Ultrasound versus microwave as green processes for extraction of rosmarinic, carnosic and ursolic acids from rosemary

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Ultrasound versus microwave as green processes for extraction of rosmarinic, carnosic and ursolic acids from rosemary

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A B S T R A C T

Ultrasound and microwave as green processes are investigated in this study, focusing on the extraction selectivity towards antioxidant extraction from rosemary leaves. Due to its richness in valuable compounds such as rosmarinic, carnosic and ursolic acids, rosemary is a reference matrix for extraction study. In this work, six alternative processes are compared: ultrasound (bath, reactor and probe), microwave (reflux under microwave, microwave under nitrogen pressure and microwave under vapor pressure). The main result of this study is that selective extraction can be achieved according to extraction techniques and therefore to the extraction process.

Keywords:
Antioxidants
Rosemary
Ultrasound
Microwave
Conventional extraction

1. Introduction

The growing demand for natural products leads to constant developments of natural extracts. In the field of food preservation, compounds such as tocopherols and flavonoids are broadly used as antioxidants [1]. Natural antioxidants are extracted from plants, more specifically from herbs or spices, where numerous compounds have been identified as potential antioxidants such as vitamins, lipids, and predominantly polyphenols [1]. Due to its polyphenol composition, rosemary can be considered as a reference matrix for the production of natural antioxidant extracts [1,2].

Rosemary (*Rosmarinus officinalis* L.) is native to the Mediterranean region. Rosemary belongs to the Lamiaceae family and possesses needle-like leaves which contain a powerful fragrance and polyphenolic compounds: phenolic acids such as rosmarinic acid (RA) and caffeic acid; phenolic diterpenes such as carnosic acid (CA), carnosol and triterpenoids such as ursolic acid (UA) (Fig. 1). This polyphenol profile induces antioxidant [3–5], antibacterial [5,6] and antimutagenic properties to rosemary extracts. RA and CA are more specifically used in the food industry as natural antioxidants [7,8]. Apart from the antioxidant properties of rosemary compounds, UA is another valuable natural compound which is studied for its pharmacological effects (e.g., antitumor property [9]).

Throughout literature, at a laboratory scale, extraction of RA and CA from rosemary leaves has been investigated using different technologies: conventional solvent extraction [10], microwave [11–13], ultrasound assisted extraction [12,14–16], supercritical and subcritical fluid extraction [16–18] pressurized liquid extraction [18,19], deodorization by Instant Controlled Pressure drop [20] or extraction with ionic liquids [21]. Table 1 details the experimental conditions of the mentioned processes. Some extraction processes, particularly conventional ones, are sometimes accompanied by several drawbacks, such as the use of harmful solvents, degradation of compounds of interest due to high temperature, long extraction time, difficulty to implement or high economic and energetic costs. That way, during the last few years, concepts of "Green chemistry" and "eco-extraction" emerged [22,23]. Extraction processes have been studied to be more energy saving, safe for users and environmental friendly than yesterday, without reducing extraction efficiency. Intensification of extraction processes taking in account those different aspects should become a new challenge for the design of extraction processes.

Due to their chemical structure, CA and RA are conventionally extracted by methanol and acetone [15,24,25]. Other solvents have been used such as ethanol and water or a mixture of both [24]. Considering sustainable and safe extraction, there is a major interest

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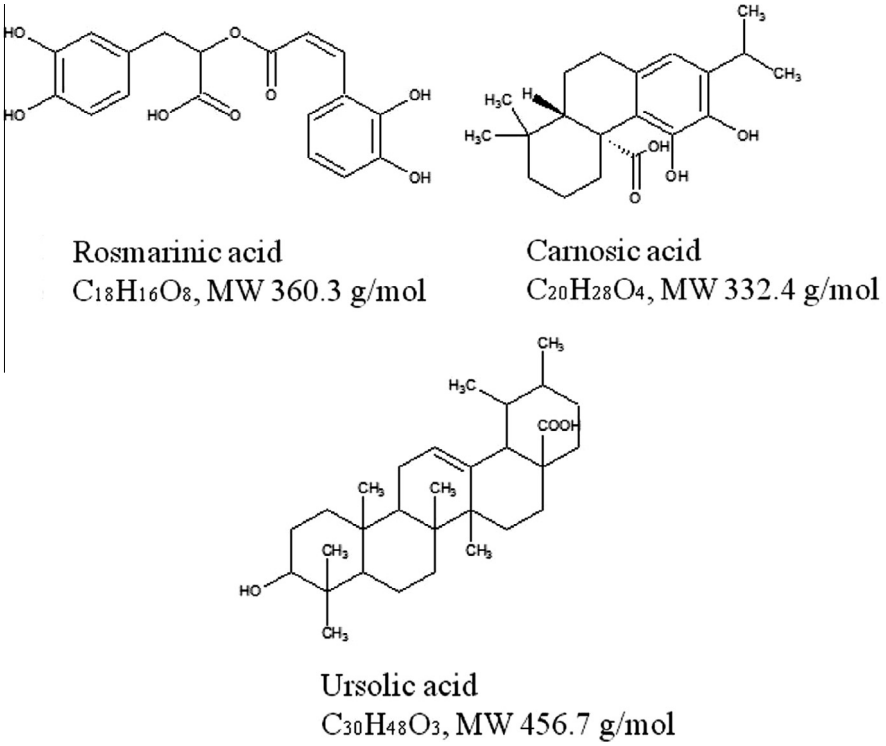

Fig. 1. Chemical structures of major antioxidants in rosemary.

Table 1
Examples of extraction conditions of RA, CA and CO from rosemary.

Extraction techniques	Extracted compounds	Solvents	Experimental conditions	Analysis	Refs.
USAE	RA, CA	Water, ethanol	Ratio 1:6 (w/w) P_{US} = 300 J/g T = 40 °C t = 7 min	TPC, DPPH, HPLC–UV	[11]
CSE	RA, CA, CO	Methanol/water 80/20 (v/v)	Ratio 1:1 (w/v) t = 2 min	HPLC–UV	[10]
DIC pre-treatment + CSE	RA, CO	Ethanol/water 80/20 (v/v)	Ratio 1:1 (w/v) t = 2 min	HPLC–UV	[20]
PLE	RA, CA, CO	Water, ethanol	T = 50–200 °C t = 20 min	TPC, DPPH, UPLC–MS	[17]
MW pre-treatment + CSE	RA, CA	Ethanol/water 80/20 (v/v)	Ratio 1:10 (w/v) P_{MW} = 8 W/g t_{MW} = 15 min t_{CSE} = 4 min	HPLC–UV	[12]
SWE	CA, CO	Subcritical water	T = 25–200 °C t = 30 min	DPPH, LC–MS	[26]
SFE	CA, CO	Supercritical CO_2	P = 355 bar T = 100 °C t = 20 min	HPLC–UV, MS	[15]
IL	RA, CA	[C_8mim]Br 1 M	Ratio 1:20 (w/v) P_{US} = 221 W $t_{soaking}$ = 2 h t_{US} = 30 min	HPLC–UV	[21]

RA: Rosmarinic Acid; CA: Carnosic Acid; CO: Carnosol; USAE: Ultrasounds Assisted Extraction; CSE: Conventional Solvent Extraction; DIC: Deodorization by Instant Controlled Pressure Drop; PLE: Pressurized Liquid Extraction; MW: Microwaves; SWE: Subcritical Water Extraction; SFE: Supercritical Fluid Extraction; IL: Ionic Liquids.

in the use of a mixture of ethanol and water as an extraction solvent, each of these solvents being classified as GRAS solvents.

Aiming at intensification of extraction taking into account concept of "Green chemistry", a comparative study is carried out between ultrasound (US) and microwave (MW) to extract RA, CA and UA from rosemary leaves. To evaluate those innovative processes, results are compared to conventional solid/liquid extraction (reflux extraction and maceration). Within the objectives of green extraction [22,23], all extractions were performed in a mixture of ethanol/water (90/10, v/v). Results are compared quantitatively on the basis of extraction yield and on the contents of RA, CA and UA in the extracts. Ultimately, the processes assessed are compared according to the energy consumption required to achieve extraction.

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2. Materials and methods

2.1. Plant material and chemicals

Rosemary (*R. officinalis* L.) was provided by Naturex. The batch used was collected in Morocco in 2013 and previously hydrodistillated by the supplier. In this study, only leaves were used and were ground for 10 s before extraction using a coffee grinder (Severin, France). Initial moisture content was 8.93 ± 0.01% and initial content in rosemary in RA and CA is 0.21% and 1.70% respectively.

For the extraction solvent, only demineralized water and absolute ethanol (ACS reagent, VWR, France) were used.

2.2. Extraction procedures

Ten different extraction processes were applied in this study: four conventional processes (reflux 30 min, reflux 5 h, grinding 3 min followed by reflux 30 min, and maceration) and six innovative (US bath, US reactor, US probe, reflux under MW, MW under nitrogen pressure and MW under vapor pressure). Those processes are illustrated in Fig. 2.

All the extractions were performed using a solid/liquid ratio of 1/20 (m/v). The extraction solvent was ethanol/water mixture in a proportion of 90/10 (v/v). After extraction, the solvent was separated from the matrix by filtration to vacuum using a filter paper. The extract was concentrated until dryness by solvent evaporation under vacuum (Laborota 4001, Heidolph, Germany).

2.2.1. Heat reflux extraction (HRE)

Rosemary leaves were submitted to reflux for two durations: 30 min and for 5 h. Extraction was done at boiling temperature (78 °C). To evaluate a potential effect of a fine dispersion in the solvent prior to extraction, the same experimental conditions were applied to rosemary leaves previously ground into the solvent during 3 min with a homogenizer (Ika T25 digital Ultra-Turrax, Germany). Each experiment was performed in triplicate.

2.2.2. Maceration procedure

Rosemary leaves were submitted to conventional maceration in double jacket reactor during 30 min. Extraction temperature was maintained at 40 °C using a cryostat (Ministat 125, Huber, Germany). Matrix was homogenized into the solvent with a motorized stirrer (Ika Labortechnik RW16 basis, Germany). Each experiment was performed in triplicate.

2.2.3. Ultrasound assisted extraction procedure and apparatus

2.2.3.1. Ultrasound bath. Ground rosemary leaves were immersed into the solvent and the mixture was introduced in an ultrasonic bath (Prolabo, Labover, France) during 30 min. Temperature of water bath was maintained at 40 °C and checked during extraction with an external temperature probe. Each experiment was performed in triplicate.

2.2.3.2. Ultrasound reactor. Ground rosemary leaves were immersed into the solvent and submitted to US during 30 min using an US reactor (150 W, Pex1, REUS, France). Matrix was homogenized into the solvent with a motorized stirrer (Ika Labortechnik RW16 basis, Germany). Extraction temperature was kept constant at 40 ± 1 °C using a cooling system (Ministat 125, Huber, Germany) connected to the double jacket of the reactor. Each experiment was performed in triplicate.

2.2.3.3. Ultrasound probe. Rosemary leaves were placed in a double jacket reactor with the solvent and the whole was submitted to US (1 kW, UIP 1000 hdT, Hielscher Ultrasonics GmbH, Germany) during 30 min. US were applied to the system using a sonotrode immerged in the solvent approximately 2 cm. Extraction temperature was measured with an external sensor and controlled at 40 ± 1 °C with a cryostat (Ministat 125, Huber, Germany). Each experiment was performed in triplicate.

2.2.4. Microwave assisted extraction procedures and apparatus

2.2.4.1. Microwave assisted extraction under pressure (nitrogen pressure). Rosemary leaves were packaged in gauze in order to be totally immersed in the solvent. The whole was placed in a reactor. Aiming at temperature homogeneity, the reactor was immersed in 700 mL of distilled water and introduced in the microwave cavity. Extraction was performed using a high performance microwave reactor (1.2 kW, UltraClave, Milestone, Italy). Before starting extraction, oxygen in the apparatus was flushed using a nitrogen flow. Pressure was reached using a nitrogen flow and temperature was reached with a microwave heating.

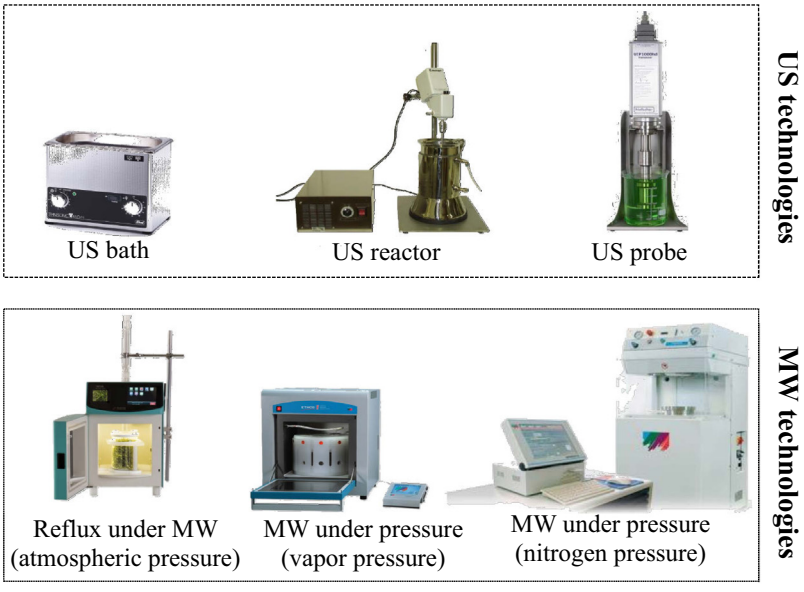

Fig. 2. Equipments assessed for the ultrasound and microwave assisted extraction study.

Extraction temperature was set at 70 °C and initial pressure at 100 bar. Pressure and temperature were controlled by external sensors. At the set temperature, extraction was performed during 30 min. Microwave power was not fixed, it varied as a function of temperature, firstly to reach the set temperature and then to keep it constant during the extraction step. Each experiment was performed in triplicate.

2.2.4.2. Microwave assisted extraction. Rosemary leaves were immersed into the solvent and submitted to microwave during 30 min, using a MW reactor (900 W, EOS-GR, Milestone, Italy) and a reflux apparatus. Microwave power was fixed at 210 W (1 W/g). Extraction was done at boiling temperature (78 °C) and atmospheric pressure. Each experiment was performed in triplicate.

2.2.4.3. Microwave assisted extraction under pressure (vapor pressure). Rosemary leaves were packaged in gauze in order to be totally immersed in the solvent, and placed in a closed Teflon reactor. The whole was introduced in a MW reactor (1 kW, Ethos 1, Milestone, Italy) and heated using microwave until the fixed temperature. Extraction was done at 125 °C and at 150 °C during 30 min. Microwave power was fixed at 300 W but it varied depending on temperature, firstly to reach the set temperature and then to keep it constant during the extraction step. Temperature and pressure were measured with external sensors. Each experiment was performed in triplicate.

2.3. HPLC analysis

Analysis of rosmarinic, carnosic and ursolic acid were done by HPLC (Agilent 1100, France) equipped with a DAD detector. Specific analytical procedures are described below. They were developed and validated in our internal laboratory. Each analysis was performed in triplicate.

2.3.1. Rosmarinic acid analysis

The column used was a C18 column (5 µm, 4.6 mm × 250 mm, Zorbax SB, Agilent Technologies, France). The mobile phase was composed of 32% acetonitrile and 68% water with 0.1% TFA (v/v) and the flow rate was set at 1 mL/min. The column oven temperature was 20 °C and the run time was 10 min. 5 µL were injected. Rosmarinic acid was detected at a wavelength of 328 nm.

2.3.2. Carnosic acid analysis

The column used was a C18 column (1.8 µm, 4.6 mm × 50 mm, Zorbax Eclipse XBD-C18, Agilent Technologies, France). The mobile phase was isocratic and composed of 0.5% H_3PO_4 (in water)/acetonitrile (35/65, v/v), and the flow rate was set at 1.5 mL/min. The column oven temperature was 25 °C. 5 µL were injected. Carnosic acid was detected at a wavelength of 230 nm.

2.3.3. Ursolic acid analysis

The column used was C18 column (3 µm, 4 mm × 150 mm, All C18, Agilent Technologies, France). The mobile phase was isocratic and composed of acetonitrile: 0.1% H_3PO_4 in water (90/10, v/v) and the flow rate was set at 0.6 mL/min. The column oven temperature was 30 °C. Run time was 15 min. 5 µL were injected. Ursolic acid was detected at a wavelength of 210 nm.

2.3.4. Carnosol analysis

The column used was a C18 column (1.8 µm, 4.6 mm × 50 mm, Zorbax Eclipse XBD-C18, Agilent Technologies, France). The mobile phase was isocratic and composed of 0.5% H_3PO_4 (in water)/acetonitrile (35/65, v/v), and the flow rate was set at 1.5 mL/min. The column oven temperature was 25 °C. 5 µL were injected. Carnosol was detected at a wavelength of 230 nm.

2.4. Calculations

In order to assess each extraction process, extraction yield, purity and content in each of the studied compound were calculated. Each mass included in equations below is expressed in dry weight. The extracts resulting from extraction were concentrated to dryness.

$$\text{Extraction yield (w/w, \%)} = \text{weight of extract/weight of rosemary leaves} \times 100 \quad (1)$$

$$\text{Purity (w/w, \%)} = \text{weight of RA, CA or UA/weight of extract} \times 100 \quad (2)$$

$$\text{Content in RA, CA and UA (w/w)} = \text{purity} \times \text{weight of extract (mg)/weight of rosemary leaves (g)} \quad (3)$$

Estimation of specific carbon emissions resulting from electrical consumption is determined considering that 1 kWh = 800 g CO_2 [26]. Energy consumption of each process was measured using an electrical meter (Cost Control, La Crosse Technology, France).

3. Results and discussion

3.1. Comparison of extraction processes in terms of extraction yield

Extraction yields obtained by heat reflux extraction (HRE), maceration, ultrasound and microwave assisted extraction are presented in Fig. 3. Regarding HRE extractions, it can be identified that most of the extraction is achieved within 30 min. Increasing the duration of HRE up to 5 h does not lead to a drastic increase of the extraction yield (20% against 19% for 5 h and 30 min extraction duration respectively). Moreover, adding a preliminary step of homogenization prior to HRE does not improve significantly the yield (18.8 ± 0.2% and 19.0 ± 0.5% respectively). Maceration at 40 °C for 30 min results in a much lower extraction yield (10.0 ± 0.3%). These differences in yield between HRE and maceration are attributed to the temperature difference during extraction.

For ultrasound assisted extraction, extraction temperatures were maintained at 40 °C. It can be identified that similar extraction yield are obtained for extraction performed with the ultrasound reactor and for the US probe (18.1 ± 2.3% and 18.8 ± 2.2% respectively). Lower yields are obtained with the ultrasound bath (13.1 ± 0.1%). Those results may be explained by a low ultrasonic power delivered by the bath compared to the ultrasonic reactor and the probe. Compared to HRE, it can be noted that equivalent yields are achieved at 40 °C using ultrasound assisted extraction.

Microwave assisted extraction was performed at 70 °C and higher temperatures (boiling temperature, 125 °C and 150 °C). For these extractions (Fig. 3), increasing temperatures lead to an increase of the extraction yield, the highest yield (25.2%) being reached at 150 °C. It is a classical observation in extraction that extraction yields increase with increasing temperatures.

If the extraction yields gives an indication of a process performance, the composition of the extracts has to be studied to assess the selectivity of extraction.

3.2. Processing impact towards the extraction selectivity of rosmarinic, carnosic and ursolic acids

The extracts compositions are compared in Fig. 4, according to HRE, ultrasound and microwave assisted extraction. It can be noted

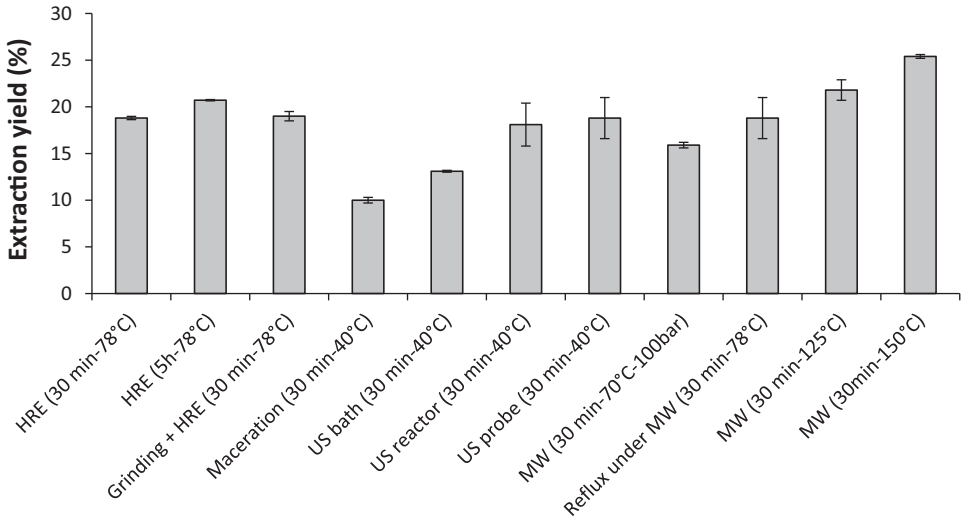

Fig. 3. Comparison of extraction yields according to HRE, maceration, ultrasound and microwave assisted extraction.

that among the three compounds of interest, CA and UA (from 5.5 to 15.4 mg/g rosemary and 20.5–35.3 mg/g rosemary respectively) are predominantly extracted on RA (from 0.4 to 2.2 mg/g rosemary), no matter the extraction technology. These proportions can be attributed to the extraction solvent used, since RA is more soluble in water whereas CA and UA are more soluble in ethanol [27]. Differences among the extraction are however noticed according to the extraction process used.

Modification of the composition of extracts obtained by HRE could be noticed. The extraction of RA seems to be enhanced with extraction duration from 30 min to 5 h (from 1.4 ± 0.1 to 2.1 ± 0.1 mg/g rosemary, Fig. 4). Extraction being performed at the boiling point of the solvent, RA does not appear to be a thermo-sensitive compound. Some authors indicate that an increase of temperature favors extraction until a critical value [18,28]. The opposite tendency is obtained for CA, which concentration decreases with the increase of the extraction duration at high temperature. The tendency observed tend to show a degradation of carnosic acid with temperature. However, different conclusions are obtained throughout literature: either enhanced extraction with temperature (from 100 to 200 °C using pressurized water extraction [18,29]) while others report a degradation with mild temperatures in stability studies (40 °C or less; [30,31]). Overall, it seems that temperature is not the sole factor impacting on the extraction of carnosic acid. Homogenization prior to extraction does not lead to an enhanced extraction for RA, CA and UA (Fig. 4), however, RA is more rapidly extracted.

For ultrasound assisted extraction, temperature was lower (40 °C) than HRE. It can be identified that the level of RA in the extracts (from 0.2 to 1 mg/g rosemary, Fig. 4) is much lower than for HRE. Among the US technologies, sonication by the US probe during 30 min appears as the most efficient process for the extraction of CA and UA. This effect may be explained by a more effective treatment due to specific ultrasonic power delivered using the US probe. Within a shorter duration of extraction and lower extraction temperature, the yields obtained are higher: for CA, contents are 15.4 ± 1.8 mg/g and 13.2 ± 0.2 mg/g for US probe and 5 h HRE respectively.

With experiments performed using microwave assisted extraction, two parameters are examined: effect of pressure and

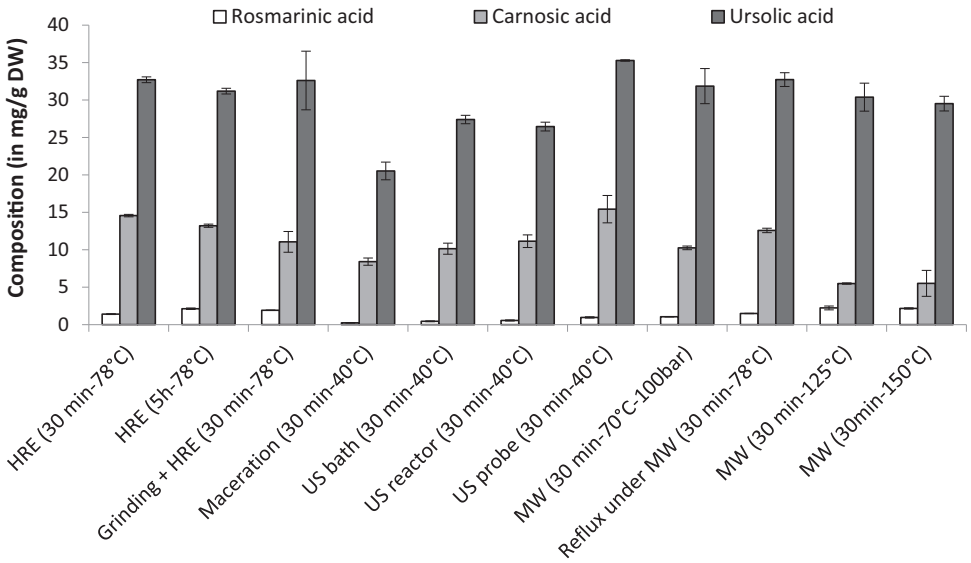

Fig. 4. Comparison of contents in RA, CA and UA in the extracts according to the process assessed.

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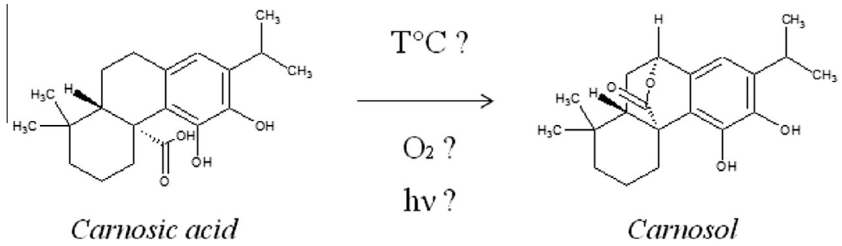

Fig. 5. Hypothesis of the conversion of carnosic acid into carnosol.

Table 2
Purities of CA and carnosol in the extracts obtained by the different processes.

Extraction process	Experimental conditions (temperature–extraction duration)	Purity of compounds in extracts	
		CA (%)	Carnosol (%)
HRE	78 °C to 30 min	7.75 ± 0.01	2.22 ± 0.02
HRE	78 °C to 5 h	6.38 ± 0.08	2.10 ± 0.01
Grinding + HRE	78 °C to 30 min	5.32 ± 0.58	2.09 ± 0.07
Maceration	40 °C to 30 min	8.41 ± 0.71	2.50 ± 0.12
US bath	40 °C to 30 min	7.73 ± 0.63	2.26 ± 0.16
US reactor	40 °C to 30 min	6.27 ± 0.92	2.02 ± 0.27
US probe	40 °C to 30 min	8.21 ± 0.00	2.37 ± 0.03
Ultraclave 100 bar	70 °C to 30 min	6.45 ± 0.02	2.24 ± 0.03
Reflux under MW	78 °C to 30 min	6.19 ± 0.49	1.93 ± 0.10
MW under pressure (vapor pressure)	125 °C to 30 min	2.53 ± 0.17	3.36 ± 0.07
	150 °C to 30 min	2.17 ± 0.66	2.04 ± 0.02

HRE: Heat Reflux Extraction; US: Ultrasound; MW: Microwave; DW: Dry Weight.

Table 3
Energy consumption and carbon emissions of the different extraction processes.

Extraction process	Experimental conditions (temperature–extraction duration)	Energy consumption (kWh/kg extract)	Carbon emissions (kg CO_2/kg extract)
HRE	78 °C to 30 min	94	75
HRE	78 °C to 5 h	850	680
Grinding + HRE	78 °C to 30 min	94	75
Maceration	40 °C to 30 min	79	63
US bath	40 °C to 30 min	15	12
US reactor	40 °C to 30 min	39	31
US probe	40 °C to 30 min	23	19
Ultraclave 100 bar	70 °C to 30 min	154	123
Reflux under MW	78 °C to 30 min	85	68
MW under vapor pressure	125 °C to 30 min	171	137
	150 °C to 30 min	157	125

HRE: Heat reflux extraction; US: Ultrasound; MW: Microwave.

temperature. Comparing extraction at 100 bars and 70 °C (MW) and 30 min HRE (Fig. 4), it was noticed that high pressure does not enhance compounds extraction. RA yields obtained with MW process at 125 °C and 150 °C (2.2 ± 0.1 mg/g) are equivalent to the reference one (2.1 ± 0.1 mg/g for 5 h HRE). RA was increasingly extracted at high temperatures (from 1.5 mg/g at 78 °C to 2.1 mg/g at 150 °C, Fig. 4), as for HRE extractions. Microwave assisted extraction appears to be are more adapted for extraction of RA. A decrease of CA is noted for all extraction assisted by microwave. A degradation of CA was noticed with the increase of temperature: concentration decreased from 12.6 ± 0.3 mg/g for reflux under MW (30 min to 78 °C) to 5.5 ± 1.7 mg/g for MW (30 min to 150 °C). Since CA was degraded at high temperatures, it may have been transformed in degradation products. Additionally, the extraction yield reached the highest level using an extraction temperature of 150 °C (25.4%, Fig. 3).

3.3. Investigation on the conversion of CA into carnosol

Factors such as temperature or light can induce a degradation of rosemary antioxidants into several compounds. A conversion of CA into carnosol (Fig. 5) is reported by several authors [31,32]. Additionally, carnosol also has antioxidant properties [33,34].

In order to assess if CA was converted into carnosol during extraction, we examined the purities of CA and carnosol in the extracts (Table 2). When extraction is performed at 40 °C or at boiling temperature (78 °C), the proportions of CA are higher than carnosol. The extracts obtained by MW at 125 °C contain more carnosol than CA: 3.36 ± 0.07% and 2.53 ± 0.17% respectively (Table 2). At 150 °C, purities in CA and carnosol are very similar: 2.17 ± 0.66% and 2.04 ± 0.02% for CA and carnosol respectively. It can be concluded that the decrease of CA in extracts does not result in a systematic increase of carnosol. Other minor degradation derivatives

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of carnosic acid are epirosmanol [35], 7-methyl-epirosmanol [36] and probably rosmanol 9-ethyl ether [37]. Other degradation products of carnosic acids such as rosmanol and rosmaridiphenol are also generated from carnosic acid during process. Moreover, it has to be underlined that carnosol naturally occurs in rosemary leaves [4], which could explain the concentration of carnosol found at lower extraction temperatures (Table 2). Globally, our results indicate that higher pressure and intensification through microwave and US probe favors a higher ratio of carnosol compared to carnosic.

3.4. Energy consumption

An energy consumption monitoring of the different experiments was performed. Table 3 indicates the measures obtained per process. It appears clearly that 5 h reflux is the most energy-consuming technique (850 kWh/kg extract) and consequently the process with the highest carbon emissions associated (680 kg CO_2/kg extract). It is mainly due to the long extraction duration (5 h). US processes applied during 30 min present the lowest values compared to MW processes and HRE: US probe resulted in an energetic consumption of 23 kWh/kg extract and 19 kg CO_2/kg extract. MW treatments also show reduced values compared to HRE, with few differences between MW processes.

This energetic assessment was carried out at laboratory scale, allowing a comparison on the basis of the sole process. For upscaling and industrial considerations, a Life Cycle Assessment (LCA) could be established to obtain the energetic and environmental profiles of different products or processes [19,38]. That way, LCA could be a tool for industrial decision-making since it could determine which process is the most eco-friendly and economical.

4. Conclusions

This study was carried out to compare different processes for extraction of rosmarinic, carnosic and ursolic acids from rosemary leaves. The main conclusion is that selective extraction of rosmarinic and carnosic acids can be achieved by modification of the extraction technique and procedure. The use of intensified extraction processes at different extraction temperatures enabled to achieve similar yields compared to conventional extraction processes (heat reflux extraction and maceration). It has been demonstrated that carnosic and ursolic acids extraction is enhanced using ultrasound processes whereas microwave are more adapted to extract rosmarinic acid. Moreover, this study revealed that ultrasound and microwave technologies are good alternatives to conventional processes regarding energy consumption and carbon emissions at laboratory scale. Further research are required to investigate the choice of the most appropriate technology for scale up and industrialization [39].

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