Resource-Efficient Technologies 3 (2017) 252–262

Contents lists available at ScienceDirect

Resource-Efficient Technologies

journal homepage: www.elsevier.com/locate/reffit

Research paper

Development of a green procedure of citrus fruits waste processing to recover carotenoids

Meryem Boukroufa[a], Chahrazed Boutekedjiret[a,*], Farid Chemat[b]

[a] Laboratoire des Sciences et Techniques de l'Environnement (LSTE), Ecole Nationale Polytechnique, 10 Avenue Hacène Badi, BP 182, El Harrach, 16200 Alger, Algeria
[b] Avignon University, INRA, UMR408, GREEN Extraction Team, F-84000, Avignon, France

ARTICLE INFO

Article history:
Received 27 October 2016
Revised 20 August 2017
Accepted 21 August 2017
Available online 1 September 2017

Keywords:
Citrus waste
Green processing
Microwave
Ultrasound
Bio-refinery
Essential oil
D-limonene
Carotenoid

ABSTRACT

In this study, an original and green procedure of processing waste of the citrus fruits was developed using the concept of bio-refinery, innovative techniques "ultrasound" and "micro-wave", and a green solvent "limonene" to recover carotenoids. Essential oil extraction was performed by Solvent Free Microwave Extraction (SFME) and compared to steam distillation (SD). The essential oil yields were comparable for both processes: 4.02 ± 0.23% for SFME and 4.16 ± 0.05% for SD. After that, carotenoid extraction from citrus peels was performed by ultrasound-assisted extraction (UAE) and conventional extraction (CE) using D-limonene obtained starting from essential oil, as a solvent, and then compared to n-hexane extract. Response surface methodology (RSM) using central composite designs (CCD) approach was launched to investigate the influence of process variables on the ultrasound-assisted extraction (UAE). The statistical analysis revealed that the optimized conditions of ultrasound power, temperature and time were 208 $W\,cm^{-2}$, 20 °C and 5 min giving carotenoid content of 11.25 $mg\,L^{-1}$. Compared to the conventional extraction, (UAE) gave an increase of 40% in carotenoid content. The comparison to n-hexane extract gave no significant changes in carotenoid content. Combination of microwave, ultrasound and D-limonene obtained from a bio-refinery of a by-product of citrus fruits industry allow us to develop a very good environmental green approach giving high added values compounds, with a saving of time, and a complete valorisation of waste.

1. Introduction

Carotenoids are pigments which present two or several units of hydrocarbons. They are synthesized by the photosynthetic organisms, certain no photosynthetic bacteria and mushrooms [1].

More than 600 carotenoids have been identified in nature and about 40 are present in the human diet [2] and found in citrus fruit peels such as oranges since 1937 by Zechmeister and Tuzson [3].

Global orange production for 2013/14 according to USDTA is estimated as 51.8 million metric tons and 2.0 million metric tons are used for orange juice production. Citrus processing engenders high quantities of citrus by-product which account for about 50% of their weight [4,5]. They are composed mainly of skins, pulp, and seeds [6]. Regarding their composition, they represent a source of essential oils (D-limonene), sugars, pigments, fat, acids, insoluble carbohydrates, enzymes, flavonoids, pectin [7–10].

Valorisation of these by-products offers several benefits: environmental protection, economic gains, and improvement of the lives of the consumers thanks to the use of natural products.

Citrus essential oil extracted from citrus by-product can be used in several kinds of food, such as flavoring ingredients in food or pharmaceuticals, essentially for its anti-inflammatory and antibacterial effects [11–14]. It is also used in the preparation of toilet soaps, perfumes and cosmetics [15].

Citrus essential oils are generally characterized by its key component: D-limonene. This monoterpene hydrocarbon is present in citrus essential oils, corresponding to more than 95% [16–18]. Commonly used in fragrance and flavor for years, it is used, over the last years, as cleaning/degreasing agent in industry and household applications [19].

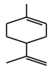
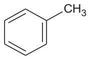
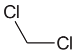
Research of greener biodegradable and non-dangerous solvents have become a major concern for research academy and industry considering the fact that n-hexane, one of the main constituents of extraction grade hexane, is sourced from fossil resources and reg-

---

* Corresponding author.
E-mail address: chahrazed.boutekedjiret@g.enp.edu.dz (C. Boutekedjiret).

http://dx.doi.org/10.1016/j.reffit.2017.08.007

Table 1
Relevant properties of D-limonene and some petrochemical solvents.

Properties	D-limonene	n-hexane	Toluene	Dichloromethane
Chemical structure				
Empirical formula	$C_{10}H_{16}$	C_6H_{14}	$C_6H_5CH_3$	CH_2Cl_2
Molecular weight	136.23	86.18	92.14	84.93
Boiling point (°C)	175.5	69	111	39.8–40.0
Heat of vaporization (kJ/kg)	353	334	351	28.6
Density (g/mL)	0.8411	0.660	0.867	1.325
Toxic	No	Yes	Yes	Yes
Renewable	Yes	No	No	No
Environmental impact	Low	High	High	High

istered under the REACH Regulation as a category 2 reprotoxic and as a category 2 aquatic chronic toxic. Green solvents have to meet the fundamental principles of Green Chemistry and in this context, bio-based derived chemicals such as limonene show a great potential [20].

Recent studies have proven that D-limonene, which is considered as GRAS (Generally Recognized as Safe) solvent by the US Food and Drug Administration, can be regarded as a valuable alternative for traditional solvents used for extraction such as methyl ethyl ketone, acetone, toluene, glycol ethers, and numerous fluorinated and chlorinated solvents, which emit polyaromatic hydrocarbons (PAHs) or fumes from volatile organic compounds (VOCs) [21]. Relevant properties of D-limonene and some petrochemical solvents are reported in Table 1 [22–24].

ß-carotene, with α-carotene, ß-cryptoxanthin, lycopene, lutein, and zeaxanthin are the main carotenoids found in citrus fruits [25]. They constitute an important source of vitamin A in many diets and may protect from development of degenerative diseases such as macular degeneration, cancer, and heart disease [26].

Conventional bio active's extraction techniques (e.g. maceration and Soxhlet) are solvent and energy consuming. Consequently, with the development of the Green Chemistry concept, green engineering and the bio-refinery concepts during the past few years, environmentally friendly techniques became more and more attractive. Therefore, the researchers have to implement the most eco-friendly and efficient extraction processes. Many researches have been carried out such as the intensification of the conventional processes by adding pre-treatments to improve the yields and quality of the extracts [27,28], or the development of new extraction techniques. Microwave-assisted extraction, ultrasound assisted extraction, supercritical fluid extraction, electro-technologies, instantaneous controlled decompression, Ohmic heating-assisted hydro distillation, gas assisted mechanical expression, are the most famous innovative techniques developed recently. These technologies have largely proved their efficiency for extraction of natural products [29–35]

In addition to the development of the extraction processes, the search for renewable alternative solvents to replace petrochemical solvents is of topicality [20].

In this paper a combination of a bio-refinery concept with an innovative green processes integrating microwaves and ultrasounds assisted extraction was investigated for carotenoid extraction from orange peels using D-limonene as an alternative bio-solvent to n-hexane.

2. Materials and methods

2.1. Plant material

Orange (*Citrus cinensis* L.osbeck) peels were collected locally after juice extraction. The initial moisture content of orange peel for the essential oil extraction and carotenoids extraction was determined using the moisture analyzer apparatus Kern DBS 60-3. The measured moisture content was 59.0 ± 0.3% and 5.9 ± 0.1% respectively.

2.2. Protocol treatment

Orange peels obtained after juice extraction were subjected to an essential oil extraction which was carried out by solvent free microwave extraction (SFME) and steam distillation (SD). Whereas carotenoid extraction from orange peels was performed by ultrasound-assisted extraction (UAE), and conventional extraction (CE), using D-limonene and n-hexane as a solvent. Solvent extracts were then analyzed by spectrophotometry. The performed protocol is shown in Fig. 1.

2.3. Solvent free microwave extraction (SFME) apparatus and procedure

The SFME extraction was performed at atmospheric pressure in a microwave oven with adjustable output power varying from 200 W to 700 W and 2450 KHz working frequency (Fig. 2). The principle and apparatus of this process are described in previous studies [36,37]. For each experiment, 500 g of fresh plant material was heated using a fixed power without adding any solvent or water. The applied microwaves heat the vegetable matrix causing the evaporation of interstitial water and the release of the essential oil. The mixture essential oil - vapor is continuously condensed in a cooling system outside the microwave cavity and recovered in a Clevenger receiving. Condensed water was refluxed to the extraction vessel in order to maintain uniform humidity rate of the plant. The extraction was performed until no more essential oil was obtained. Essential oil was collected then dried under anhydrous sodium sulfate and stored at 4 °C.

The studied extraction parameters are the microwave power and the extraction time. Microwave power and temperature are interrelated. It is known that the temperature is controlled by incident microwave power that controls the amount of energy provided to the matrix, which is turned into heat energy [38]. However, for this study we were interested in the influence of the microwave power on the oil yield and the kinetics of extraction.

The optimal microwave power was determined by a parametric study in which 500 g of fresh plant material was heated with a variable output power (200, 300, 400, 500, and 700 W) without any addition of solvent or water. The optimal extraction time was determined by a kinetic study.

2.4. Steam distillation (SD) apparatus and procedure

Vapour flow is a significant parameter in SD process; a high flow can involve the creation of a by-pass, what reduces contact between the vapour and the plant and thus decreasing the essential oil yield. A low flow would be insufficient to extract the to-

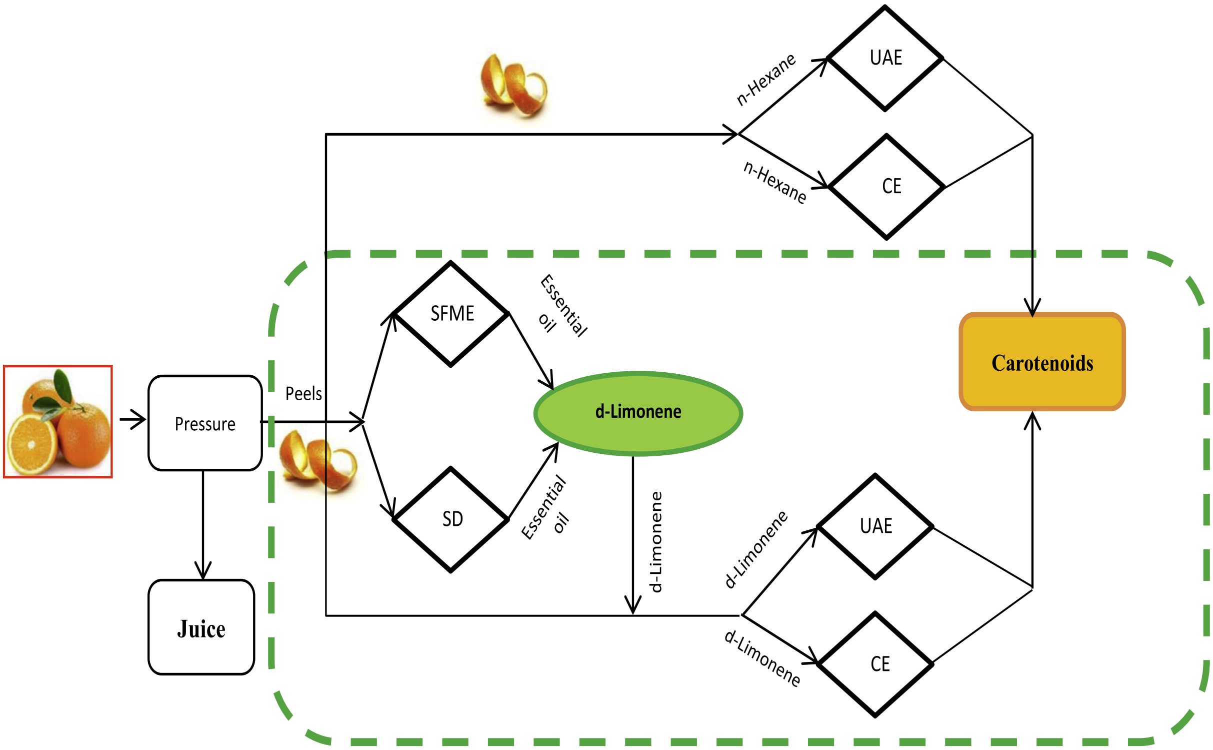


Fig. 1. Protocol treatment of orange peels.

tality of essential oil. Thus, it is necessary to determine the value of the steam flow which makes it possible to obtain the best essential oil yield. For this reason, several flow rates (8, 12, 14, 16 and 18 $g\,min^{-1}$) were tested. For each experiment, 500 g of fresh plant material was used. The vapor produced by the steam generator, charged with essential oil, crosses the plant and then passes through the condenser to a receiving Florentine flask. Essential oil is collected then dried with anhydrous sodium sulfate and stored at 4 °C. Optimal extraction time was also determined by the kinetic study of the process.

For a rigorous comparison between SFME and SD processes, the same glassware has been used. The tests were performed using 500 g of fresh plant material and an optimal microwave power of 500 W for SFME, and an optimal steam flow of 16 $g\,min^{-1}$ for SD, previously determined by a preliminary study.

2.5. Ultrasound assisted extraction (UAE)

Ultrasound-assisted extractions (UAE) of carotenoid were performed with an ultrasonic homogenizer UP 200 Ht (Hielscher, Contes, France) working at a frequency of 26 kHz and a maximum output power of 200 W. The diameter of the horn used in all experiments was 7 mm. The tests were carried out at ambient temperature and atmospheric pressure while the extraction temperature was controlled using the double layered mantle by cooling/heating systems (Fig. 3).

The real ultrasounds power, taking into account the power fraction, converted to heat dissipated in the medium was evaluated by calorimetric measurements and expressed as ultrasonic intensity (UI) [39].

2.6. Extraction procedure of carotenoid

Carotenoid extraction was performed by ultrasound-assisted extraction (UAE) and conventional extraction (CE) using D-limonene as a solvent, and then compared to n-hexane extract.

First, a parametric study of UAE was done to determine the value of the solid/solvent ratio and the field of variation of the ultrasound intensity. The values investigated for solid/solvent ratio are 1:10 and 2:10 $g\,mL^{-1}$. The ultrasonic intensity varies from 52 to 208 $W\,cm^{-2}$. After that, UAE optimisation by response surface methodology (RSM) was launched to investigate the influence of the operating variables, namely temperature, ultrasonic intensity and extraction time. The impact of the ultrasounds on the carotenoid extraction was evaluated by a comparative study between CE and UAE. For that reason, CE was performed using the optimum values of temperature and extraction time obtained by RSM study, and the optimum solid/solvent ratio obtained by a parametric study. The particle size previously determined by a preliminary study was fixed at 2 cm^2 for all tests [9].

2.7. Experimental design

In order to measure UAE performance of carotenoid content, a response surface methodology (RSM) was conducted. Central Composite Design (CCD) was used to achieve maximal information about the process from a minimum number of possible experiments. The multivariate study allows the identification of interaction between variables and provides a complete exploration of the experimental studied field. In this study, a Central composite face-centered (CCF) experimental design to determine the optimal conditions was used to optimize the process with three levels (−1, 0 and +1) for each factor. In this design, the star points are at the center of each face of the factorial space, thus $\pm\alpha = \pm 1$. Operational parameters investigated are temperature **T** (°C) (A), ultrasonic intensity **UI** ($W\,cm^{-2}$) (B) and time **t** (min) (C)**.** The limit values of the ultrasonic intensity were chosen as function of the results of parametric study. Temperature was fixed between 20 °C and 50 °C in order to check a possible degradation of carotenoid. Time extraction was fixed between 5 min and 35 min. The coded levels and the natural values of the factors are shown in Table 2. A total of 18 different combinations including four replicates of

Table 2
Central composite design with their observed response.

Run	Coded variables			Decoded variables			Response
	A	B	C	Temperature (°C)	UI[a] (W cm^{-2})	Time (min)	Carotenoid content (mg L^{-1})
1	+1	−1	−1	50	52	5	3.10
2	0	0	−1	35	130	5	6.10
3	0	−1	0	35	52	20	5.05
4	+1	+1	+1	50	208	35	6.25
5	−1	−1	−1	20	52	5	2.65
6	0	0	+1	35	130	35	8.25
7	−1	+1	+1	20	208	35	6.50
8	0	0	0	35	130	20	9.20
9	−1	0	0	20	130	20	7.20
10	+1	+1	−1	50	208	5	9.20
11	0	0	0	35	130	20	9.10
12	0	+1	0	35	208	20	10.70
13	+1	0	0	50	130	20	6.30
14	0	0	0	35	130	20	9.15
15	**−1**	**+1**	**−1**	**20**	**208**	**5**	**11.25**
16	0	0	0	35	130	20	9.00
17	+1	−1	+1	50	52	35	4.35
18	−1	−1	+1	20	52	35	3.00

[a] UI: ultrasonic intensity.

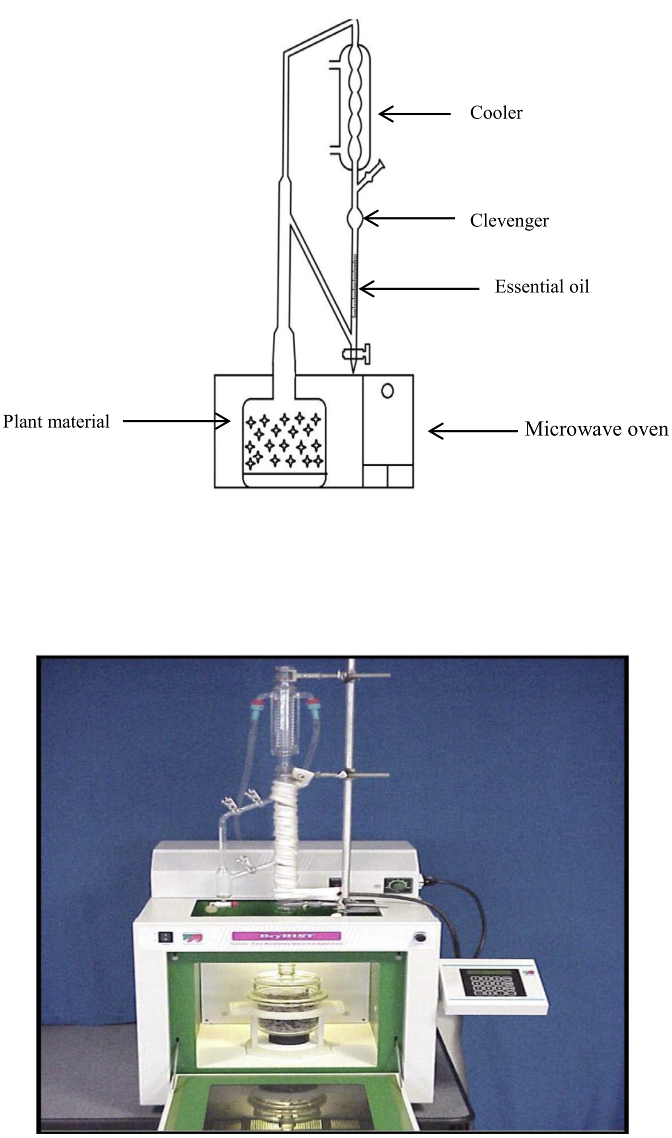


Fig. 2. Solvent Free Microwave Extraction (SFME) apparatus.

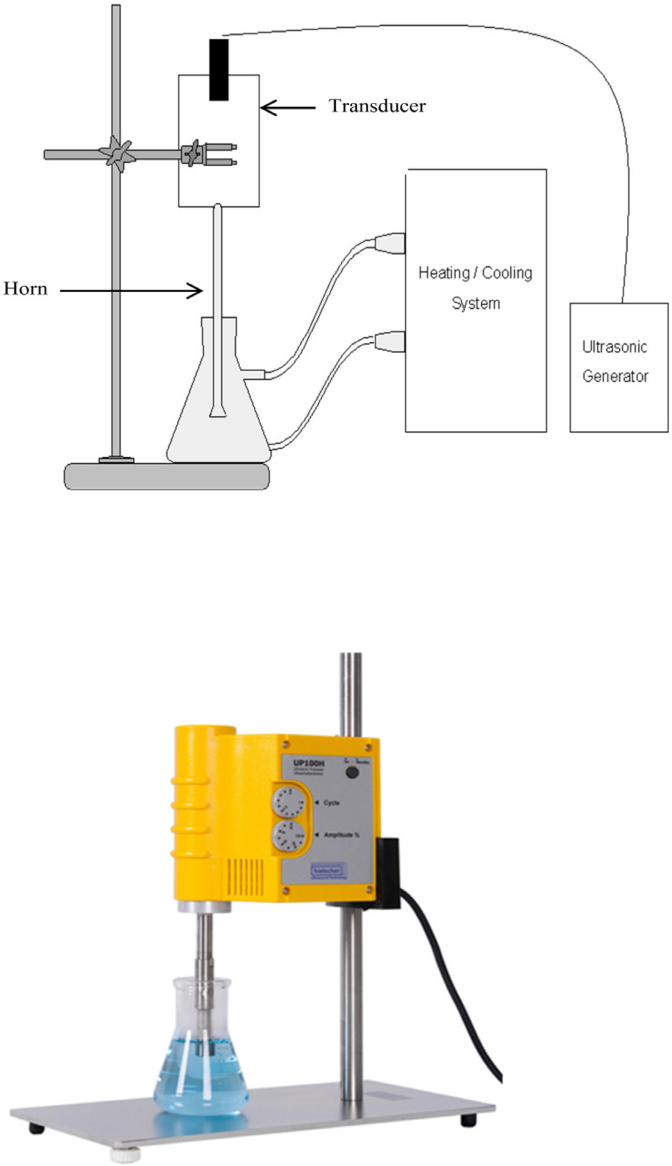


Fig. 3. Ultrasound apparatus.

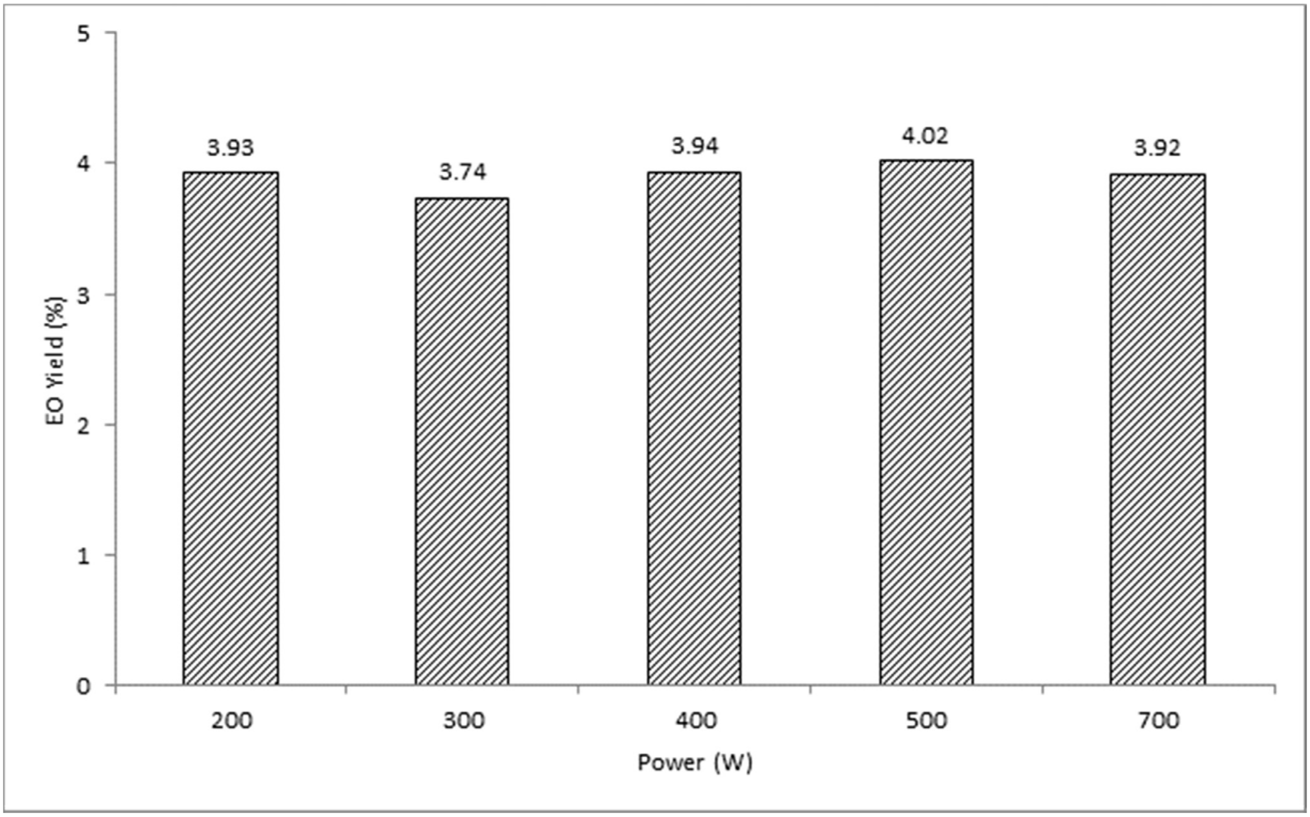


Fig. 4. Yield profiles as a function of power for SFME extraction of essential oil from orange peels.

center point, each designated by the coded value 0, were chosen in random order according to a CCF configuration for three factors. The selected optimisation parameter was carotenoid content ($Y_{carotenoid}$) expressed as mg $_{ß\text{-carotene}}$ L^{-1}. The experimental design was constructed and the experimental results were processed by using the software STATGRAPHICS PLUS (Version 5.1, Statistical Graphics Corporation, Rockville, USA, 2000). For all tests, a solid-liquid ratio was fixed at 1/10, value determined by a parametric study.

2.8. Carotenoids determination

Carotenoid content was determined by a Spectrophotometric method. After extraction, samples were filtered and the absorbance was measured at 450 nm against a blank prepared from particular solvent (D-limonene and n-hexane). Carotenoid concentration was then obtained by the beer lambert law using a calibration curve prepared using ß-carotene standard. Carotenoid content was then expressed as mg of ß-carotene L^{-1} [40].

3. Results

3.1. Essential oil extraction

3.1.1. Parametric study of SFME essential oil extraction

Because of the complexity of the vegetable matter, an appropriate microwave irradiation power is important in order to make sure that the complete essential oil was collected without incur alteration or destruction. Indeed, low powers do not allow recovering the totality of essential oil, whereas high power can cause a degradation of thermo labile compounds, what results in a reduction in yield, and also it can destroy the vegetable matter caused by a high localized heating [38].

The results of the parametric study of orange peels essential oil extraction by SFME (Fig. 4) showed that the extraction yield is approximately the same for all power ratings. The difference lies only in time necessary to recover the totality of essential oil. Fig. 5 illustrates the evolution of the total extraction time according to the microwaves power. Also, it does show that the extraction time decreases according to the increase in the microwaves power and stabilizes at around 22 min for a power of 500 W. Thus, for the low powers, time necessary to heat the vegetable matter and to cause the bursting of the essential oil sites is more significant. For the higher powers, heating of the vegetable matter requires shorter time. Consequently, the extraction is carried out more quickly.

3.1.2. Comparative study between SFME and SD essential extraction

Essential oil yields obtained by SFME and SD processes are comparable: 4.02 ± 0.23% for SFME against 4.16 ± 0.05% for SD. A difference is observed only in the extraction time. Indeed, steam distillation known as the most common method of essential oil production takes 240 min to recover all essential oil, whereas 22 min are enough with SFME process. Therefore, SFME process is ten times faster than conventional SD which offers a gain of time of more than 91%. This reduction of time is due to the mass and heat transfer which occurs from inside the plant cell to the outside, while these two transport phenomena are in the opposite direction in conventional process [38]. A synergistic combination of the mass and heat transfer gradients working in the same direction in SFME process makes the temperature increase in shorter time due to the volumetric heating effect depending on the microwave power, the thing that results in a reduction of the extraction time [38]. This advantage, in terms of reducing time, was also reported by other studies such as Filly et al. [41], for extraction of essential oil from aromatic herbs (30 min with SFME vs 120 min with hydrodistillation (HD)), Xiao-Lin Qi et al. for extraction of essential oil from

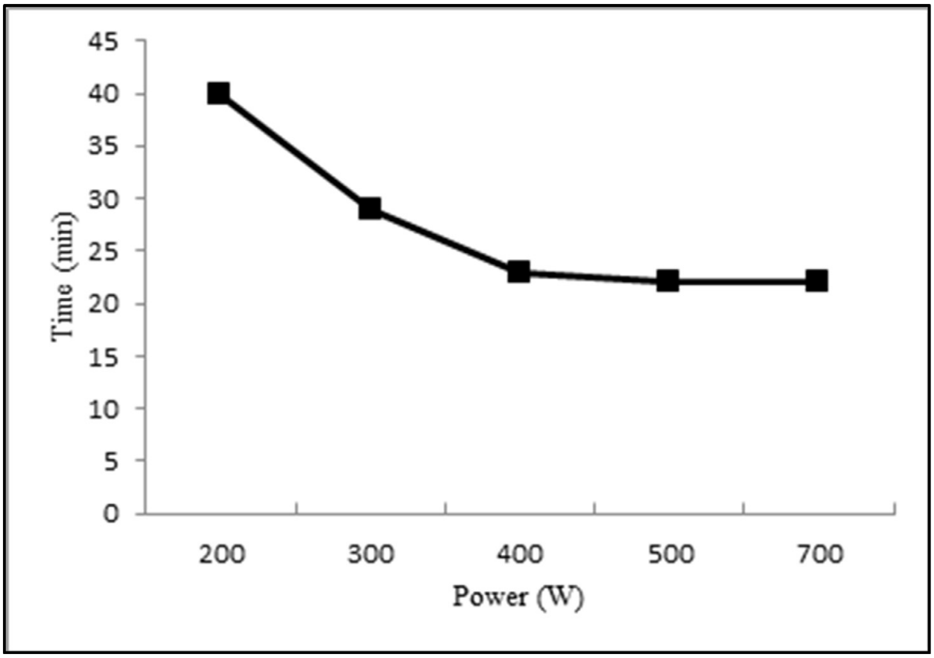


Fig. 5. Evolution of the extraction time by SFME as a function of microwaves power.

pigeon pea leaves (44 min with SFME vs 300 min with HD) [42], Sahraoui et al. for essential oil extraction using microwave steam distillation (MSD)), have seen the extraction time switches from 3 hours for SD to 6 min for MSD for *Citrus* essential oil, and 6 min for MSD vs 40 min for SD for lavender essential oil extraction [17,43].

As we can see in Fig. 6, the extraction kinetics of essential oil for both SFME and SD processes has the same extraction profile characterized by three distinct phases. However, extraction speeds are different and depend on the process used. Step 0 representing the heating phase from room temperature to 100 °C, is faster for SFME with only 4 min against 40 min for SD. The first step (Step 1) in which 63.68% of essential oil is recovered for SFME and 61.53% for SD is represented by a rapid increase in the yield which characterizes the extraction of the oil located at the surface of vegetable particles. This phase was achieved in only 4 min for SFME vs 37 min for SD. Step 2 is characterized by a second increasing line realised into 14 min for SFME and 163 min for SD. This phase corresponds to the intern diffusion of the essential oil from the midst of the particles towards the external medium involved by the heating of the interstitial water of the plant. The oil amount extracted represents 36.32% for SFME and 38.46% for SD of the global yield. The end of the extraction process marked by a horizontal line corresponds to Step 3.

3.2. Carotenoid extraction

Based on the bio-refinery concept, limonene obtained from orange peels essential oil was used as an alternative solvent for carotenoid extraction to replace n-hexane witch is petroleum solvent.

3.2.1. Effect of ultrasonic intensity on carotenoid content

In order to determine the effect of ultrasonic intensity applied for the carotenoid extraction, different values of ultrasonic intensity ranged from 52 to 208 W cm^{-2} were investigated. The results are shown in Fig. 7.

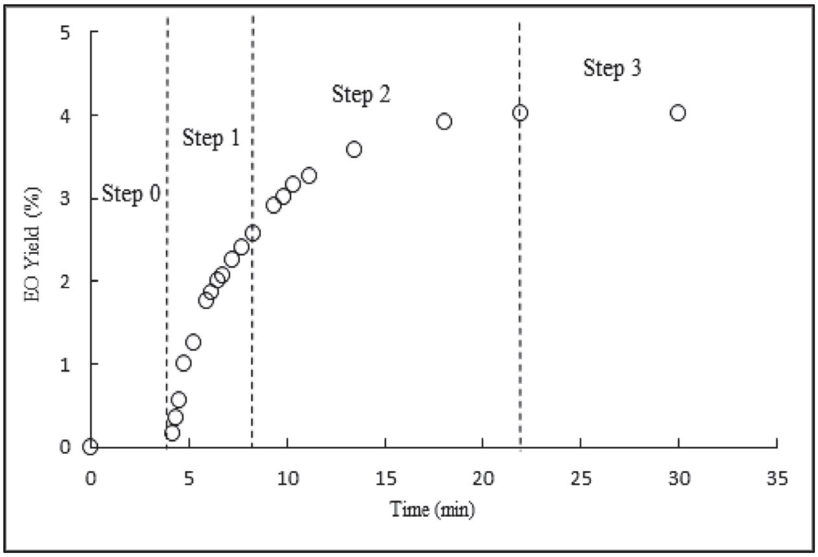
We notice an increase of carotenoid concentration with the increasing of the ultrasonic intensity ranging from 52 to 195 W cm^{-2}. This rapid increase is due the cavitation phenomenon induced by the physical processes created by ultrasound. This phenomenon creates, enlarges and implodes micro bubbles of gases dissolved in the liquid by the compression and decompression of molecules that constitute the medium [44]. The collapse of the cavitation bubble induce a transitory hot spot with elevated localized temperature and pressure, which can accelerate dramatically the chemical reactivity into the medium [44]. The energy transferred for cavitation phenomenon increases with an increasing in electrical acoustic intensity, which facilitate the disruption of cell wall of the matrix and accelerate the extraction process. The same data were observed by several authors for the extraction of carotenoid using ultrasound [2,45–47].

When the ultrasound intensity is higher than 195 W cm^{-2}, we notice a dramatic decrease of about 40% in carotenoid content. This phenomenon could be explained by the formation of a greater number of cavitation which hampers the propagation of shock waves. The bubbles may coalesce to form bigger ones and implode faintly which can cause the reduction of cavitation effect [45,47].

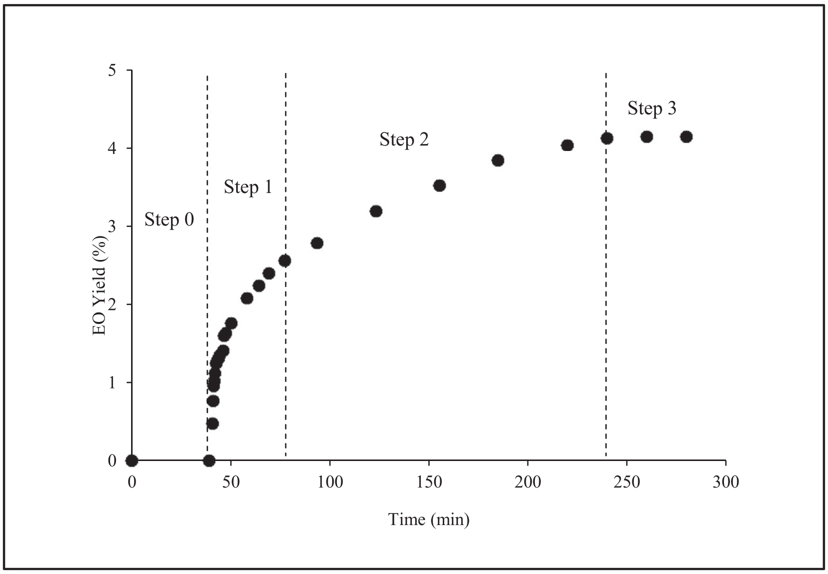
3.2.2. Effect of solid/solvent ratio

The study of the impact of the solid/solvent ratio on the carotenoid content, prove that for the tested values (1:10 and 2:10 (g mL^{-1})), the solid/solvent ratio does not have a particular impact on the extraction yield (23 and 24 mg L^{-1}, respectively), probably because of the saturation of solvent. Li et al. found in the extraction of ß-carotene from carrots using sun flower oil as green solvent that the concentration of ß-carotene tripled as the carrot to oil ratio increased from 1:10 to 2:10 and was limited when the ratio rose up to 3:10 [40]. Conversely, Sun et al. reported that the extraction yield of the all-trans-ß-carotene from citrus peels obtained by UAE increased as the solid/solvent ratio did increase from 1:30 to 3:30, and decreased when the ratio increased from 3:30 to 5:30 [47].

The difference observed between our results and those reported in the literature may be due to the nature of solvents used and the solubility of the carotenoid in these solvents. The value of 1/10 was selected for all the tests.



(a)



(b)

Fig. 6. Comparison between SFME (a) and SD (b) extraction of essential oil from orange peels.

3.2.3. Statistical analysis for UAE extraction of carotenoid

The coded and decoded (natural) values of independent variables and carotenoid yields obtained for different trials of the experimental design protocol are shown in Table 2. These results were analyzed statistically through Statgraphics software in order to obtain the optimal UAE parameters.

The statistical significance of the factors' effects (linear, quadratic and interactions between variables) on UAE of carotenoid from citrus peels was determined by p-value and illustrated by the standardized Pareto Chart diagram (Fig. 8).

Positive and negative effects of the factors in the response variables are represented by horizontal bars and the vertical line tests the significance of the effects at the 95% confidence level. The Pareto Chart diagram revealed four significant effects. Linear effect of ultrasonic intensity (UI) is the most affecting factor, followed by the cross product term (T.time) and (UI.T.time), and finally the quadratic effect of temperature (TT).

The quantitative effects of process variables and their interactions on the measured response can be described by a mathematical relationship obtained with statistical software Statgraphics. This mathematical correlation link the response studied (carotenoid yield) and key variables in the model and described by the polynomial equation as follows:

$$\begin{aligned} Y_{\mathrm{Carotenoids}} = & -0.9705 + 0.0502\ \mathrm{T} + 0.0099\ \mathrm{IU} + 0.0280\ \mathrm{t} \\ & -0.0006\ \mathrm{T}^2 - 0.0001\mathrm{T}\ \mathrm{IU} - 0.0001\ \mathrm{T}\ \mathrm{t} \end{aligned}$$

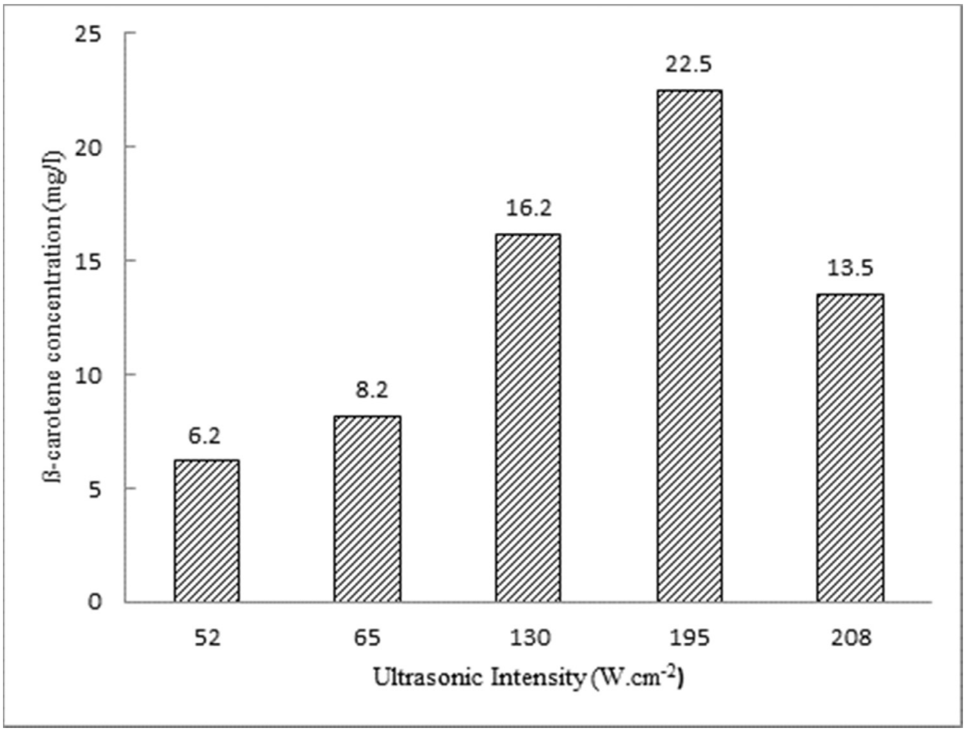


Fig. 7. Effect of Ultrasonic Intensity on carotenoid content.

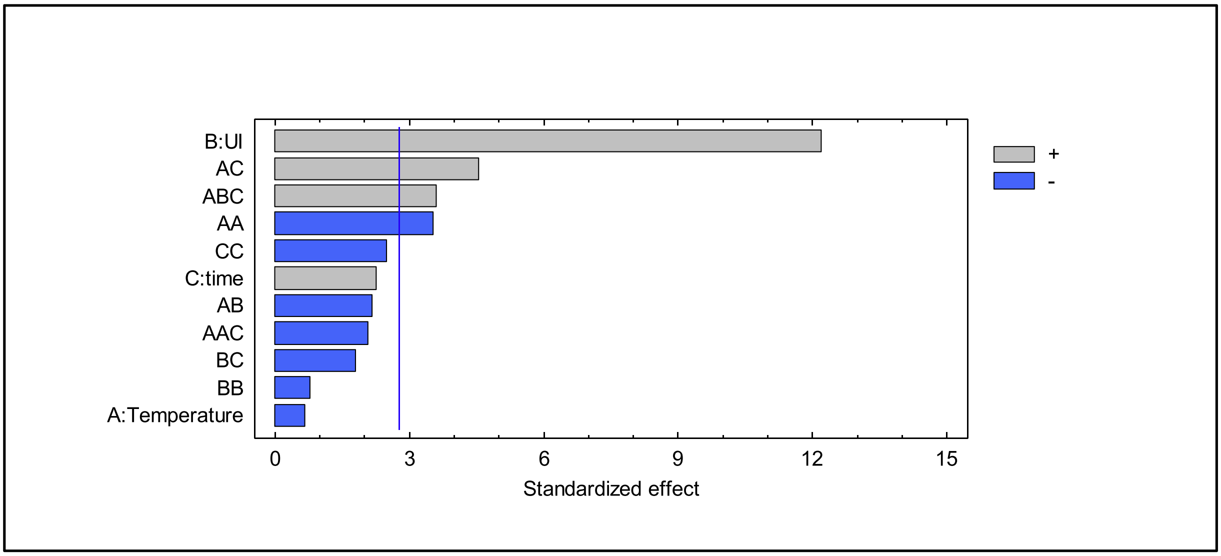


Fig. 8. Standardized Pareto Chart for carotenoid content.

$$-0.000003\ \mathrm{IU}^2 - 0.0002\ \mathrm{IU}\ t - 0.0004\ t^2 + 0.000004\ \mathrm{T}\ \mathrm{IU}\ t.$$

ANOVA gave a coefficient of determination (R^2) of 95.69%, for carotenoid determination, and an adjusted coefficient of determination (R^2_{adj}) of 89.53%, which indicate a close agreement between experiment and predictive values.

Taking into account only a significant influence of the parameters one can assume that:

$$Y_{\mathrm{Carotenoids}} = -0.9705 + 0.0099\ \mathrm{IU} - 0.0006\ \mathrm{T}^2 - 0.0001\ \mathrm{T}\ t + 0.000004\ \mathrm{T}\ \mathrm{IU}\ t.$$

The surface plots obtained for carotenoid content as a function of temperature, ultrasonic intensity and time illustrated in (Fig. 9), showed that the optimum values for the three parameters for carotenoid maximization were 208 W cm⁻² for the ultrasonic intensity, 20 °C for temperature and 5 min for the sonication time, giving the highest carotenoid yield of 11.31 mg L^{-1}.

The experimental conditions obtained a closer optimum values (run 15: 20 °C, 208 W cm⁻² and 5 min, $Y_{\mathrm{Carotenoids}} = 11.25$ mg L^{-1}, see Table 1), we notice that the values predicted by the model and the experimental values are very close.

3.2.4. Comparison between UAE and CE of carotenoid

In order to evaluate the impact of ultrasound-assisted on the extraction of carotenoid using limonene as green solvent, a comparison study was carried out between ultrasound and conventional extractions in optimized conditions obtained from

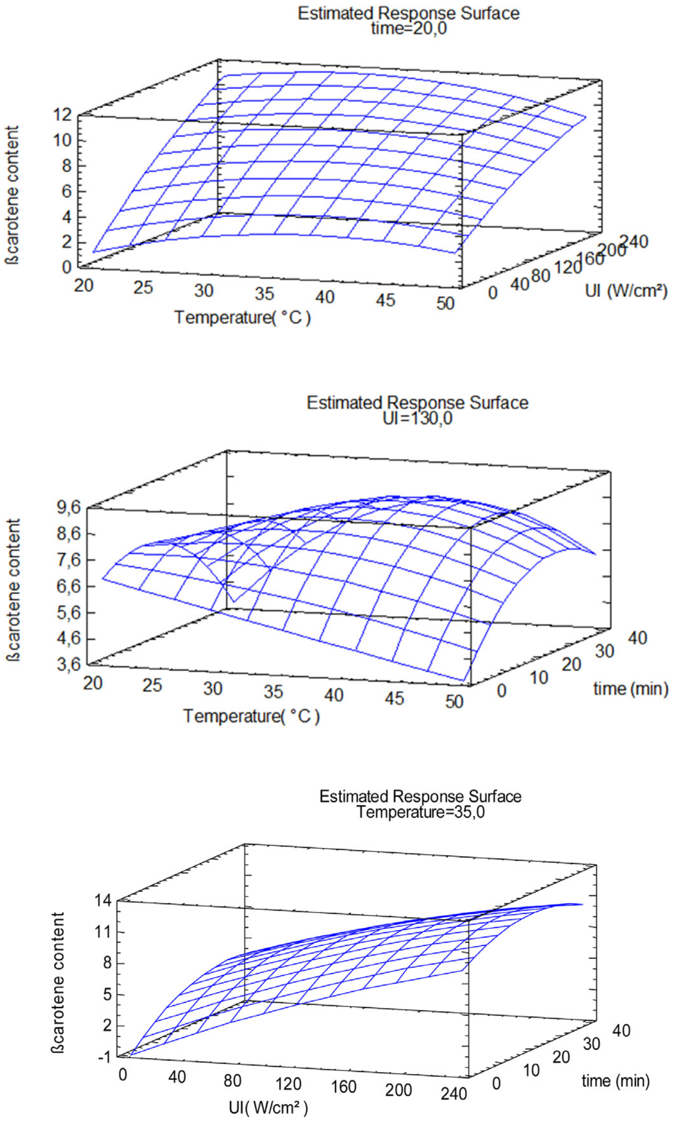


Fig. 9. Response Surface Plots for carotenoid content depending on the temperature, the ultrasonic intensity and time.

the response surface methodology but without using ultrasounds (T = 20 °C, t = 5 min).

Comparison of kinetic of UAE and CE extraction presented in Fig. 10 shows a clear improvement of UAE extraction representing by an increase from 4.17 mg L^{-1} for CE to 6.9 mg L^{-1} for UAE which represents an augmentation of 40%. This intensification is due to the propagation of ultrasound pressure waves through the solvent which causes cavitation phenomenon. The implosion of cavitation bubbles generates micro-turbulence, high-velocity inter-particle collisions and perturbation in micro-porous particles of the matrix which accelerates the eddy diffusion and internal diffusion [48]. The same observations were reported by Sun et al. for extraction of all-trans-ß-carotene from citrus peels who show the yield triplicate in 20 min extraction using ultrasound and dichloromethane as extraction solvent [47]. Xu et al., noticed that the yield doubled using ultrasound in 15 min extraction for the extraction of all-trans-lycopene from red grapefruit [46].

3.2.5. Comparison between limonene and hexane as solvent for UAE and CE

To sum up, an ideal alternative solvent should not be emitting VOC, be of low toxicity for humans, have a limited impact on environment (be eco-friendly), be obtained from renewable resources, have a high solvation power, be easy to recover, and not significantly change a process set up [20]. On the basis of these criteria and in order to prove the effectiveness of limonene as green solvent for the extraction of carotenoids, results were compared to a control using hexane as solvent extraction. As we can see in Fig. 11, the carotenoid content is practically the same for both solvent which indicate that limonene can easily use as a valuable replacement for traditional solvents in the extraction of bioactive compound. Numerous studies were done in this field like Virot et al., described a green and original alternative procedure for fats and oils determination in oleaginous seeds using D-limonene as extracting solvent, and compared to n-hexane extraction. No significant difference was noticed between both extracts which indicates that the use of D-limonene as green solvent instead of n-hexane is effective and valuable. On the other hand, recycling of solvents used shows that n-hexane allows the recovery of only 50% of solvent against 90% for the D-limonene [49].

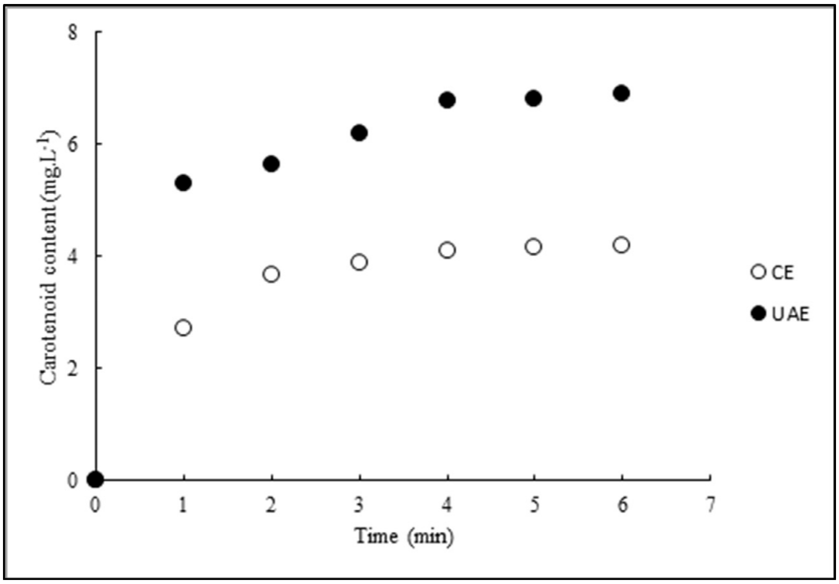


Fig. 10. Comparison between conventional (CE) and ultrasound assisted extraction (UAE).

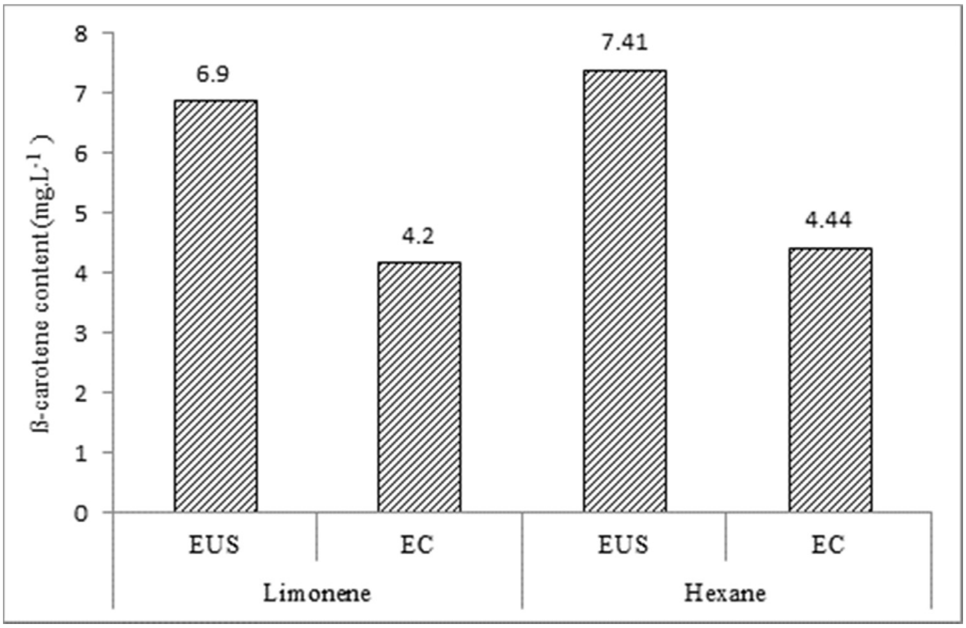


Fig. 11. Comparison between D-limonene and n-hexane as solvent extraction for conventional (CE) and ultrasound assisted extraction (UAE).

The same observations were noticed by Chemat-Djenni et al. for extraction of lycopene with a green method using D-limonene as a substitute for dichloromethane [50].

4. Conclusion

In this study a combination of microwave, ultrasound and D-limonene obtained from a bio-refinery of a by-product of citrus fruits industry was investigated for the extraction of carotenoids of orange peels. The results showed that D-limonene, a main compound of orange peels essential oil can be developed by its use as a bio-solvent for the extraction of carotenoids that are of great importance for food industry. D-limonene gave results comparable with those obtained with n-hexane, which enables it to be a potential alternative solvent. Moreover, the application of the ultrasounds made it possible to increase significantly the carotenoid yields. This procedure constitutes a very good environmental green approach, and allows a saving of time, and a complete valorisation of waste. Indeed, SFME process is ten times faster than conventional SD which offers a gain of time of more than 91%. UAE provides an increase of 40% in carotenoid yield; and 90% of limonene used for carotenoid extraction were recycled.

References

[1] G. Britton, F. Khachik, Carotenoids in food, in: G. Britton, H. Pfander, S. Liaaen–Jensen (Eds.), Carotenoids Volume 5: Nutrition and Health, Birkhäuser Basel, Basel, 2009, pp. 45–66.
[2] Y. Sun, G. Ma, X. Ye, Y. Kakuda, R. Meng, Stability of all-trans-β-carotene under ultrasound treatment in a model system: effects of different factors, kinetics and newly formed compounds, Ultrason. Sonochem. 17 (2010) 654–661.
[3] A.L. CURL, Apo-10′-violaxanthal, a new carotenoid from valencia orange peels, J. Food Sci. 32 (1967) 141–143.
[4] USDA, Citrus: World Markets and Trade, Agriculture – Foreign Agricultural Service, USDA, US, 2011.
[5] FAO, Citrus Fruit: Fresh and Processed – Annual Statistics, Food and Agriculture Organization of the United Nations, US, 2010.
[6] R. Braddock, By-products of citrus fruit, Food Technol. 49 (1995) 74–77.
[7] V. Bampidis, P. Robinson, Citrus by-products as ruminant feeds: a review, Animal Feed Sci. Technol. 128 (2006) 175–217.
[8] H. Bagherian, F.Z. Ashtiani, A. Fouladitajar, M. Mohtashamy, Comparisons between conventional, microwave-and ultrasound-assisted methods for extraction of pectin from grapefruit, Chem. Eng. Process. 50 (2011) 1237–1243.
[9] M.K. Khan, M. Abert-Vian, A.-S. Fabiano-Tixier, O. Dangles, F. Chemat, Ultrasound-assisted extraction of polyphenols (flavanone glycosides) from orange (Citrus sinensis L.) peel, Food Chem. 119 (2010) 851–858.
[10] P. Putnik, D. Bursać Kovačević, A. Režek Jambrak, F.J. Barba, G. Cravotto, A. Binello, J.M. Lorenzo, A. Shpigelman, Innovative "Green" and novel strategies for the extraction of bioactive added value compounds from citrus wastes—a review, Molecules 22 (2017) 680.
[11] M.R. Zahi, H. Liang, Q. Yuan, Improving the antimicrobial activity of D-limonene using a novel organogel-based nanoemulsion, Food Contr. 50 (2015) 554–559.
[12] L. Espina, M. Somolinos, S. Lorán, P. Conchello, D. García, R. Pagán, Chemical composition of commercial citrus fruit essential oils and evaluation of their antimicrobial activity acting alone or in combined processes, Food Contr. 22 (2011) 896–902.
[13] K. Fisher, C. Phillips, Potential antimicrobial uses of essential oils in food: is citrus the answer? Trends Food Sci. Technol. 19 (2008) 156–164.
[14] S. Droby, A. Eick, D. Macarisin, L. Cohen, G. Rafael, R. Stange, G. McColum, N. Dudai, A. Nasser, M. Wisniewski, Role of citrus volatiles in host recognition, germination and growth of Penicillium digitatum and Penicillium italicum, Postharvest Biol. Technol. 49 (2008) 386–396.
[15] S. Raeissi, S. Diaz, S. Espinosa, C. Peters, E. Brignole, Ethane as an alternative solvent for supercritical extraction of orange peel oils, J. Supercritical Fluids 45 (2008) 306–313.
[16] A. Badee, S.A. Helmy, N.F. Morsy, Utilisation of orange peel in the production of α-terpineol by Penicillium digitatum (NRRL 1202), Food Chem. 126 (2011) 849–854.
[17] N. Sahraoui, M.A. Vian, M. El Maataoui, C. Boutekedjiret, F. Chemat, Valorization of citrus by-products using microwave steam distillation (MSD), Innov. Food Sci. Emerging Technol. 12 (2011) 163–170.
[18] M.A. Ferhat, B.Y. Meklati, F. Chemat, Comparison of different isolation methods of essential oil from citrus fruits: cold pressing, hydrodistillation and microwave 'dry'distillation, Flavour Fragrance J. 22 (2007) 494–504.
[19] T. Toplisek, R. Gustafson, Cleaning with D-limonenes: a substitute for chlorinated solvents, Precis Clean 3 (1995) 17–20.
[20] F. Chemat, Alternative Solvents For Natural Products Extraction, Springer, 2014.
[21] S. Chemat, V. Tomao, F. Chemat, in: Limonene As Green Solvent For Extraction of Natural Products, Green Solvents I, Springer, 2012, pp. 175–186.
[22] J.A. Riddick, W.B. Bunger, T.K. Sakano, Organic Solvents: Physical Properties and Methods of Purification, John Wiley and Sons, New York, 1986.
[23] R.J. Braddock, Handbook of Citrus By-Products and Processing Technology, John Wiley and Sons, New York, 1999.
[24] R.A. Clara´, A.C.G.m. Marigliano, H.N. Soĺimo, Density, viscosity, and refractive index in the range (283.15 to 353.15) K and vapor pressure of α-pinene, D-limonene,(±)-linalool, and citral over the pressure range 1.0 kPa atmospheric pressure, J. Chem. Eng. Data 54 (2009) 1087–1090.
[25] N.I. Krinsky, E.J. Johnson, Carotenoid actions and their relation to health and disease, Molecular Aspects Med. 26 (2005) 459–516.
[26] F. Granado, B. Olmedilla, I. Blanco, E. Rojas-Hidalgo, Carotenoid composition in raw and cooked Spanish vegetables, J. Agri. Food Chem. 40 (1992) 2135–2140.
[27] T. Allaf, V. Tomao, K. Ruiz, K. Bachari, M. ElMaataoui, F. Chemat, Deodorization by instant controlled pressure drop autovaporization of rosemary leaves prior to solvent extraction of antioxidants, LWT-Food Sci. Technol. 51 (2013) 111–119.
[28] X. Sui, T. Liu, C. Ma, L. Yang, Y. Zu, L. Zhang, H. Wang, Microwave irradiation to pretreat rosemary (Rosmarinus officinalis L.) for maintaining antioxidant content during storage and to extract essential oil simultaneously, Food Chem. 131 (2012) 1399–1405.

[29] F. Lebovka, N. Vorobiev, E. Chemat, Enhancing Extraction Processes in the Food Industry, CRC Press, 2011.

[30] N. Sahraoui, M. Hazzit, C. Boutekedjiret, Effects of microwave heating on the antioxidant and insecticidal activities of essential oil of Origanum glandulosum Desf. obtained by microwave steam distillation, J. Essent. Oil Res. 29 (2017) 420–429.

[31] N. Sahraoui, C. Boutekedjiret, in: Innovative Process of Essential Oil Extraction: Steam Distillation Assisted by Microwave, Progress in Clean Energy, 1, Springer, 2015, pp. 831–841.

[32] M. Bouras, M. Chadni, F.J. Barba, N. Grimi, O. Bals, E. Vorobiev, Optimization of microwave-assisted extraction of polyphenols from Quercus bark, Ind. Crops Products 77 (2015) 590–601.

[33] Z. Zhu, Q. Guan, M. Koubaa, F.J. Barba, S. Roohinejad, G. Cravotto, X. Yang, S. Li, J. He, HPLC-DAD-ESI-MS 2 analytical profile of extracts obtained from purple sweet potato after green ultrasound-assisted extraction, Food Chem. 215 (2017) 391–400.

[34] S.M.B. Hashemi, N. Nikmaram, S. Esteghlal, A.M. Khaneghah, M. Niakousari, F.J. Barba, S. Roohinejad, M. Koubaa, Efficiency of ohmic assisted hydrodistillation for the extraction of essential oil from oregano (Origanum vulgare subsp. viride) spices, Innov. Food Sci. Emerging Technol. 41 (2017) 172–178.

[35] M. Koubaa, F.J. Barba, H. Mhemdi, N. Grimi, W. Koubaa, E. Vorobiev, Gas assisted mechanical expression (GAME) as a promising technology for oil and phenolic compound recovery from tiger nuts, Innov. Food Sci. Emerging Technol. 32 (2015) 172–180.

[36] M.E. Lucchesi, F. Chemat, J. Smadja, Solvent-free microwave extraction of essential oil from aromatic herbs: comparison with conventional hydro-distillation, J. Chromatograph. A 1043 (2004) 323–327.

[37] F. Chemat, J. Smadja, M.E. Lucchesi, European Patent Demand, 2003.

[38] P.C. Veggi, J. Martinez, M.A.A. Meireles, Fundamentals of microwave extraction, in: F. Chemat, G. Cravotto (Eds.), Microwave-Assisted Extraction for Bioactive Compounds: Theory and Practice, Springer US, Boston, MA, 2013, pp. 15–52.

[39] M. Boukroufa, C. Boutekedjiret, L. Petigny, N. Rakotomanomana, F. Chemat, Bio-refinery of orange peels waste: a new concept based on integrated green and solvent free extraction processes using ultrasound and microwave techniques to obtain essential oil, polyphenols and pectin, Ultrasonics Sonochem. 24 (2015) 72–79.

[40] Y. Li, A.S. Fabiano-Tixier, V. Tomao, G. Cravotto, F. Chemat, Green ultrasound-assisted extraction of carotenoids based on the bio-refinery concept using sunflower oil as an alternative solvent, Ultrasonics Sonochem. 20 (2013) 12–18.

[41] A. Filly, X. Fernandez, M. Minuti, F. Visinoni, G. Cravotto, F. Chemat, Solvent-free microwave extraction of essential oil from aromatic herbs: from laboratory to pilot and industrial scale, Food Chem. 150 (2014) 193–198.

[42] X.-L. Qi, T.-T. Li, Z.-F. Wei, N. Guo, M. Luo, W. Wang, Y.-G. Zu, Y.-J. Fu, X. Peng, Solvent-free microwave extraction of essential oil from pigeon pea leaves [Cajanus cajan (L.) Millsp.] and evaluation of its antimicrobial activity, Ind. Crops Products 58 (2014) 322–328.

[43] N. Sahraoui, M.A. Vian, I. Bornard, C. Boutekedjiret, F. Chemat, Improved microwave steam distillation apparatus for isolation of essential oils: comparison with conventional steam distillation, J. Chromatograph. A 1210 (2008) 229–233.

[44] D. Pingret, A.-S. Fabiano-Tixier, F. Chemat, Degradation during application of ultrasound in food processing: a review, Food Contr. 31 (2013) 593–606.

[45] S. Dey, V.K. Rathod, Ultrasound assisted extraction of β-carotene from Spirulina platensis, Ultrason. Sonochem. 20 (2013) 271–276.

[46] Y. Xu, S. Pan, Effects of various factors of ultrasonic treatment on the extraction yield of all-trans-lycopene from red grapefruit (Citrus paradise Macf.), Ultrason. Sonochem. 20 (2013) 1026–1032.

[47] Y. Sun, D. Liu, J. Chen, X. Ye, D. Yu, Effects of different factors of ultrasound treatment on the extraction yield of the all-trans-β-carotene from citrus peels, Ultrason. Sonochem. 18 (2011) 243–249.

[48] S. Shirsath, S. Sonawane, P. Gogate, Intensification of extraction of natural products using ultrasonic irradiations—a review of current status, Chem. Eng. Process. 53 (2012) 10–23.

[49] M. Virot, V. Tomao, C. Ginies, F. Visinoni, F. Chemat, Green procedure with a green solvent for fats and oils' determination: microwave-integrated Soxhlet using limonene followed by microwave Clevenger distillation, J. Chromatograph. A 1196 (2008) 147–152.

[50] Z. Chemat-Djenni, M.A. Ferhat, V. Tomao, F. Chemat, Carotenoid extraction from tomato using a green solvent resulting from orange processing waste, J. Essent. Oil Bearing Plants 13 (2010) 139–147.