 to 7.77 mg/100 g DW for the control extraction and from 7.49 to 14.08 mg/100 g DW for the extraction assisted by manosonication. These contents are within the range of values reported in the literature by other authors who

have investigated the extraction of carotenoids from dry tomato waste in different solvents and solvent mixtures at different temperatures [37, 38]. It was observed that the maximum CEY obtained (7.77 mg/100 g DW) for the control extraction at the highest extraction temperature and percentage of hexane was similar to the minimum CEY (7.49 mg/100 g DW) obtained for the extraction assisted by manosonication at the lowest extraction temperature and percentage of hexane. Therefore, a benefit of the ultrasound-assisted extraction of carotenoids from tomato waste is the possibility of decreasing the extraction temperature and percentage of hexane without affecting CEY.

In order to identify and quantify the potential advantages of the application of ultrasound under pressure in terms of increasing the extraction yield and reducing the extraction temperature or concentration of hexane in the solvent mixture, RSM was used. The application of a multiple regression analysis to the independent and response variables shown in Table 2 for the control extraction and for the extraction assisted by manosonication resulted in two second-order polynomial equations, the coefficients of which are given in Table 3. For the model that describes the control extraction, the backward regression ($p < 0.05$) procedure eliminated the interaction terms of the temperature and percentage of hexane and the quadratic term of the percentage of hexane, whereas, when extraction was conducted with manosonication, the interaction terms of temperature and percentage of hexane were eliminated.

Table 3 also shows the results of the analysis of variance for the significant terms of the models. The statistical analysis indicated that both models were adequate to estimate CEY as a function of the two independent factors investigated. The overall significance of the models was high, denoted by the calculated *F*-values and the corresponding low probability values ($p < 0.01$). The determination coefficient (R^2) for each model was 0.94, which means that only 6% of the total response variation

remained unexplained by the models obtained. Finally, for both models, the *p* value ($p < 0.001$) agreed with the goodness of the fit of the mathematical equations to the experimental data.

To illustrate the influence of the extraction temperature and concentration of hexane in the solvent mixture, response surface plots were obtained using the corresponding regression models (Table 3) for the control extraction (Fig 3A) and manosonication-assisted extraction (Fig 3B). Temperature was the most significant parameter on CEY for both the control and ultrasound-under-pressure-assisted extractions. However, the square of extraction temperature was also a significant term in the equation that described the manosonication-assisted extraction. The presence of these square terms in the equation means that, in the range of temperatures investigated, when the extraction temperature changed, its effect on CEY was non-linear. The effect of temperature on extraction yield observed in this study is characteristic in solid-liquid extraction processes of intracellular compounds from plant materials. At higher temperatures, the solubility of the material being extracted and its diffusivity increase and, as a result, higher extraction yields are obtained [39]. Because the interaction of the treatment temperature with the concentration of hexane in the solvent mixture was not significant, the effect of the temperature was independent on the composition of the solvent mixture used for extraction, as shown in Figure 2. The effect of increasing the temperature from 25 ºC to 45 ºC on the enhancement of CEY was similar for both extraction procedures. An increase of 20 ºC in the temperature extraction increased CEY by 60%, but CEY was always higher when ultrasound under pressure was applied during extraction.

The linear term of the hexane concentration was included in the model for both control and manosonication-assisted extractions, indicating that CEY improved as the

concentration of hexane in the solvent increased. However, for manosonication-assisted extraction, the quadratic term of the hexane concentration was also significant. The negative sign of this square term indicates that, in the range of hexane concentrations investigated, there is a maximum value from which the increment of hexane concentration does not significantly increase CEY. For the control, an increase in the hexane concentration from 25% to 75% increased CEY by 30%. However, in the extraction assisted by ultrasound under pressure, an increase in the hexane concentration from 25% to 50% increased CEY by 75%, whereas the effect of additional increments was insignificant. Therefore, according to the obtained models, in the range of experimental conditions investigated, the optimal solvent hexane/ethanol mixture for the extraction of carotenoids from tomato pomace was different for the control extraction (75/25) and for the extraction assisted by ultrasound under pressure (58/42). Extraction efficiency of solvents depends not only on the solubility of the compound of interest but also on the penetration or diffusion of the solvents into the solid matrix [25, 40]. The lower concentration of hexane required in the solvent mixture in the extraction assisted by ultrasound under pressure could be due to the fact that the mechanical effects of cavitation provide a better penetration of hexane into the dry tomato pomace.

3.4. HPLC Characterization of Carotenoids Extracted from Dry Tomato Pomace

Extracts obtained in the extractions conducted in the optimization experiment were analyzed by reverse-phase HPLC. Figure 3 shows, as an example, chromatogram profiles detected at 472 nm for extracts obtained in a control extraction (45º C and 50/50 hexane/ethanol) and an extraction assisted by ultrasound under pressure (45º C and 50/50 hexane/ethanol). Similar chromatograms profiles were obtained for the extracts obtained in the different experimental conditions investigated. As shown in Figure 3, all obtained extracts contained lycopene and ß-carotene, which are the main

carotenoids in tomato waste. It was observed that the application of ultrasound under pressure during extraction did not affect the extraction of a selected carotenoid, and no evidence of carotenoid degradation was observed. All the extracts analyzed contained between 88% and 95% of lycopene. It has been reported that the isomerization of all-trans lycopene to cis-lycopene occurs when extraction is conducted at temperatures of around 60 ºC or higher, the amount depending upon the solvent [21, 41]. However, HPLC analysis of all extracts obtained in our study did not reveal any peaks that would indicate the isomerization of lycopene. Our results are in agreement with those obtained by Eh and Teoh [13], who also observed an insignificant lycopene isomerizarion when this compound was extracted from freeze-dried tomatoes by ultrasound-assisted extraction in a nitrogen atmosphere at temperatures below 50 ºC.

4. Conclusions

Manosonication-assisted extraction has been shown to be a promising technology for extracting carotenoids from dried tomato pomace at relatively short extraction times as compared with other extraction procedures. It has been demonstrated that an increase in external pressure drastically increased the effect of ultrasound on CEY, with this effect linearly related to the ultrasonic power delivered to the extraction medium. The possibility of decreasing the percentage of hexane without affecting CEY and the fact that the treatment did not cause the degradation of extracts are key benefits of the manosonication-assisted extraction of carotenoids from tomatoes. The possibility of implementing the application of ultrasound-under-pressure treatment on an industrial scale and the energetic cost of the process are aspects that require further research before the technology can be introduced to improve extraction.

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Figure captions.

Fig. 1. Influence of the hydrostatic pressure (A) (□) 0 kPa, (▲) 50 kPa and (◊) 100 kPa; and the vibration amplitude of ultrasonic horn (B) (■) 58 μm, (▽) 76 μm and (○) 94 μm on the ultrasound assisted carotenoid extraction yield (CEY) from dry tomato pomace along the time. Figure 1A also illustrates the extraction curve obtained when the extraction was conducted without application of ultrasound (●).Error bars correspond to standard error.

Fig. 2. Influence of the ultrasonic power delivery to the treatment media on the maximum carotenoid extraction yield (CEY) from dry tomato pomace.

Fig. 3. Response surface plots of the carotenoid extraction yield (CEY) from dry tomato pomace as function of temperature and percentage of hexane for control extraction (A) and manosonication assisted extraction (B) after 6 minutes of extraction.

Fig. 4. HPLC chromatograms of carotenoid profiles of extracts at 472 nm for control (A) and manosonicaition assisted extraction (B). 1: all-trans-lycopene; 2: β-carotene. Temperature: 45ºC, 50/50 hexane/ethanol. Extraction time: 6 minutes.

Table 1. Y_{max} and k values from the fitting of Eq. (1) to the carotenoid extraction curves of Fig. 1A and Fig. 1B.

Pressure (kPa)	**Amplitude (μm)**	**Y_{max} (±CI 95%)***	**k (±CI 95%)***	**R^2**
0	0	6.95 ± 0.32a	0.40 ± 0.08a	0.96
	76	11.32 ± 0.70b	0.47 ± 0.11a	0.94
50	58	14.62 ± 2.02c	0.35 ± 0.15a	0.90
	76	15.06 ± 1.01c	0.31 ± 0.04a	0.97
	94	18.34 ± 1.18d	0.29 ± 0.05a	0.96
100	76	17.37 ± 1.14d	0.32 ± 0.05a	0.97

* Different letters show significant differences (p<0.05)

Table 2. Carotenoid extraction yield (CEY) resulting for control extraction and manosonication assisted extraction (50 kPa; 76 μm) at different temperatures and % of hexane in a mixture hexane/ethanol.

		CEY (mg/100g dw)	
Temperature (º C)	**Hexane (%)**	**Control (±CI 95%)**	**Manosonication (±CI 95%)**
25	25	3.54 ±0.25	8.43 ±0.26
	50	4.83 ±0.43	9.40 ±2.51
	75	5.54 ±0.95	9.13 ±0.10
35	25	5.05 ±0.33	7.49 ±1.39
	50	5.26 ±0.49	10.57 ±1.39
	75	5.66 ±0.32	10.05 ±2.30
45	25	6.22 ±0.07	10.80 ±0.24
	50	7.64 ±0.57	14.08 ±3.55
	75	7.77 ±0.14	13.59 ±1.06

Table 3. Coefficients, F-values and p- values of the ANOVA analysis for the quadratic model developed to describe the influence of the temperature (T) and percentage of hexane (H) on the carotenoid extraction yield from dry tomato pomace.

	Control			**Manosonication**		
	Coefficient	**Fvalue**	**pvalue**	**Coefficient**	**F_{value}**	**p_{value}**
Intercept	7.09			13.53		
H	0.028	23.87	0.0018	0.28	17.95	0.0055
T	-0.31	82.05	<0.0001	-0.83	64.81	0.0002
H^2	—	—	—	-0.0024	16.97	0.0062
T^2	0.0063	8.87	0.0206	0.015	15.80	0.0073
Model		38.26	0.0001		27.16	0.0006
R^2		0.94			0.94	
R^2-adj		0.92			0.9	
RMSE		0.35			0.58	
Lack of fit		0.85			2.04	

Figure 1.

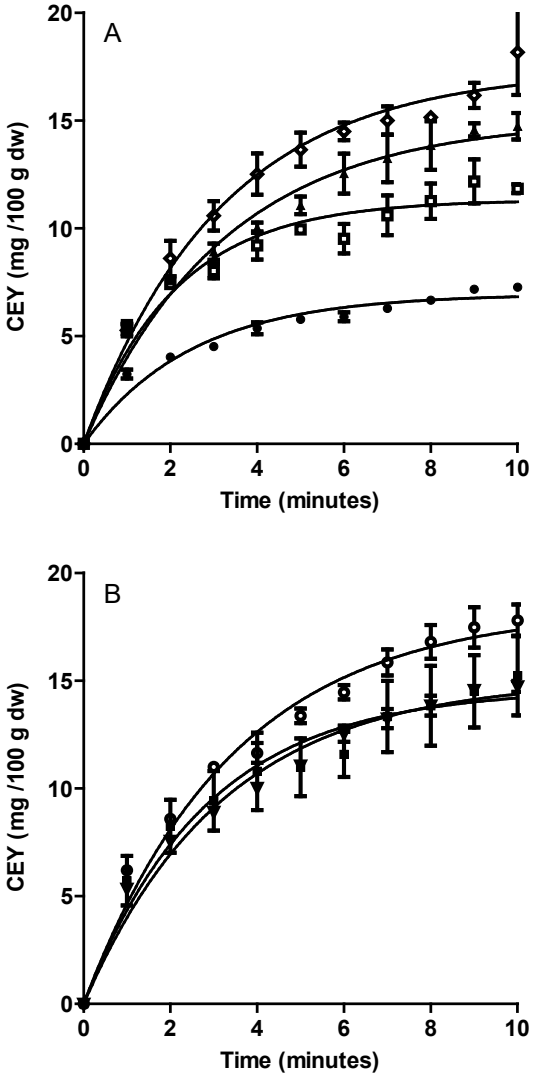


Figure 2.

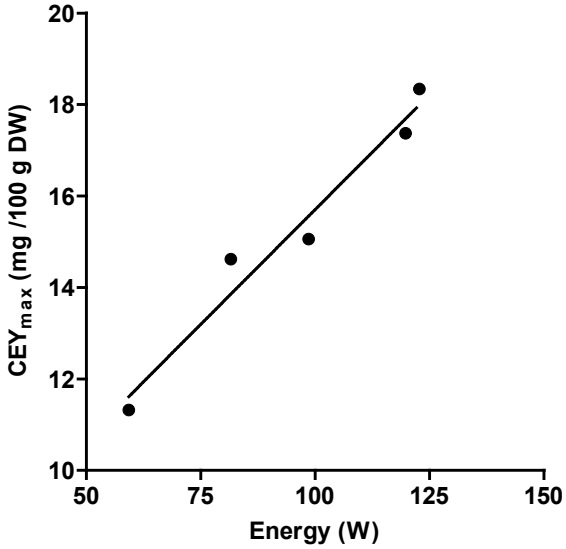
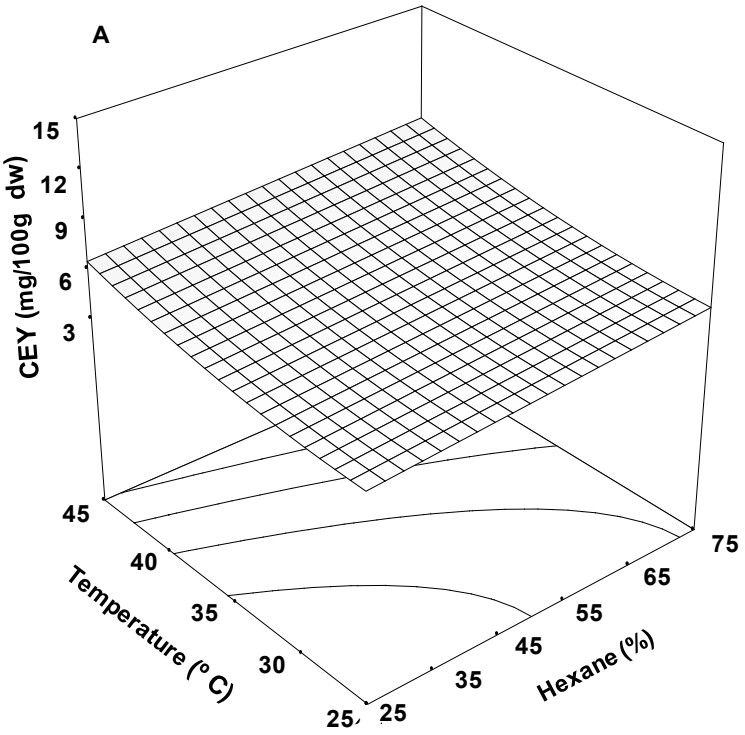


Figure 3.


A
CEY (mg/100g dw)
15
12
9
6
3
45
40
35
30
25
Temperature (° C)
25
35
45
55
65
75
Hexane (%)

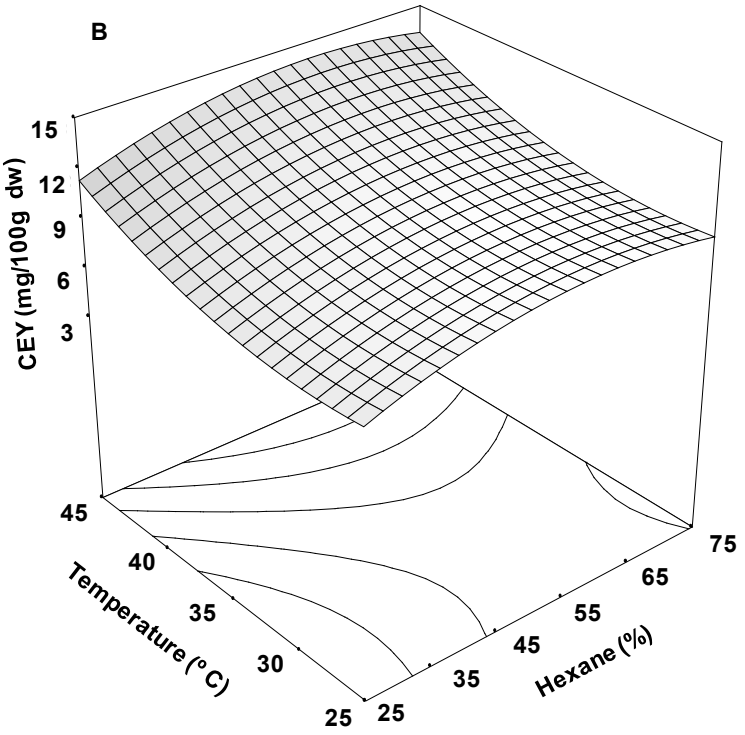
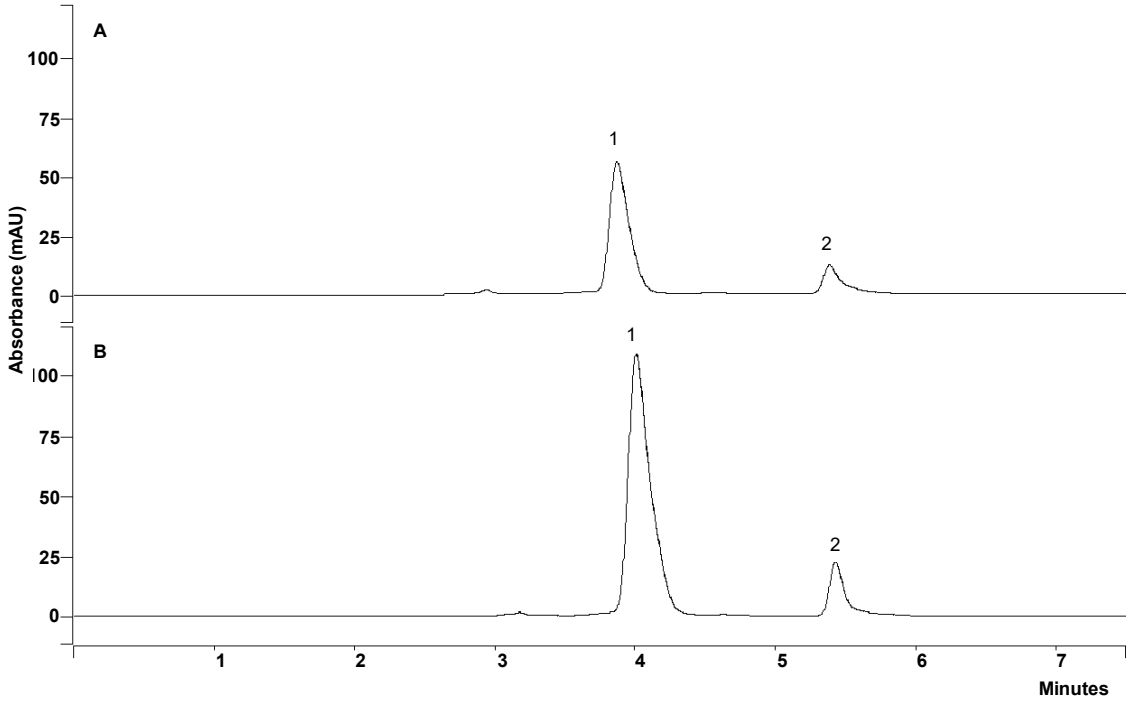

B
CEY (mg/100g dw)
15
12
9
6
3
45
40
35
30
25
Temperature (° C)
25
35
45
55
65
75
Hexane (%)

Figure 4.


A
100
75
50
25
0
1
2
Absorbance (mAU)
B
100
75
50
25
0
1
2
1
2
3
4
5
6
7
Minutes