Microwave Pretreatment of Vegetable Materials to Increase the Extraction Yield of Natural Products

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It was designed and built a laboratory experimental installation (LEI) for the microwave pretreatment of vegetable materials. To study the influence of microwave pretreatment on the total phenolic content (TPC), a conventional extraction of polyphenols from treated and untreated fresh sea buckthorn leaves was performed. For short extraction times, the amount of phenolic compounds was higher for the extracts obtained from treated leaves, but a long pretreatment time (28 s) led to a decrease in TPC. The qualitative analysis showed that the chemical composition is not affected by the microwave pretreatment.

Keywords: microwave pretreatment, polyphenols, sea buckthorn

A general definition of green chemistry is developing strategies to reduce or to eliminate the use and generation of hazardous substances. An example of this concept is represented by the sustainable transformation of biomass into building blocks (such as ethanol, lactic acid, levulinic and succinic acids, 5-hydroxymethilfurfural, etc.) [1-3]. Considering the latter, the concept of green extraction can be defined as follow: developing extraction techniques which will allow the use of alternative solvents and renewable natural products and ensure a safe and high quality of extract [4].

Bioactive compounds from different parts of medicinal plants (flowers, leaves, fruits, peels, seeds) are beneficial for human health. Due to their biological activity, polyphenolic compounds are among the most important bioactive molecules [5].

Sea buckthorn (genus *Hippophae*) is a small tree of *Elaeagnaceae* family which grows in different regions of Europe and Asia [6,7]. All parts of sea buckthorn shrub are valuable because they contain a variety of bioactive compounds with medicinal and nutritional potential [8-12].

Sea buckthorn leaves are rich in polyphenolic compounds such as: catechins, p-coumaric acid, caffeic acid, gallic acid, quercetin, kaempferol, isorhamnetin, myricetin, and their glycosides [13,14]. Distribution of phenolic compounds in plants is not uniform. Insoluble compounds are found in the cell walls and soluble components are within the plant cell vacuoles. The external layers of plants contain higher amounts of polyphenols than those located inside the plant parts [15,16].

Extraction of polyphenols from plant tissues is accomplished by conventional methods (maceration, Soxhlet extraction) or using unconventional methods such as microwave assisted extraction, ultrasound assisted extraction [17], supercritical fluid extraction, etc. The efficiency of the extraction process is influenced by the interactions between sample matrix and bioactive molecules as well as by the diffusion of solvent through the plant matrix [18].

Since the structure of the material is a key factor influencing the extraction efficiency, any means to modify the structure to enhance the extraction is attractive [19]. It is indeed recognized that sample pretreatment significantly affects the microstructure of a plant material and the release of nutrients [20,21]. The extraction efficiency of polyphenols from plant material can be improved by applying a microwave pretreatment. During the microwave pretreatment, the water present within the cells of the vegetable material evaporates, generating a high pressure against the walls of the cell. Due to the high pressure, the cell walls are destroyed and the compounds migrate easier from the plant cells [22].

The purpose of this work was to study the effect of microwave pretreatment in order to improve the extraction yield of polyphenols from sea buckthorn leaves prior to conventional extraction.

Experimental part

Materials

Fresh sea buckthorn leaves (*HippophaeRhamnoides L.*) were harvested at the beginning of June 2015 at Hofigal S.A. in Bucharest. Folin - Ciocalteu reagent (Merck), ethanol, and sodium carbonate were analytical purity grade. For the HPLC quantification of phenolic compounds, the following standards were used: gallic acid, caffeic acid, chlorogenic acid, catechin, ferulic acid, p-coumaric acid, and rutin from Sigma-Aldrich.

Microwave pretreatment equipment

A laboratory experimental installation (LEI) was specially designed and built for microwave pretreatment of plants before the extraction of bioactive compounds. It consists mainly of the following units (fig. 1): a magnetron (MAG) of 2.45 GHz with adjustable output power (0-700 W) generated as 10 ms pulses at 50 Hz repetition rate; a microwave launcher (MWL) which contains magnetron antenna; a transition waveguide to fit MWL to WR430 standard waveguide; a transition from WR430 to WR340 (SAIREM); a ferrite rectangular circulator (FRC-ENGLAND/ F1152-12); a dual directional rectangular coupler (SAIREM/ Model BCT 236**)** for forward and reflected power monitoring; a three stub tuner (SAIREM/Model AI 35 609R) for impedance matching; a rectangular to cylindrical mode transducer; an elongate cylindrical cavity (ECC) of ~ 9 cm inner diameter and ~ 100 cm length, in which is installed along its axis a quartz tube (guidance tube) containing continuously circulating plant samples. The ECC is adapted

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to operate in the TM_{01n} mode, where *n* is an integer in the range of 0-5. The resonant TM_{010} mode is definitely preferred for heating materials axially disposed within ECC [23]. The ECC is provided with a microwave injection waveguide (IW) coupled there to propagate the electric field component of microwave power substantially parallel to the axis of the ECC. The ECC wall has in the middle an aperture (iris), interposed between its juncture with IW, which has a proper cross-sectional area to maintain resonance within the ECC during its operation as a dielectric heating. A conventional resonance cavity for TM_{010} mode at 2.45 GHz having a diameter of 9.04 cm can excite any cavity length (see mode chart for right-circular-cylindrical cavity [24]), but the diameter value is relatively critical to obtain a resonance. However, in the case of elongated cylindrical cavity, the TM_{010} mode can exist at 2.45 ± 0.05 GHz, for certain cavity lengths such as 14.22 cm, 23.87 cm, 42.4 cm, 52.07 cm, 88.9 cm, 99.82 cm (for our ECC), 109.22 cm, without excitation of the TE modes (these modes develop no axial electric field and do not contribute to heating the dielectric material running axially in the cavity) [20]. Also, the ECC tuning can be achieved by providing a telescoping quartz tube internal of guidance tube and movable into or out of the ECC to determine sharp resonance selection [23]. The ECC ends are closed off with microwave chokes of length greater than l/4 in order to minimize energy radiation from the cavity. The plants are stored inside of cylindrical glass ampoule for microwave assisted pretreatment. In these installations, the conventional operation of the MAG supplied by an L.C. single-phase half-wave doublers (L.C. HWD) was modified in order to permit the use of a manually or PC-controlled electronic regulator for the microwave power adjustment and remote control [25]. The MAG power controller was specifically designed and built to ensure a continuous variable power for magnetron as well as on-line measurement of magnetron average current. Also, several electronic units are added for microwave exposure time presetting as well as for presetting the sample travel (forward and back) velocity inside ECC. The sample motion starts and interrupts simultaneously with microwave switch on and switch off, respectively. The plant sample temperature is determined by a fiber-optic-thermometer. By changing the movement speed of the glass tube containing the sample and the microwave power, different temperature profiles can be achieved.

Extraction procedure

The extraction of phenolic compounds was performed using a heating plate equipped with a temperature controller unit and magnetic stirring. The extraction was carried out in triplicate, in a water bath, using a mixture of 50% ethanol in water, at a temperature of 60°C and 900 rpm stirring rate. For each experiment a 20:1 ratio of solvent to plant was used. Individual experiments were performed considering the following extraction times: 300, 450, and 600 s. After the extraction, the mixture was centrifuged at 3000 rpm for 5 min at room temperature and the fresh supernatant was further analyzed.

Microwave pretreatment procedure

Microwave pretreatment was performed using the laboratory experimental installation described in figure 1. Fresh sea buckthorn leaves, sliced in small pieces (1-2 mm), were introduced in a vial (5 mL) and subjected to pretreatment, using 1 or 2 g of plant. The experiments were carried out at different microwave powers (different current intensities: 100, 150, 200, 250, and 290 mA) and different pretreatment times (20 and 28 s). The density of the vegetable material in the glass vial was about the same in all experiments. For each experiment the temperature profile was recorded using a temperature sensor with optic fiber (OpSens, model PicoPowerSens 62) connected to the computer. The average specific power was subsequently calculated. After the microwave pretreatment, the plant material was directly subjected to conventional extraction. Microwave pretreatment was performed in triplicate for each extraction.

Analysis

The analysis methods for total phenolic content and HPLC determination of major phenolic compounds are described in our previous work [26].

Results and discussions

Determination of specific absorption rate (SAR)

The influence of microwave pretreatment on the polyphenols extraction was studied by recording the temperature profile (fig. 3). The temperature increase during the initial period is used to calculate the *SAR* values around the antenna using eq. (1), where *SAR* [W/kg] is the specific absorption rate, *c* [J/(kg·K)] the specific heat of fresh plant, ΔT [K] the change in temperature after the power is applied for a Δt [s] period.

$$SAR = c\frac{\Delta T}{\Delta t} \quad (1)$$

This period has to be short enough to ensure an almost adiabatic heating process and a linear form for temperature

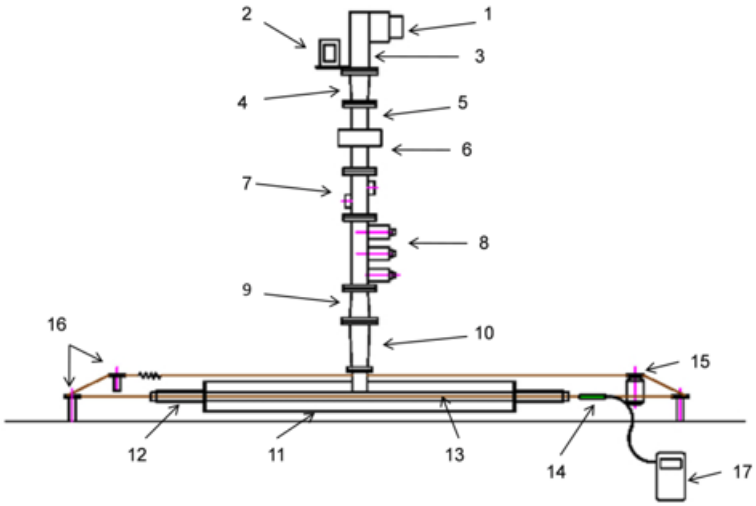

Fig. 1. Scheme of the laboratory experimental installation (LEI): 1. 2.45 GHz magnetron (0-700 W); 2. Magnetron filament supply transformer (high voltage insulated); 3. MW launcher (MWL); 4. Transition waveguide from MWL to WR430 waveguide; 5. Transition from WR430 to WR340 waveguide; 6. Ferrite rectangular circulator; 7. Ferrite rectangular circulator; 8. Three stub tuner; 9. Transition from WR340 to WR430 waveguide; 10. Rectangular to cylindrical mode transducer; 11. Elongated cylindrical cavity; 12. Microwave choke; 13. Quartz tube; 14. Glass ampoule containing plant; 15. Motor + speed reducer; 16. Teflon wire guide; 17. Fiber optic thermometer

increase, with a rate proportional to local *SAR*. *SAR* values were determined for the total heating time (average values) and for a short heating period where the temperature increase was maximum (maximum value).

Influence of microwave pretreatment

To study the influence of microwave pretreatment on the polyphenols extraction from fresh sea buckthorn leaves, experiments with microwave pretreated and untreated plant were performed. The influence of SAR on the total phenolic content is shown in figure 2. The temperature profile was recorded for each microwave pretreatment stage (fig. 3).

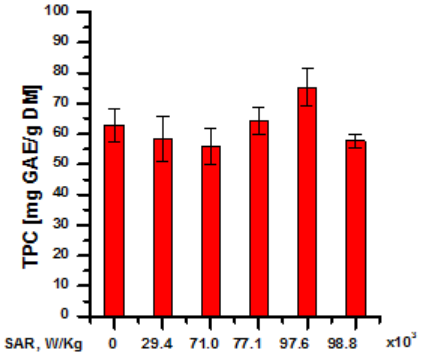

Fig. 2. Influence of microwave pretreatment on the conventional extraction (300 s extraction time, 20 s pretreatment time)

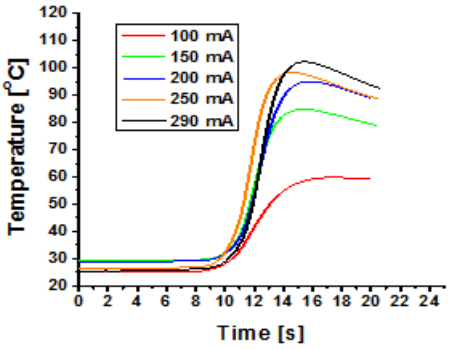

Fig. 3. Temperature profiles

The amount of extracted polyphenols increases for a higher *SAR* value and implicitly for a higher pretreatment temperature. This can be explained by a rapid heating of intracellular water and polar compounds from plant cells during microwave irradiation. Thus, the water evaporates and the inside cell pressure increases causing the rupture of the cell's wall. Further, the access of solvent in the plant cell is facilitated and the compounds of interest diffuse outside the plant tissue more easily.

A high pretreatment temperature improves the amount of phenolic compounds. However, the TPC decreases at a *SAR* value of 98.8 W/kg (fig. 2). This can be explained by polyphenols degradation at higher temperatures. At longer extraction time the difference between the results obtained for the treated and untreated plant is smaller (fig. 4).

Influence of microwave pretreatment time

The microwave pretreatment time of fresh leaves represents another parameter that influences the amount of polyphenols extracted. As shown in figs. 5 and 6, a longer

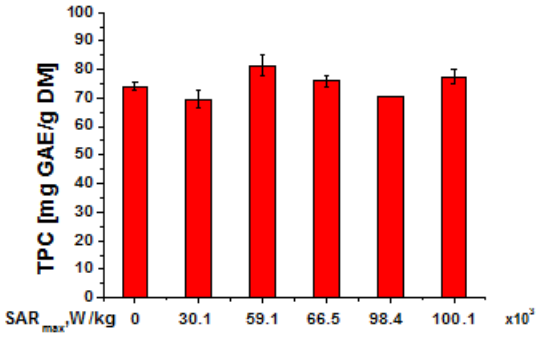

Fig. 4. Influence of the microwave pretreatment on the conventional extraction (600 s extraction time, 20 s pretreatment time)

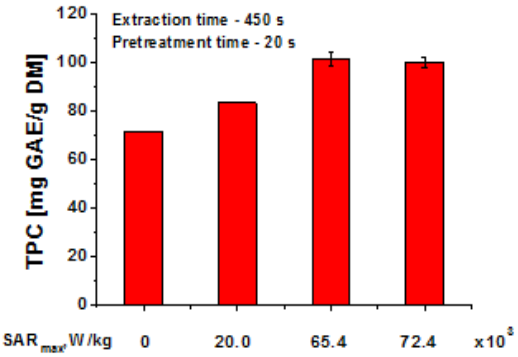

Fig. 5. Influence of microwave pretreatment time on the conventional extraction

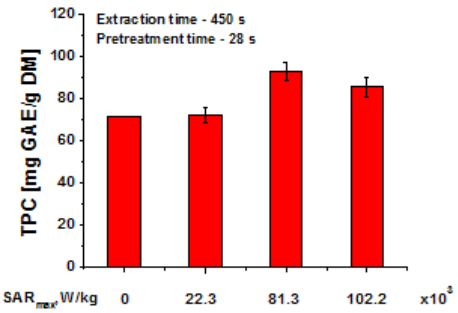

Fig. 6. Influence of microwave pretreatment time on the conventional extraction

pretreatment time leads to a decrease in TPC. Also, the maximum temperature increases with the pretreatment time. The decrease in TPC can be explained by the fact that, for a long pretreatment time, internal micro-environments with high temperatures can be created where polyphenols degradation can occur.

HPLC analysis of sea buckthorn extracts

The chemical composition of sea buckthorn extracts was identified by HPLC analysis. The results are summarized in table 1. The highest concentration of the identified phenolic compounds was found for the microwave pretreated samples. Catechin, gallic acid, caffeic acid, and rutin are the most abundant polyphenols in sea buckthorn leaves. The amount of catechin, caffeic

Compound	Retention time [min]	Extraction without MW pretreatment [mg/g DM]	Extraction with MW pretreatment [mg/g DM]
Gallic acid	5.7	0.376	0.836
Catechin	7.9	1.882	3.607
Caffeic acid	12.3	0.365	0.428
Rutin	32.8	0.323	0.109

Table 1
IDENTIFICATION AND QUANTIFICATION OF POLYPHENOLS FROM SAMPLES OBTAINED FROM EXTRACTION WITH MICROWAVE PRETREATMENT (SAR 65.4 IN FIG.5) AND WITHOUT PRETREATMENT

and gallic acids increased for the microwave pretreated leaves extracts, while the amount of rutin slowly decreased. Catechin was the most abundant polyphenol in all samples and the highest concentration was found in the microwave pretreated sample (3.607 mg/g DM in table 1).

Conclusions

Microwave pretreatment is an efficient method to improve the polyphenols extraction yield. The microwave pretreatment of fresh sea buckthorn leaves leads to the disruption of the plant cell walls and, further the polyphenols are released more easily from the vegetal material. The conventional extraction from pretreated leaves led to a higher TPC compared to the extraction without previous pretreatment. However, the conventional extraction efficiency after microwave pretreatment increased for short extraction times (about 20%). The difference of TPC between the treated and untreated samples for long extraction times was low (about 3-6%). Regarding the influence of the pretreatment time on the extraction efficiency, the TPC decreased with approximately 10 mg GAE/g DM for a pretreatment time of 28 s. This is explained by a specific power threshold where the degradation of phenolic compounds occurs.

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