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Tehnici neconvenționale pentru separarea compușilor valoroși din plante
Nonconventional techniques for the separation of valuable compounds from
plants

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Nonconventional techniques for the separation of valuable compounds from plants

Thesis abstract

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Keywords: microwave assisted extraction, conventional extraction, polyphenolic compounds, sea buckthorn, coaxial antenna, batch extraction process, semicontinuous extraction process, microwave hydrodiffusion and gravity, microwave hydrodistillation, rosemary, thyme, ginger, microwave pretreatment, essential oil, extraction efficiency, energy consumption.

Note: In this document, the notation of chapters, subchapters and figures and tables are the same as those in the thesis.

OBJECTIVES AND PAPER STRUCTURE

The aim of this research was to develop new methods and to improve the existing methods of active principles extraction from plants (polyphenolic compounds and essential oils).

The main objectives of this study are: microwave assisted extraction of polyphenols from sea buckthorn leaves and microwave assisted extraction of essential oils from aromatic herbs (rosemary, thyme and ginger).

The paper contains two parts, namely: the first part presents a literature study on the subject, while the second part deals with the original experimental part.

The literature study is further structured into two chapters. **Chapter I** presents the literature referring to the extraction of polyphenols from sea buckthorn leaves and details information about the selected raw material (sea buckthorn leaves), the classification of polyphenols and their location in sea buckthorn leaves, and the conventional methods and innovative for extracting them. Also, the mechanism of polyphenol extraction is presented, together with a comparison between conventional and microwave assisted extraction.

Chapter II presents a literature study on the extraction of essential oils from aromatic herbs. It includes the description of the selected plants (rosemary, thyme and ginger), the classification of essential oils and their location in the plant structure. Also, conventional and innovative extraction methods for essential oils are presented.

The second part of thesis (the original one) is also divided into two chapters.

In **Chapter III** the research performed for the extraction of polyphenols from sea buckthorn leaves is presented. This chapter is further subdivided into three subchapters, which describe the batch and semicontinuous microwave assisted extraction of polyphenols from sea buckthorn, considering the influence of various parameters on the process. Furthermore, an innovative extraction method using coaxial antennas is presented. This method improves the extraction process due to the use of an efficient cooling and stirring system and also due to the presence of non-uniform heating in the extraction cell, thus providing mild working conditions.

In **Chapter IV** the research performed on the extraction of essential oils from aromatic herbs is presented. This chapter is also divided into two subchapters, which describe the study of an integrated process of extracting rosemary and thyme essential oils by microwave hydrodiffusion and by gravity method, simultaneously with leaves pretreatment for the subsequent extraction of polyphenols. This pretreatment improves the polyphenols extraction process. Further, the extraction of ginger essential oil by microwave hydrodiffusion and gravity method is presented, focusing on energy considerations.

INTRODUCTION

With increasing energy prices and the drive to reduce CO₂ emissions, it is necessary to find new technologies and new process strategies which reduce energy use and maximize valorization of raw materials for economic sustainability.

Biorefinery is a concept defined as the sustainable processing of biomass into a spectrum of marketable products and energy. The Biorefinery is an industrial facility that covers an extensive range of combined technologies aimed at full sustainable transformation of biomass into building blocks (such as ethanol, furfural, lactic acid, glycerol, levulinic and succinic acids, 5-hydroxymethylfurfural, etc. (Gavrila et al. 2017a; Gavrila et al. 2017b)) simultaneous with energy, specialty chemicals and materials, and biofuels production (Rombaut et al. 2014).

Such a concept can be applied to any industrial production, for example extraction of natural products used as food supplements or in pharmaceuticals, medicine and agriculture industries. The term of natural products refers to a very wide range of chemical compounds derived and isolated from biological sources and it is necessary to know how to access them and to understand their uses. Natural products include: an entire organism (e.g., a plant, an animal, or a microorganism), a part of an organism (e.g., leaves or flowers of a plant, an isolated animal organ), an extract of an organism or part of an organism, and exudates, and pure compounds (e.g., alkaloids, flavonoids, glycosides, terpenoids, etc.) (Sarker et al. 2006). Although plants contain a wide variety of chemical constituents, secondary metabolites are usually found in low concentration, makes their recovery and purification very challenging (Zhang et al. 2011).

Among the natural products found in herbs are also polyphenolic compounds and essential oils.

Polyphenols are compounds containing one or more aromatic rings with one or more hydroxyl groups. They are found in a wide variety of plants and are the most abundant secondary metabolites (Salas et al. 2010). Polyphenols are rarely found in free form, most of them being isolated in conjugated forms, most often having a linked glycosidic groups (Lattanzio et al. 2006). Phenolic compounds in plants include phenolic acids, phenolic alcohols, flavonoids, tannins, stilbens and lignans (Naczek et al. 2006; Dai et al. 2010).

Essential oils are complex mixtures of volatile compounds found in a wide variety of aromatic herbs and are very important in plant physiology and ecology. They can be classified into two main groups: hydrocarbons and oxygenated compounds. Hydrocarbons include terpenes, sesquiterpenes and diterpenes, and oxygenated compounds include esters, aldehydes, ketones, alcohols, phenols, oxides, acids and lactones. Some essential oils may also contain nitrogen or sulfur compounds (Chemat et al. 2013; El Asbahani et al. 2015)

A general definition of green chemistry is developing strategies to reduce or to eliminate the use and generation of hazardous substances. Considering the latter, the concept of green extraction can be defined as follow: developing extraction techniques, which will reduce energy consumption, allow for the use of alternative solvents and renewable natural products, and ensure a safe and high quality of extract (Rombaut et al. 2014).

In the field of extraction at industrial scale, conventional processes have some major drawbacks, such as insufficient recovery of extracts, extensive extraction time, high energy consumption, etc. Thus, green extraction processes should focus on intensification: an effective energy use, increased mass transfer, reduced equipment size, and reduction of processing steps (Rombaut et al. 2014).

Microwave assisted extraction (MAE) is one of the most advanced and promising techniques for the extraction of bioactive compounds from plants (Chumnanpaisont et al. 2014). MAE is one of the fastest extraction methods with a very high efficiency compared to conventional ones. Also, in the case of MAE, heating occurs rapidly, thermal gradients are reduced, the equipment is small (Alupului et al. 2012), energy consumption is low (Wang et al. 2010), the extraction time is reduced and the solvent consumption is low (Chen et al. 2007; Chemat et al. 2015).

Microwave hydrodiffusion and gravity (MHG) is a new technique for the extraction of bioactive compounds from plants developed by Chemat et al. (Vian et al. 2008). This extraction method combines microwave heating with gravity at atmospheric pressure. Based on a simple principle, this technique does not involve the addition of water or other solvent. The heating of the intracellular water allows the rupture of the plant cells containing the bioactive compounds. All components, together with intracellular water, are released and transferred from the inside of plant matrix to the outside. This is the physical phenomenon of hydrodiffusion that allows the extract to drop by gravity (Chemat et al. 2017).

CHAPTER III – POLYPHENOLS EXTRACTION FROM SEA BUCKTHORN LEAVES

III.2. Materials and methods

The Sea Buckthorn leaves (*Hippophae Rhamnoides* L.) were harvested in the summer of 2014 at Hofigal S.A. in Furculesti.

Batch and semicontinuous microwave assisted extractions were performed using a Biotage®Initiator microwave oven. In the case of semicontinuous extraction, the microwave equipment was adapted so as to introduce continuously the solvent into the reactor.

The microwave assisted extraction of polyphenols using coaxial antenna was performed on innovative experimental equipment (IEI-1) provided with MW-TEM mode in coaxial structure. The equipment is based on batch operation of a cell containing vegetable sample for polyphenols extraction (fig. III.2).

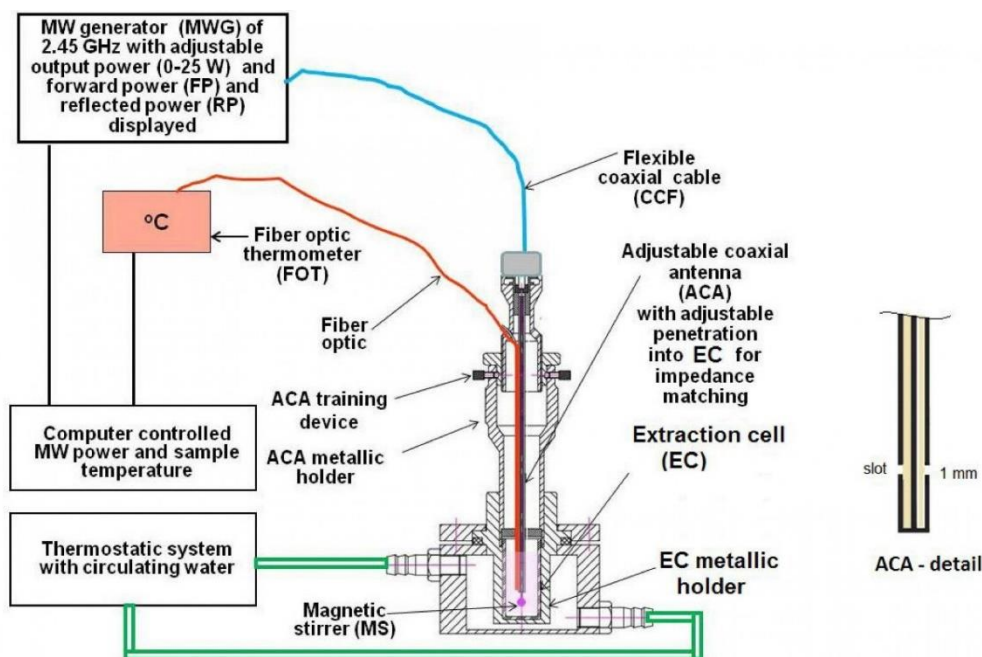


Fig. III.2. Scheme of the experimental equipment (IEI-1) designed for the polyphenols extraction using coaxial antenna.

For all extraction methods, the experiments were performed in triplicate using a 20/1 (v/w) ratio of solvent to plant.

The batch microwave assisted extraction of polyphenolic compounds was performed at different temperatures, extraction times, microwave powers and stirring rates. The extractions were carried out in a mixture of ethanol and water at different concentrations of ethanol. The extractions were performed using the two types of reactors shown in fig. III.3. Comparative conventional extractions, using the same conditions, were performed.

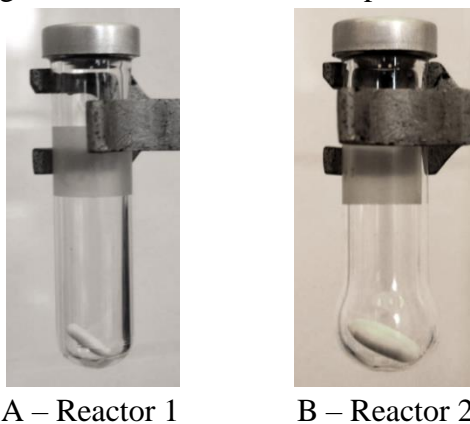
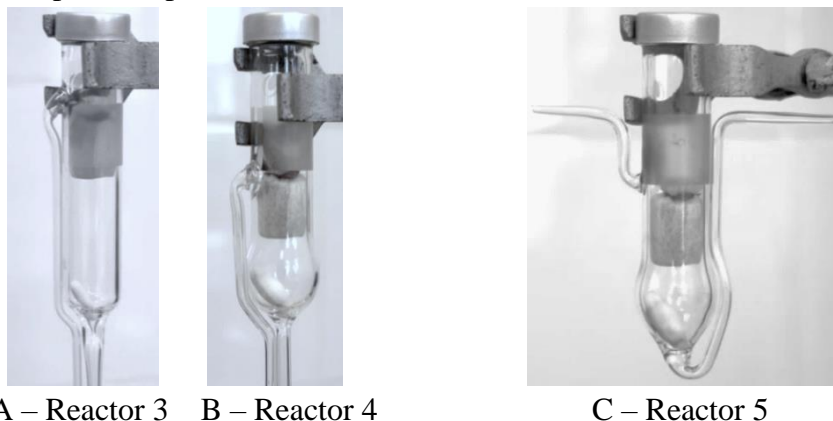


Fig. III.3. Types of reactors

The semicontinuous MAE of polyphenolic compounds was performed using a standard reactor modified in order to achieve a semicontinuous process. The experiments of MAE were carried out using two extraction procedures: pre-heating the solvent at a temperature of 60°C before entering into reactor and introducing the solvent into reactor at room temperature. The experiments were performed using different types of reactors presented in fig. III.4 (fig. III.4A

and III.4B present the reactors used for semicontinuous MAE, meanwhile fig. III.4C presents the reactor used for semicontinuous conventional extraction). Comparative conventional extractions, using the same conditions, were performed. During all experiments, the temperature was monitored. The temperature profile was similar for both methods.



A – Reactor 3 B – Reactor 4

C – Reactor 5

Fig. III.4. Types of reactors used for semicontinuous extraction (reactors 3 and 4 – MAE; reactor 5 – conventional extraction)

In order to determine the efficiency of semicontinuous extraction, information about the total amount of polyphenols in sea buckthorn leaves is required. Thus, a batch multiple extractions were performed using the same extraction conditions as in the semicontinuous process. After 8 cycles the total amount of polyphenols was 216.37 mg GAE/g DM.

The semicontinuous extractions were performed using a mixture of 50% ethanol in water, at a temperature of 60°C, different microwave powers and stirring rates. The experiments were carried out at different solvent flow rates and different residence times respectively. For each experiment, 8 fractions of extract were collected.

In the case of MAE of polyphenols using coaxial antenna, the experiments were performed using a mixture of 50% ethanol in water, at a temperature of 75°C, different stirring rates, and different extraction times. The experiments were carried out at different microwave powers (0 and 25 W) for all extraction times. In order to maintain a constant temperature value, a thermostatic system with circulating water was used. In order to obtain the same temperature profile for both methods of extraction (conventional and MAE), the conventional heating was achieved with hot water in a thermostatic system.

The extracts were analyzed by Folin-Ciocalteu and DPV assays in order to determine the total content of polyphenols (TPC). The extracts were also analyzed by TEAC method in order to determine the antioxidant capacity. The extracts composition (main polyphenolic compounds) was performed by HPLC method.

III.3. Microwave assisted batch extraction of polyphenols from sea buckthorn leaves

III.3.1. Influence of different parameters on the MAE process

a) Influence of stirring rate on the MAE of polyphenols

One of the studied parameters was the influence of stirring rate on the extraction of polyphenolic compounds. The results are shown in fig. III.8 (Asofiei et al. 2016).

The increase of the stirring rate, from 300 rpm to 900 rpm, led to a threefold increase in the total phenolic content. In addition, at a stirring rate of 300 rpm, a longer extraction time determines a slow increase of TPC. For this reason, a low stirring rate requires very long extraction times in order to achieve an efficient extraction. Moreover, it is noted that for higher stirring rate (900 rpm) a longer extraction time (over 200 s) leads to a slight decrease in TPC. Thus, it is desirable to use an extraction method in which stirring allows the complete extraction of polyphenols at relatively short extraction times (Asofiei et al. 2016).

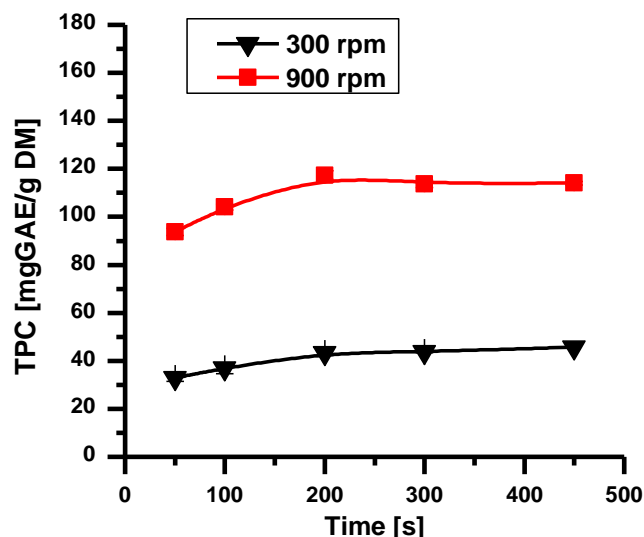


Fig. III.8. Influence of stirring rate on the TPC for batch MAE (50% ethanol in water, temperature of 60°C, reactor 1) (Asofiei et al. 2016).

b) Influence of reactor type on the MAE of polyphenols

The extraction process is more effective if the stirring rate is higher. A method for improving the mixing of the extraction medium is to change the geometry of the reactor. Thus, the reactor was modified in order to allow the use of a bigger magnetic stirrer. For this study, the extraction of polyphenolic compounds was carried out using two types of reactors (normal Biotage reactor - 1 and a modified reactor – 2, as shown in fig. III.3). The two reactors make possible the extraction of polyphenols using the same volume of solvent (5 mL). The results of the influence of the reactor type on the polyphenols concentration are shown in fig. III.9 (Asofiei et al. 2016).

Changing the geometry of the reactor and using a larger stirrer improved the extraction process of polyphenols, as shown in fig. III.9. Although, at short extraction times, the polyphenols concentration is about the same for both reactors, at longer times (after 200 s) the

TPC is significantly higher (around 20%) for modified reactor. Given the fact that the highest amounts of polyphenols were obtained for the modified reactor, the following studies of polyphenols extraction from sea buckthorn leaves were carried out using this reactor (Asofiei et al. 2016).

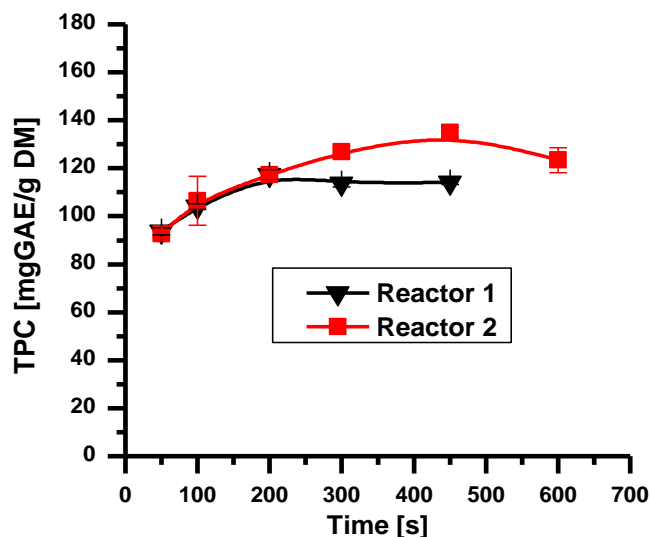


Fig. III. 9. Influence of reactor type on the TPC for batch MAE (50% ethanol in water, temperature of 60°C, stirring rate of 900 rpm) (Asofiei et al. 2016).

c) *Influence of ethanol concentration on the MAE of polyphenols*

The efficiency of polyphenols extraction can also be influenced by their solubility in the solvent. The solubility parameter of a liquid (δ), defined as the square root of the cohesive energy density, is a quantity that allows the estimation of several thermodynamic properties of the solutions. The cohesive energy density is defined as the ratio of the energy of vaporization to the molar volume, both referred to the same temperature. The concept of solubility parameter refers to formulating an expression for the partial molar energy of the mixture, or for the particular case of a zero volume change, for the heat of the mixing of two liquids. This method of estimating the solubility parameter allows estimation of both δ and molar volume for both liquids and compounds with large molecular masses, requiring only knowledge of the chemical structure of the compound (Fedors 1973).

Table III.1 shows Hildebrand solubility parameters of polyphenols found in sea buckthorn leaves.

Table III. 1. Hildebrand solubility parameters of phenolic compounds found in sea buckthorn leaves (*weighted average was calculated considering the percentage concentrations resulted from HPLC analysis of MAE extracts. Single HPLC analysis was performed)

Solvent	
Ethanol	26.1
Water	48
50% Ethanol	37.1

Phenolic compound	
Gallic acid	39.08
Catechin	32.72
Epicatechin	32.72
Epigallocatechin	33.35
Caffeic acid	31.74
Rutin	42.70
Coumaric acid	27.79
Ferulic acid	29.68
Kaempferol-3-O-glucoside	41.24
Izoramnetin-3-O-glucoside	42.87
Quercetin	40.98
Kaempferol	37.88
Isoramnetin	36.23
Weighted average[*]	37.1

The influence study of solvent on the extraction process was achieved by using different concentrations of ethanol in water (fig. III.10).

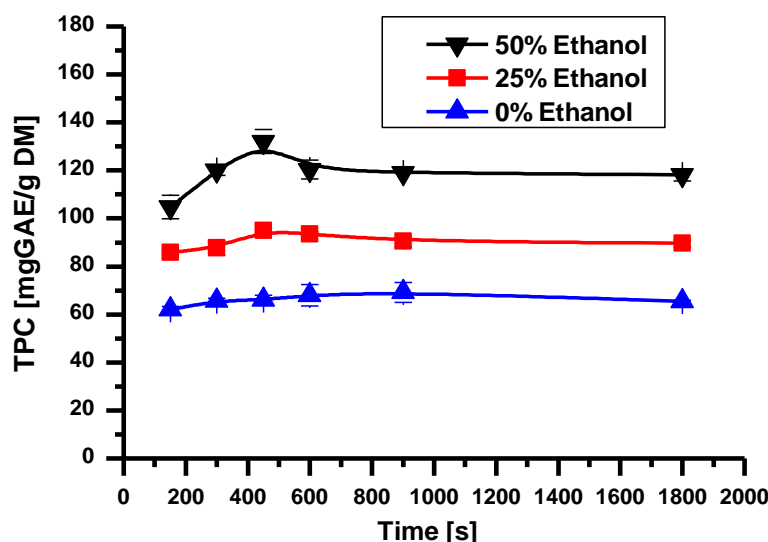


Fig. III. 10. Influence of ethanol concentration on the TPC for batch MAE (temperature of 60°C, stirring rate of 900 rpm, reactor 2) (Asofiei et al. 2016).

The increase of ethanol concentration in the extraction solvent mixture increases the TPC, as shown in fig. III.10. Thus, the use of a 25% ethanol concentration in water results in achieving polyphenol concentrations with approximately 30 mgGAE/g DM higher than when using only water as solvent. Moreover, increasing the concentration of ethanol in water to 50% doubles the amount of extracted polyphenols compared to the extraction without ethanol. This behavior can be explained by the difference in solubility of compounds in water and ethanol. To support this behavior, Hildebrand solubility parameters for both polyphenols and solvent were calculated. Thus, as shown in table III.1, the mixture of 50% ethanol in water and the polyphenols (their

weighted average) have the same value of Hildebrand solubility parameters. Thus, the polyphenolic compounds will be extracted more efficiently with a mixture of 50% ethanol in water than with only one of the two solvents. Therefore, the choice of the solvent to water ratio depends on the composition of polyphenols. In conclusion, a concentration of 50% ethanol in water leads in an efficient extraction of polyphenols from sea buckthorn leaves at a temperature of 60 °C (Asofiei et al. 2016).

d) Influence of temperature and time on the MAE of polyphenols

Another studied parameter on polyphenols extraction from sea buckthorn leaves is the influence of temperature on the TPC.

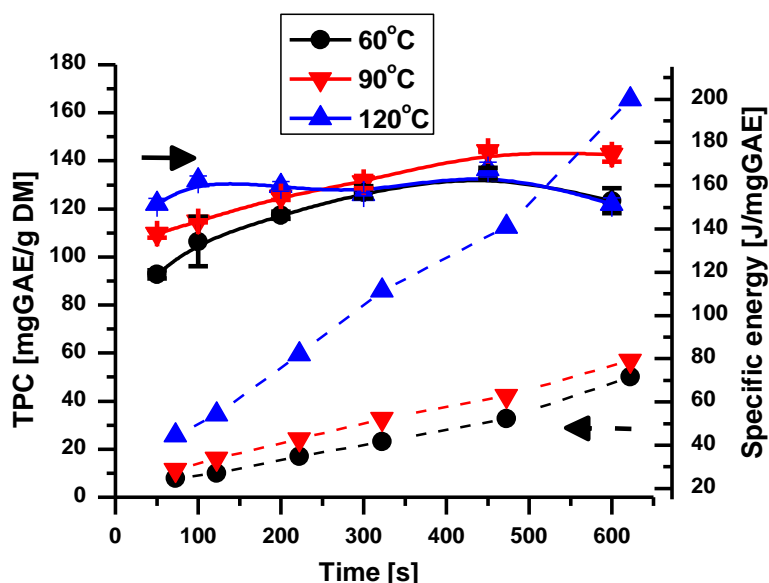


Fig. III. 12. Influence of temperature on the TPC and on the specific energy for batch MAE (50% ethanol in water, stirring rate of 900 rpm, reactor 2) (Asofiei et al. 2016).

Fig. III.12 shows the influence of extraction time and temperature on the TPC and specific energy by batch MAE of sea buckthorn leaves. As shown in fig. III.12, the lowest specific energy corresponds to the experiments performed at a temperature of 60°C. At 90°C it can be observed a slightly increase of the specific energy compared with the values obtained at 60°C. Moreover, polyphenols concentration at 90°C is higher than for extractions at 60°C or 120°C. For this reason, the extraction at 90°C can be considered more efficient regarding both TPC and specific energy.

These values of specific energy are expected to decrease when scaling up. This effect is typical for pilot and industrial installations where a higher ratio between the microwave energy absorbed by the system and the one delivered is required. In this experimental work, reactors with small volumes are used and for this reason the ratio between the required and total energy is only 1 to 4 (Asofiei et al. 2016).

III.3.2. Microwave assisted extraction vs. conventional extraction

To highlight the effect of microwaves on polyphenols extraction from sea buckthorn leaves, experiments under the same conditions, for various temperatures, both by microwave assisted extraction and conventional extraction were carried out (fig. III.13).

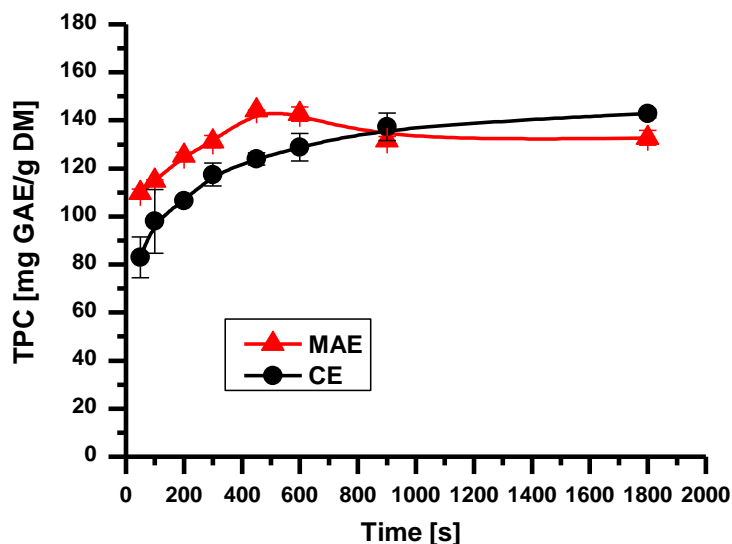


Fig. III. 13. Influence of extraction time on the TPC for batch MAE and conventional extraction (50% ethanol in water, temperature of 90°C, stirring rate of 900 rpm, reactor 2) (Asofiei et al. 2016).

The increase of the extraction time leads to an increase in the TPC, reaching a maximum after 450 s for MAE and after 1800 s for the conventional extraction (fig. III.13). However, a further increase of the extraction time leads to a decrease of the polyphenolic content. Therefore, although the maximum concentration of polyphenols is about the same for both methods, the MAE leads to reducing the extraction time (Asofiei et al. 2016).

III.3.3. TPC determination by DPV

Differential Pulse Voltammetry (DPV) can measure the ability of compounds to donate electrons. This can be related to the antioxidant capacity of polyphenolic compounds. For lower values of the oxidation peak potential, the oxidation occurs easily. Therefore, the antioxidant capacity of the compound is higher (Abou Samra et al. 2011).

Table III.3. The TPC determined by Folin-Ciocalteu and DPV methods (Asofiei et al. 2016).

Exp. no.	Experimental conditions			TPC (mg GAE/g DM)	
	Ethanol concentration [%]	Extraction time [s]	Heating type	Folin-Ciocalteu	DPV
1	50	450	Microwave	134.96	93.71±3.2
2	25	450	Microwave	95.02	70.34±2.5
3	0	450	Microwave	66.00	47.90±1.1
4	0	150	Microwave	62.13	41.40±0.8
5	50	450	Conventional	123.93	86.49±2.9

Table III.3 presents a comparison between the results of the TPC analysis performed by Folin-Ciocalteu method and by DPV. The DPV values are lower than those obtained by Folin-Ciocalteu method. This can be explained by the fact that the Folin-Ciocalteu reagent is less selective and can be also reduced by other non-phenolic compounds found in the analyzed samples (Šeruga et al. 2011).

III.3.4. Composition of sea buckthorn extracts

TPC values can be influenced by a number of other compounds with reducing character that can be extracted from plant (as shown in subchapter III.3.3). For this reason, the polyphenolic extracts were analyzed by HPLC analysis to quantify the content of polyphenols (table III.4). The phenolic compounds identified in sea buckthorn leaves are: gallic acid, catechin, caffeic acid and rutin. In this case, catechin was the predominant polyphenolic species, followed by gallic acid and rutin (Asofiei et al. 2016).

Table III. 4. Quantitative analysis of polyphenolic compounds found in sea buckthorn leaves (MAE, temperature of 90°C, stirring rate of 900 rpm, reactor 2, 50% ethanol in water) (Asofiei et al. 2016).

Exp. no.	Time [s]	Compound [mg/gDM]				TPC [mgGAE/g DM]
		Gallic acid	Catechin	Caffeic acid	Rutin	
1.	50	3.24	9.37	1.51	3.94	109.75±1.6
2.	100	3.34	10.09	1.64	3.79	114.84±0.5
3.	200	3.31	9.24	1.44	4.02	125.31±1.5
4.	300	3.13	9.02	1.4	3.01	131.14±2.6

From the results shown in table III.4 it can be noticed that the increase of the extraction time led to the increase of TPC values, but the major components content slightly decreased at long extraction time (Asofiei et al. 2016).

III.4. Semicontinuous microwave assisted extraction of polyphenols from sea buckthorn leaves

III.4.1. Influence of different parameters on MAE

a) Influence of stirring rate and solvent flow rate on the semicontinuous MAE

In order to determine the factors that influence the semicontinuous process of microwave assisted extraction of polyphenols, a series of parameters were studied. Therefore, one of the studied parameter was the influence of the stirring rate on the TPC.

The stirring rate represents an important factor in polyphenols extraction. As shown in fig. III.14, a higher stirring rate leads to increasing the TPC both in the first fraction and the total amount of polyphenols for all 8 fractions. Due to an efficient stirring of the extraction medium, a better contact between plant material and solvent is achieved and the TPC increases. In addition, a higher stirring rate causes the mechanical destruction of cell walls and further improves the access of the solvent into the cells which favours the extraction of polyphenols.

The flow rate is another parameter that influences the semicontinuous MAE of polyphenols. Decreasing the flow rate leads to an important increase of the polyphenolic content in the first fraction (from 39.34 mg GAE/g DM at 6 mL of solvent/min to 51.06 mg GAE/g DM

at 1.5 mL of solvent/min) for a stirring rate of 900 rpm (fig. III.14B). Moreover, decreasing the flow rate leads to a higher total amount of polyphenols (from 118.90 mg GAE/g DM at 6 mL of solvent/min to 127.76 mg GAE/g DM at 1.5 mL of solvent/min). This can be explained by a better extraction of polyphenols with the increase of residence time from 50 s to 200 s.

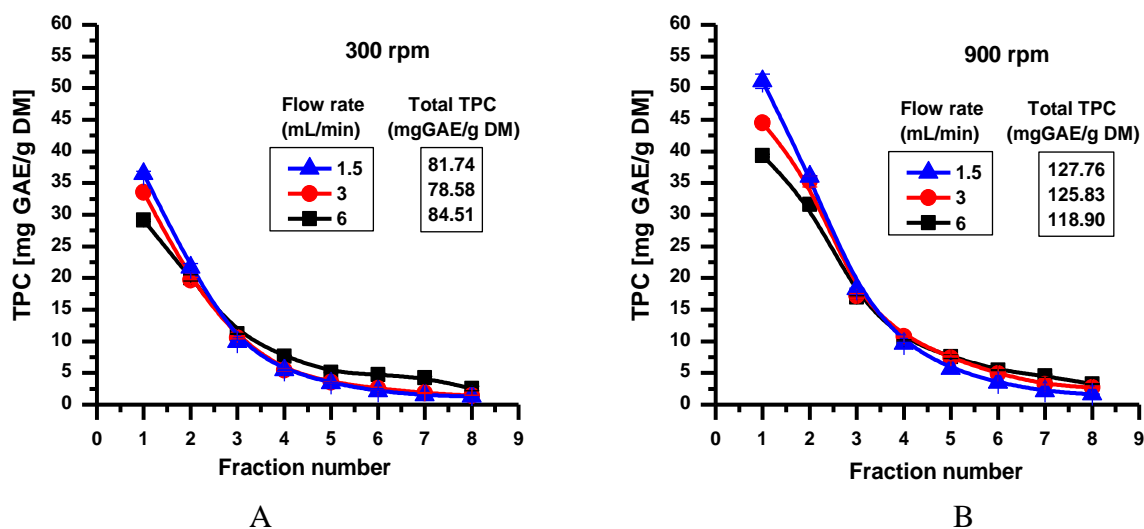


Fig. III. 14. Influence of stirring rate and solvent flow rate on the semicontinuous MAE of polyphenols (reactor 3; A – stirring rate of 300 rpm; B – stirring rate of 900 rpm).

b) Influence of reactor type on the semicontinuous MAE of polyphenols

As shown previous, increasing the stirring rate leads to a higher total amount of polyphenols (from 78.58 mg GAE/g DM at 300 rpm to 125.83 mg GAE/g DM at 900 rpm for a stirring rate of 3 mL of solvent/min). An approach to achieve an efficient mixing of the extraction medium is to use a stirrer with large dimensions. Thus, the reactor used for the extractions was modified to allow using a bigger stirrer. The reactors used for semicontinuous MAE of polyphenols from sea buckthorn leaves are shown in fig. III.4 (reactor 3 – fig. III.4A and reactor 4 – fig. III.4B). The influence of the reactor type on the semicontinuous MAE of polyphenols was studied at a flow rate of 3 mL/min for both reactors as shown in fig. III.15.

Increasing the stirring rate by using a bigger magnetic stirrer leads to a higher TPC in the first fraction (fig. III.15). Although the difference of the TPC values for the other fractions is insignificant, the total amount of polyphenols is higher for reactor 4. This increase of the TPC can be explained by a better mass transfer and by a higher average microwave power for reactor 4 compared with reactor 3.

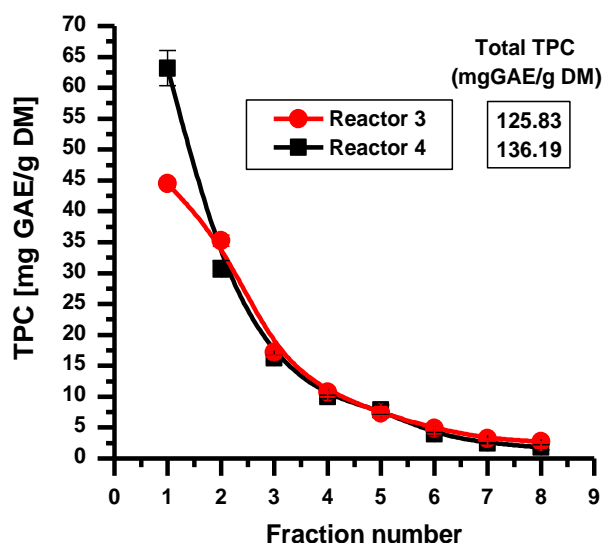


Fig. III. 15. Influence of reactor type on the semicontinuous MAE of polyphenols (stirring rate of 900 rpm, solvent flow rate of 3 mL/min).

c) Influence of solvent pre-heating on the semicontinuous MAE of polyphenols

A strategy to improve the amount of polyphenols in the first fraction for semicontinuous process is solvent pre-heating. Before the entrance into the extraction reactor which contains the sea buckthorn leaves, the solvent is heated at 60°C and then it is allowed to flow continuously through the extraction reactor. The influence of the solvent pre-heating on the TPC of each fraction at different solvent flow rates is presented in fig. III.16.

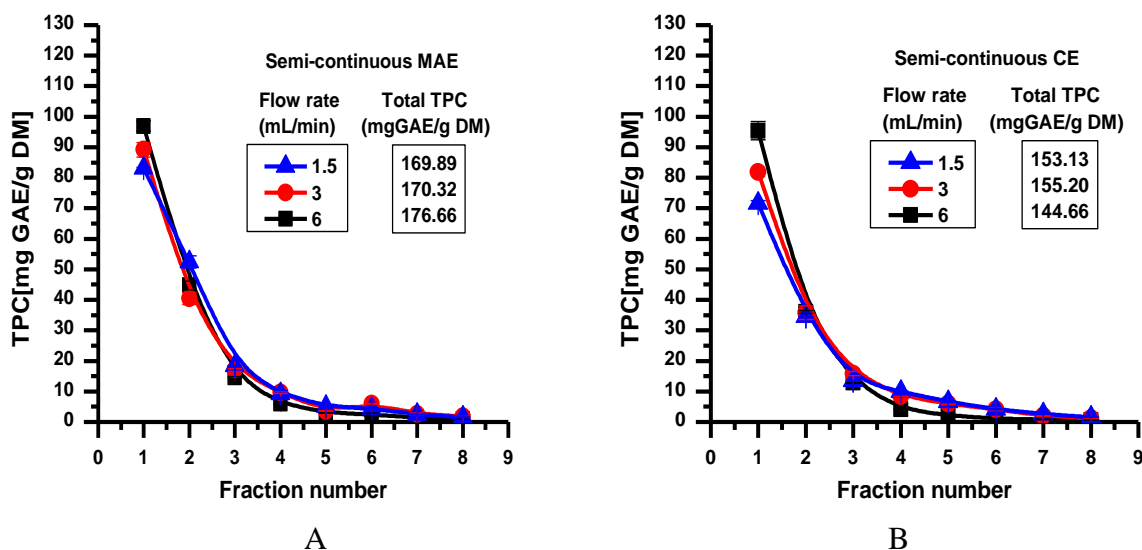


Fig. III. 16. Influence of solvent pre-heating on the semicontinuous MAE of polyphenols at a stirring rate of 900 rpm (A – MAE - reactor 4 and B – conventional extraction - reactor 5)

For a flow rate of 3 mL of solvent/min, the solvent pre-heating influences the total amount of polyphenols, increasing the TPC in the first fraction with approximately 30% and the

total amount of polyphenols with approximately 20% compared with the extraction without solvent pre-heating (fig. III.15 and III.16A). Also, the pre-heating of the solvent allows increasing the flow rate from 1.5 mL/min to 6 mL/min without affecting the extraction efficiency. This strategy of solvent pre-heating leads to extract about 80% of the total amount of polyphenols from sea buckthorn leaves (176.66 mg GAE/g DM for semicontinuous MAE and 216.37 mg GAE/g DM for batch multiple extraction – see fig. III.16A and subchapter III.2.3).

Semicontinuous conventional extractions were also performed to highlight the influence of microwave assisted process on the polyphenols extraction (fig. III.16B). The experiments were carried out in the same conditions as semicontinuous MAE with solvent pre-heating using the reactor 5 (fig. III.4C).

As shown in fig. III.16A and III.16B, the TPC have a similar behavior during all 8 fractions for both extraction methods. Thus, the total amount of polyphenols is higher for semicontinuous MAE compared with semicontinuous conventional extraction when only about 65-70% of polyphenols are extracted.

III.4.2. Antioxidant capacity of polyphenolic extracts

The antioxidant capacity was carried out for MAE and conventional extraction methods with solvent pre-heating at a flow rate of 3 mL of solvent/min and a stirring rate of 900 rpm. The antioxidant capacity of all collected fractions is in concordance with the TPC values obtained for the same extraction conditions (fig. III.16 and III.17). The antioxidant capacity is higher for MAE compared with conventional extraction for both the first fraction and also for the total amount of all 8 fractions (fig. III.17).

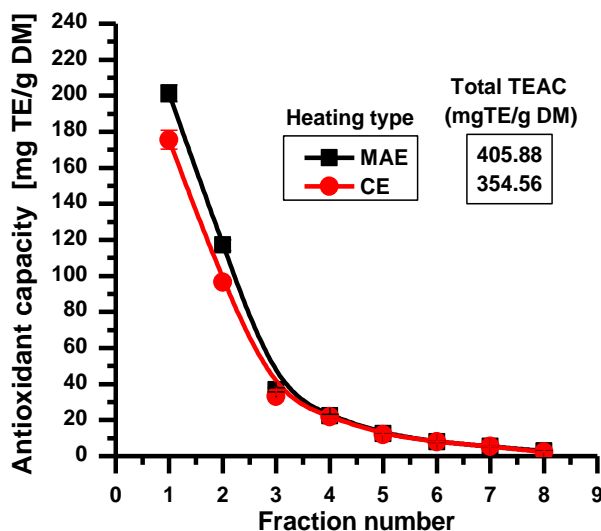


Fig. III. 17. Antioxidant capacity of the extracts for MAE and conventional extraction with solvent pre-heating at a flow rate of 3 mL of solvent/min and a stirring rate of 900 rpm.

III.4.3. Composition of sea buckthorn extracts

The quantitative analysis of the main polyphenols for sea buckthorn leaves is presented in table III.5. The HPLC analysis was performed for the first and fourth fractions of the extracts

obtained by MAE and conventional extraction with solvent pre-heating at a flow rate of 3 mL of solvent/min and a stirring rate of 900 rpm. The main components from sea buckthorn leaves for both fractions are gallic acid, catechin, *p*-coumaric and ferulic acids, rutin, kaempferol-3-*O*-glucoside, isorhamnetin-3-*O*-glucoside and quercetin. All identified components are obtained in higher quantities by semicontinuous MAE than by conventional extraction. Gallic acid was the predominant polyphenolic species followed by catechin for both analyzed fractions.

Table III. 5. Qualitative analysis of the main polyphenolic compounds for the extracts obtained by MAE and conventional extraction with solvent pre-heating (solvent flow rate of 3 mL/min, stirring rate of 900 rpm). Single HPLC analysis was performed.

Polyphenolic compound	Polyphenolic compound concentration [mg/g DM]			
	Heating type			
	MAE		Conventional extraction	
	Fraction 1	Fraction 4	Fraction 1	Fraction 4
Gallic acid	18.02	3.67	12.37	3.42
Catechin	14.62	2.43	9.86	1.62
<i>p</i> -coumaric acid	0.31	0.03	0.28	0.03
Ferulic acid	0.60	0.07	0.52	0.07
Rutin	3.37	0.28	2.69	0.22
Kaempferol-3- <i>O</i> -glucoside	2.49	0.19	2.19	0.16
Isorhamnetin-3- <i>O</i> -glucoside	2.09	0.27	1.96	0.30
Quercetin	1.64	0.93	1.24	0.88
Total	43.14	7.88	31.11	6.71

As shown in table III.5, the polyphenols compound concentration is much lower in the fourth fraction compared with first fraction for both methods (semicontinuous MAE and conventional extraction). However, the quercetin is extracted slower than the other compounds. The concentration of quercetin in the fourth fraction is about 60-70% compared with the concentration obtained in the first fraction.

III.5. Microwave assisted extraction of polyphenols from sea buckthorn leaves using a coaxial antenna

III.5.1. Influence of non-uniform heating

a) Temperature profile in the extraction vessel

All conventional and microwave assisted extractions were carried out in the same extraction vessel. Conventional heating was ensured by recirculating hot water around extraction cell metallic holder. This allowed that the heating rate of the extraction mixture to be rigorously the same as the microwave heating rate. In the case of microwave heating, water at 25°C was recirculating in the space around extraction cell metallic holder, to maintain the average extraction temperature even if the content of the extraction vessel was continuously heated by microwaves.

During the microwave heating, the temperature was measured in two different areas of the extraction vessel, one close to the coaxial antenna and the other near the extraction cell wall (see fig.III.18).

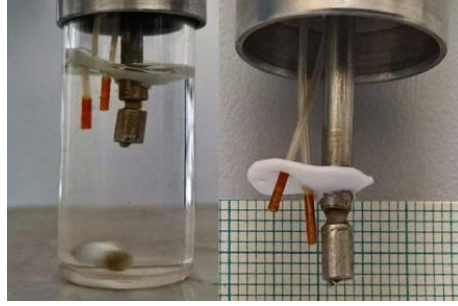


Fig. III. 18. Temperature measurement devices inside the extraction cell.

Numerical models were developed to simulate temperature and electric field profiles in Newtonian fluids during continuous microwave heating by one way coupling electromagnetism and heat transport in COMSOL Multiphysics1 v5.2a. The results are presented in fig. III.19 and III.20.

Graphic representations from fig. III.19 shows the distribution of temperature and SAR when the coaxial antenna heats the cell extraction, surrounded or not by a metal applicator. It can be seen that for a small metal applicator (20 mm diameter, 40 mm height) SAR is more concentrated, especially in the upper slot (9×10^6 W/kg maximum SAR) than if the extraction cell is not contained in a metal applicator (2.4×10^6 W/kg maximum SAR). Different SAR distribution also resulted in a different liquid temperature distribution, even if the same values for the average SAR and global heat transfer coefficient were used (Calinescu et al. 2017b).

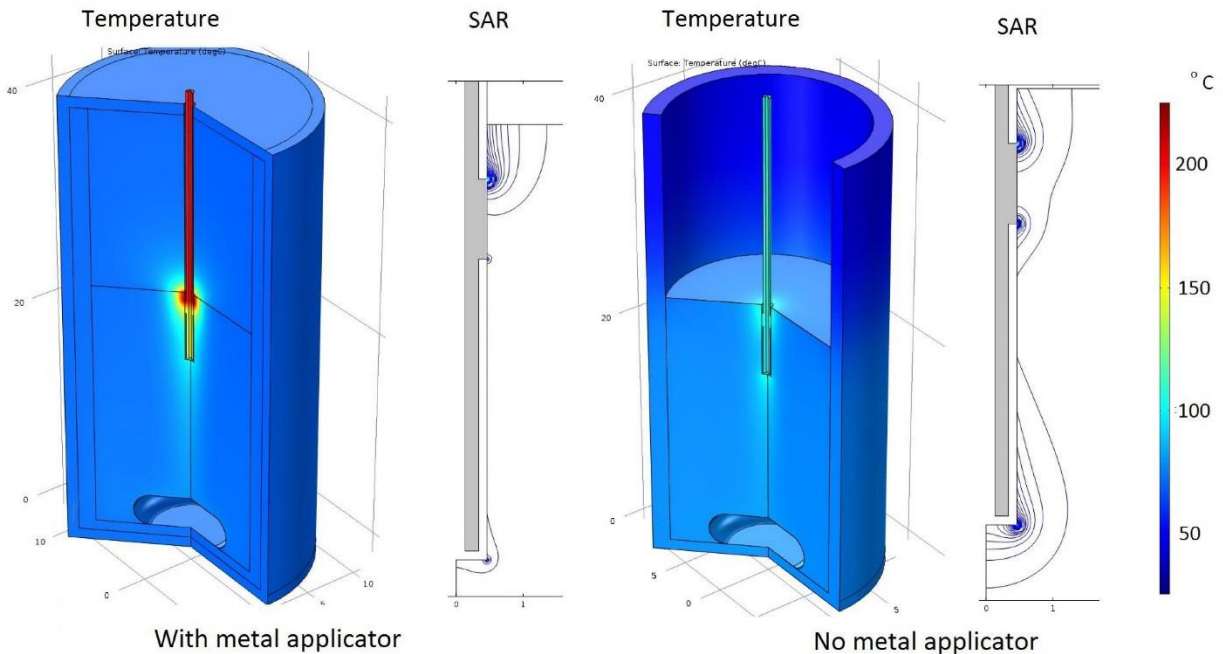


Fig. III. 19. The results obtained in COMSOL for temperature and SAR distribution in extraction cell with and without metal applicator (25 W microwave power, 350 rpm stirring rate, $10 \text{ W}/(\text{m}^2 \cdot \text{K})$ heat transfer coefficient) (Calinescu et al. 2017b).

In fig. III.20 is shown the results obtained by modeling the temperature distribution within the cell extraction contained in a metal applicator (as in the case of installation used and

presented in fig. III.2). It can be noticed that non-uniformity of heating is intensely influenced by the stirring rate. At a low stirring rate (350 rpm) the heating is quite non-uniform, while at a high stirring rate (800 rpm) the heating non-uniformity is reduced, even if the electric field and SAR non-uniformity are the same (Calinescu et al. 2017b).

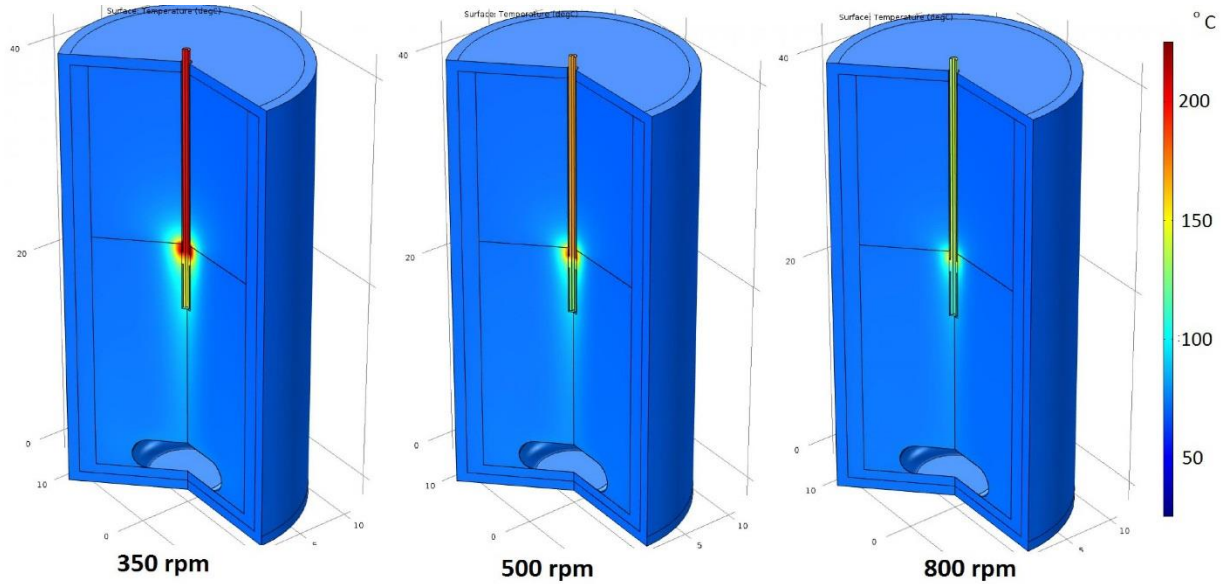


Fig. III. 10. The results obtained in COMSOL for temperature distribution in extraction cell surrounded by metal applicator for different stirring rates (25 W microwave power, $10 \text{ W}/(\text{m}^2 \cdot \text{K})$ heat transfer coefficient) (Calinescu et al. 2017b).

To verify the existence of a non-uniform heating, a series of experiments were performed using microwave power of 25 W and different stirring rates. The temperature was measured using two sensors with optical fiber mounted one close to the antenna and the other one near to the wall of the extraction vessel (fig. III.19).

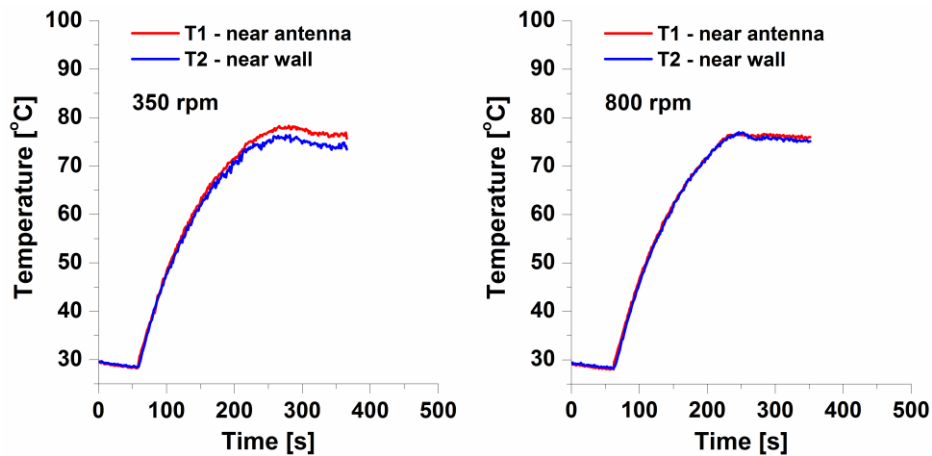


Fig. III. 11. Temperatures recorded by two temperature sensors in extraction cell (25 W microwave power) (Calinescu et al. 2017b).

The results recorded (shown in fig. III.21) confirm the existence of a temperature difference between the two measured areas. This temperature difference is not as high as that

determined by COMSOL modeling, mainly due to the difficulty of measuring the temperature in the very narrow space where modeling indicates a maximum temperature. In modeling, this area has a size of tens of micrometers, while the sensor size is 0.6 mm and his position is very difficult to fix even in the maximum temperature area. The introduction of the sensors into suspension changed significantly the flow pattern and, therefore, the temperature field, acting as secondary generators of turbulence, intensifying the mixing (Calinescu et al. 2017b).

III.5.2. Total phenolic content and antioxidant activity of the extracts

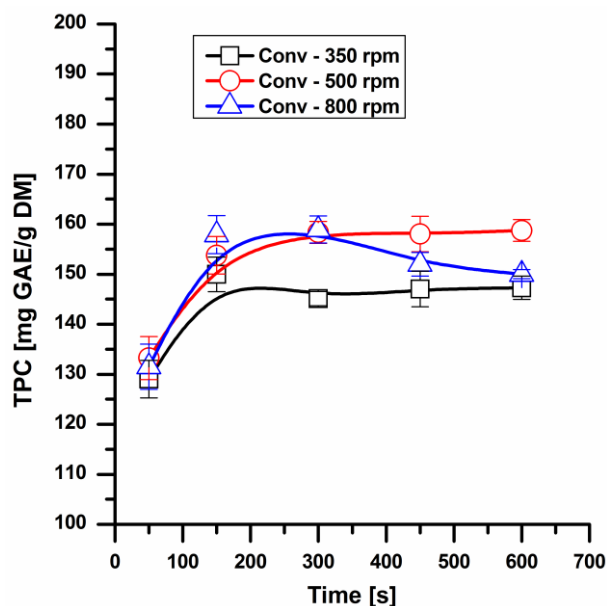


Fig. III. 12. Influence of stirring rate on the TPC for conventional extraction (Calinescu et al. 2017b).

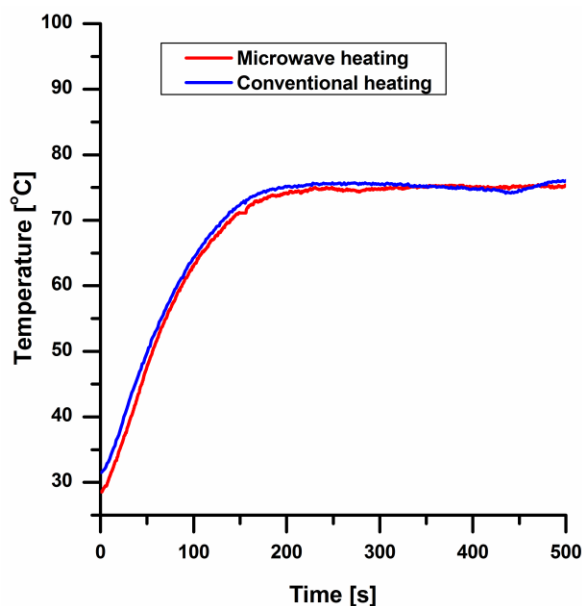


Fig. III. 13. Temperature profile for microwave and conventional heating, 500 rpm (Calinescu et al. 2017b).

For experiments at low stirring rates, sea buckthorn leaves finely grounded and sieved to a maximum size of 0.16 mm were used. For such samples the influence of the stirring rate for conventional extraction is not very important (see fig.III.22).

Conventional extractions were performed in the same extraction cell as in the case of MAE. The heating was performed by flowing hot water (80°C) through the surrounding jacket of the extraction cell. Average temperatures profiles recorded for the conventional and microwave assisted extractions were similar (see fig. III.23). In this case, a single sensor placed midway between the antenna and the cylindrical wall of the extraction vessel was used (Calinescu et al. 2017b).

A comparison between conventional extraction and microwave assisted extraction at 75°C and different stirring rates is presented in figs. III.24 and III.25. From the analysis of TPC results it can be noticed that the effect of microwaves is more important for lower stirring rates (due to non-uniform heating). The best values were obtained for 500 rpm and an extraction time of 150 s (Calinescu et al. 2017b).

Antioxidant capacity values of polyphenols are presented in fig. III.25. It can be seen the same favorable effect of non-uniform microwave heating. It confirms that the best results are obtained at 500 rpm and extraction time in the range of 150–300 s (Calinescu et al. 2017b).

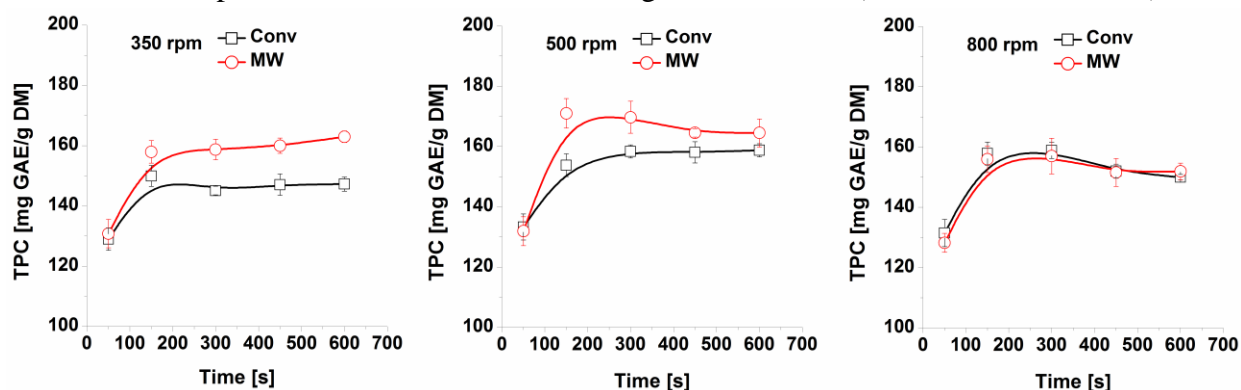


Fig. III. 14. TPC values for MAE and conventional extraction of polyphenols from sea buckthorn leaves at different stirring rates (Calinescu et al. 2017b).

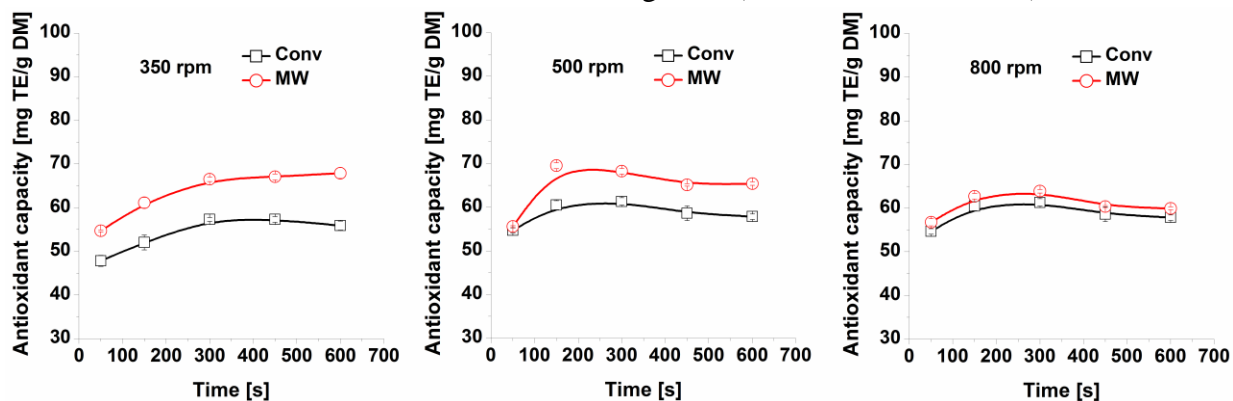


Fig. III. 15. Antioxidant capacity values for MAE and conventional extraction of polyphenols from sea buckthorn leaves at different stirring rates (Calinescu et al. 2017b).

III.5.3. Composition of sea buckthorn extracts

To confirm these results a qualitative analysis of the extracted polyphenols was performed. As shown in table III.6, MAE affects in a lesser extent the composition of the extracted polyphenols. Only the concentration of free gallic acid is significantly higher in the MAE. This can be explained by the hydrolysis of gallic acid glycosides, which is favored by microwave (Calinescu et al. 2017b).

Cumulative values for polyphenolic compounds concentration obtained by HPLC analysis are significantly lower than those obtained by direct determination of TPC. This is due to the existence of some complexes between glycosides and polyphenols, which cannot be determined by chromatographic analysis (Arimboor et al. 2008).

Table III.6. HPLC analysis of polyphenolic compounds for the experiments performed at 300 s (MW – microwave assisted extraction and CE – conventional extraction). Single HPLC analysis was performed. (Calinescu et al. 2017b).

Polyphenolic compound	Polyphenolic compound concentration [mg/g DM]	
	Heating type	
	MW	CE
Gallic acid	67.33	31.02
Catechin	22.66	24.49
Epicatechin	8.70	9.24
Epigallocatechin	18.48	26.21
<i>p</i> -coumaric acid	0.57	0.52
Ferulic acid	0.99	0.60
Rutin	5.68	5.90
Kaempferol-3- <i>O</i> -glycoside	5.07	5.16
Isorhamnetin-3- <i>O</i> -glycoside	3.77	3.80
Quercetin	2.91	2.90
Kaempferol	0.37	0.32
Isorhamnetin	0.81	0.78
Total	137.33	110.95

CHAPTER IV – ESSENTIAL OILS EXTRACTION FROM HERBS

IV.2. Materials and methods

The essential oils extraction was performed using different raw materials, such as: rosemary and thyme leaves and stems and ginger. Rosemary and thyme (fresh stems and leaves) were harvested at the beginning of October 2015 at Hofigal S.A. in Bucharest. The water content of rosemary and thyme was 65.2% and 71.5% respectively. Fresh ginger rhizomes were purchased from a local market in Bucharest. The experiments were performed using both shredded ginger rhizomes and pressed pulp resulted from mechanical pressing of ginger rhizomes. The initial water content of fresh ginger rhizome was approximately 90%. Applying a mechanical pressing to 100 g ginger rhizomes, results 75 g pressed pulp which has an initial water content of approximately 70%.

An innovative experimental installation (IEI-2) was specially designed and built for fresh plant materials treatment by microwave hydrodiffusion and gravity method (see fig. IV.1).

As shown in fig. IV.1, the innovation of IEI-2, compared with classical MHG equipment, consists of a stirring system (in order to achieve a uniform microwave irradiation and to avoid local degradation and overheating). The treatment of fresh plant leads to intracellular water and essential oil separation.

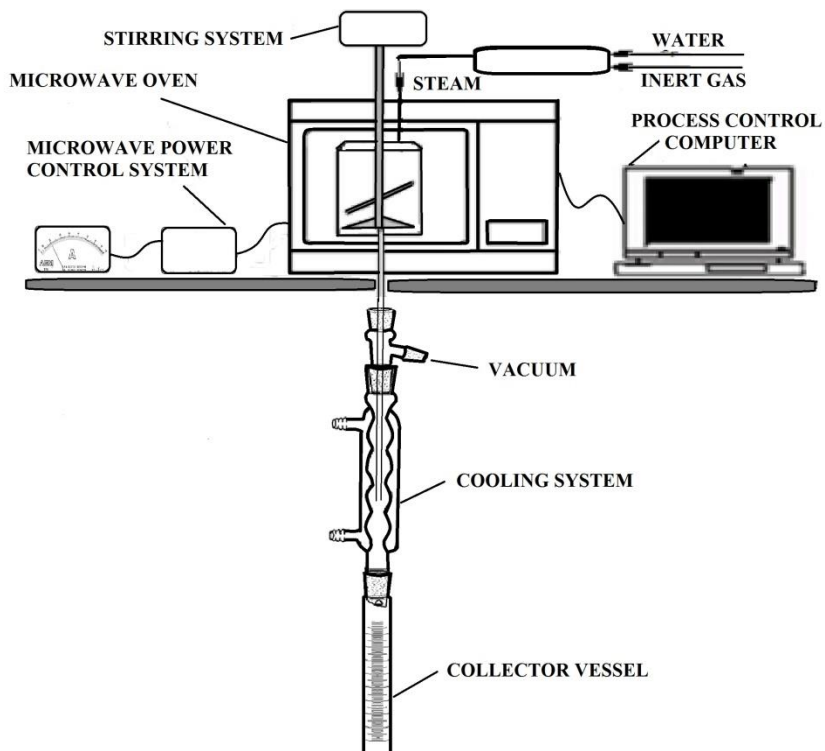


Fig. IV. 1. Innovative experimental installation – IEI-2

Comparative extraction of essential oil by microwave hydrodistillation, using a multimode microwave oven (Plazmatronika, Poland) equipped with a Clevenger type apparatus, and by conventional hydrodistillation, using a nest heater equipped with a Clevenger type apparatus.

The essential oil extraction from rosemary and thyme was performed using three methods: conventional hydro-distillation (CHD), microwave hydrodistillation (MHD), and microwave hydrodiffusion and gravity (MHG).

The extraction conditions have been chosen considering the principle that MAE is more efficient when the extraction time is shorter and the microwave specific power is higher (Li et al. 2013). Since IEI-2 equipment is provided with a stirring system, the extraction of essential oils can be performed at higher specific power which further led to better results.

Experiments at a constant power were performed (supplied power). For each extraction, the temperature was the same. Before extraction, in order to enhance the essential oil yield, distilled water was pulverized on the vegetable material.

The vegetable materials resulted from the MHG extraction of essential oils from rosemary and thyme was used to extract the polyphenols by MAE, using a monomode microwave system (Biotage Initiator). Comparative MAE of polyphenols without plants pretreatment was also performed. The experiments were carried out for the optimum extraction conditions presented in subchapter III.3.3.

The extraction of ginger essential oil was carried out by two methods: MHD and MHG. MHD was used as reference method to compare the amount of the essential oil resulted after the extraction by MHG. Due to low amounts of essential oil, its quantification from the obtained extraction mixture (water and EO) was performed by extraction with hexane.

The extractions were performed at constant power or by changing the power during the process. The maximum temperature was the same for all experiments. During the extraction temperature, microwave power and time were recorded using an operating console (software LabView).

Quantification and chemical composition determination of rosemary, thyme and ginger essential oils were performed by GS/MS analysis. TPC of rosemary and thyme leaves was determined by Folin-Ciocalteu method. Fresh ginger pulp and that resulted from MHG pretreatment were submitted to SEM analysis.

IV.3. Integrating microwave assisted extraction of essential oils and polyphenols from rosemary and thyme

IV.3.1. Rosemary and thyme essential oil extraction

a) Rosemary and thyme protocol treatment

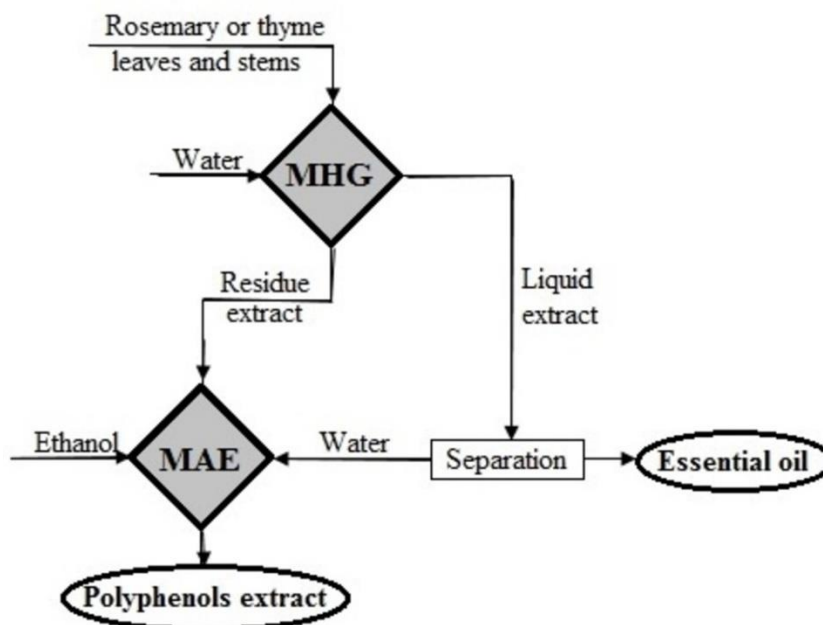


Fig. IV. 4. Protocol treatment of rosemary or thyme.

The residue sample resulted from MHG extraction of rosemary and thyme essential oil was further submitted to polyphenols extraction. The extraction was carried out with a solution

of 50% ethanol in water (residual water obtained after MHG process). The protocol treatment of rosemary and thyme is presented in fig. IV.4 (Calinescu et al. 2017a).

b) Determination of specific power, specific energy and essential oil quantity

The MHG experiments were compared with the classical extraction methods (CHD and MHD). During the experimental work, for the entire extraction interval, the temperature profile was recorded. The specific power and specific energy were also calculated.

The amount of each essential oil compound for all experiments was calculated using the equation:

$$m_{\text{compound}} = m_{\text{EO}} \times \frac{C_{\text{compound}}}{100} \times 1000 \quad (\text{IV.10})$$

Where: m_{compound} – the amount of each EO compound (mg), m_{EO} – is EO weight (g, see tables IV.3 and IV.4), C_{compound} – is concentration of each EO compound (% , see tables IV.6 and IV.7).

Further, using this equation, the amount of the compounds with boiling points below or higher than 200°C was calculated by summing up the amount of each corresponding compound.

c) Quantitative Results of EO from Rosemary and Thyme

The results obtained for EO extraction from rosemary and thyme leaves and stems are detailed in tables IV.3 and IV.4. The experimental conditions were chosen in order to improve the extraction of the bioactive compounds (sub-atmospheric pressure and addition of water). Thus, as shown in these tables, the amount of the EO for both plants is dependent on the type of extraction method: CHD, MHD, or MHG (approximately 200–300 mg EOs/100 g of plant for thyme and 700–800 mg EOs/100 g of plant for rosemary). The difference between these three methods consists in a shorter extraction time for MHG (approximately 10 min with a lower specific energy) compared with 105 min for MHD and 150 min for CHD (Calinescu et al. 2017a).

Table IV.3. Extraction conditions and results for thyme essential oil (Calinescu et al. 2017a).

Exp	Method	Added water [g]	Pressure [at]	Extraction time ^a [s]	EO [mg/100 g plant]	Specific power [W/g plant]	Specific energy [KJ/g UE]	Amount of compounds, [mg/100 g plant]	
								p.f < 200°C	p.f > 200°C
1	MHG	-	1	612	184±25	3.60	1.2	78.9	97.6
2	MHG	25.3	1	686	262±23	2.87	0.94	89.8	162.4
3	MHG	24.5	0.7	768	313±22	2.89	0.88	148.9	153.0
4	MHD	400	1	6300	299±17	0.43 ^b	4.5	129.0	156.8
5	CHD	400	1	9000	305±19	0.76 ^b	11.2	132.8	159.5

^a Extraction time means the time until the temperature of 106–108°C was achieved.

^b Specific powers for MHD and CHD (exp. 4 and 5) were calculated based on total mass weight: plant and water

As shown in tables IV.3 and IV.4, the addition of water and a slight decrease of pressure lead to a higher amount of EO for both plants. When distilled water was pulverized on the fresh plant material, the amount of the EO was higher than the extractions without the addition of

water. The water content of fresh plant materials (71.5% for thyme and 65.2% for rosemary) is not high enough to entrain all the EO constituents; especially those having relatively high boiling points (see tables IV.6 and IV.7). As expected, the water addition led to an increased amount of EO components, especially those with boiling points higher than 200°C (66% for thyme and 22% for rosemary). Reducing the pressure to 0.7 at favors an increased amount of the compounds with boiling points below 200°C (with 65% for thyme and 32% for rosemary) compared with the extraction at atmospheric pressure. The reduction of pressure favors the evaporation rate of the water inside the solid material, thus increasing the cell membrane degradation. Therefore, the EO compounds with lower molecular masses (higher mobility due to the internal energy) are extracted faster than the compounds with higher molecular masses. Consequently, for the same extraction time, the concentration of the former will be slightly higher (Calinescu et al. 2017a).

Table IV.4. Extraction conditions and results for rosemary essential oil (Calinescu et al. 2017a).

Exp	Method	Added water [g]	Pressure [at]	Extraction time ^a [s]	EO [mg/100 g plant]	Specific power [W/g plant]	Specific energy [KJ/g UE]	Amount of compounds, [mg/100 g plant]	
								p.f < 200°C	p.f > 200°C
6	MHG	-	1	579	691±32	3.60	0.3	273.6	347.3
7	MHG	23.8	1	518	762±31	2.90	0.24	270.6	423.5
8	MHG	23.4	0.7	781	825±37	2.91	0.34	357.8	398.2
9	MHD	400	1	6300	769±22	0.43 ^d	1.76	355.1	329.7
10	CHD	400	1	9000	837±24	0.76 ^d	4.08	371.8	372.9

^c Extraction time means the time until the temperature of 106–108°C was achieved.

^d Specific powers for MHD and CHD (exp. 9 and 10) were calculated based on total mass weight: plant and water

An advantage of this extraction equipment is the consumption of energy; the specific energy is lower compared with MHD and CHD methods. Although the specific power for MHG is few times higher than for classical methods, the specific energy is about 5–15 fold lower. This is owed to the extraction times which are smaller for MHG method (Calinescu et al. 2017a).

Table IV.5. Vapor pressure of water– α -pinene–thymol mixture at 95°C (total pressure = 1 at) and 85.7°C (total pressure = 0.7 at) (webbok.nist.gov).

Temperature [°C]	Total pressure [mmHg]	Vapor pressure of water, [mmHg]	Vapor pressure of α -pinene, [mmHg]	Vapor pressure of thymol, [mmHg]	Mole fraction of α -pinene	Mole fraction of thymol
95	760.3	633.6	121.2	5.5	0.1605	0.0073
85.7	533.8	444.9	85.5	3.4	0.1612	0.0064

Table IV.5 shows the calculated values of vapor pressure for a mixture of water, α -pinene (a more volatile constituent of EO) and thymol (a less volatile constituent of EO). The influence of added water and reduced pressure on the amount of thyme EO is higher than for rosemary EO

due to the smaller content of the EO found in thyme leaves (300 mg/100 g of plant for thyme compared with 800 mg/100 g of plant for rosemary) (Calinescu et al. 2017a).

d) Qualitative Results of EOs from Rosemary and Thyme

• *Thyme Essential Oil Results*

For all methods, around 30 compounds were identified by GC-MS analysis. The results are shown in table IV.6. The main constituents of thyme EO are thymol, γ -terpinene, and *p*-cymene. These compounds represent approximately 75% of the total amount of resulted EO. As shown in table IV.6, in terms of the EO composition, there is only a slight difference between the three extraction methods. However, the addition of water led to an increase of the percentage of the extracted compounds (heavier compounds especially, e.g., thymol). The composition of EO for the extraction at reduced pressure is similar with that obtained by classical methods (see exp. number 3) (Calinescu et al. 2017a).

Table IV.6. Chemical composition of thyme EO extracted by MHG, MHD, and CHD. Single GC analysis was performed (Calinescu et al. 2017a).

RT	Compound	CAS	Boiling point, [°C]	Composition, [%]				
				Exp.				
				1	2	3	4	5
9.23	α -Pinene	80-56-8	155	0.52	0.38	0.99	0.62	0.68
9.49	Camphene	79-92-5	159	0.30	0.31	0.58	0.46	0.64
9.78	Sabinene	3387-41-5	163	0.06	0.05	0.08	0.07	-
10.07	β -Pinene	127-91-3	165	1.73	1.42	2.15	2.04	2.08
10.38	α -Phellandrene	99-83-2	171	0.21	0.19	0.33	0.29	0.27
10.59	α -Terpinene	99-86-5	173	1.50	1.41	2.05	1.96	2.06
10.63	<i>p</i> -Cymene	99-87-6	177	13.90	8.62	15.48	13.98	14.37
10.81	Eucalyptol	470-82-6	176	2.43	1.04	2.61	1.72	1.18
11.25	γ -Terpinene	99-85-4	183	18.55	17.92	20.78	19.62	19.75
11.36	trans-Sabinene hydrate	17699-16-0	201	1.38	1.07	0.77	0.58	0.62
11.77	α -Terpinolene	586-62-9	187	2.14	1.73	1.66	1.74	1.82
11.85	Linalool	78-70-6	198	0.18	0.14	0.09	0.07	0.08
12.51	Camphor	76-22-2	204	0.70	0.09	1.28	1.47	0.94
12.93	Borneol	507-70-0	213	0.70	1.88	0.86	1.25	1.13
13.09	Terpinen-4-ol	562-74-3	209	0.23	0.23	0.22	0.31	0.36
13.24	α -Terpineol	98-55-5	219	0.37	0.21	0.32	0.29	0.21
13.43	Verbenone	1196-01-6	227	-	-	0.22	0.33	0.11
13.79	Thymol methyl ether	1076-56-8	214	0.12	0.30	0.12	0.87	0.34
13.95	Isothymol methyl ether	31574-44-4		-	0.13	-	0.43	0.50
14.50	Thymol	89-83-8	232	41.73	52.02	39.43	41.28	42.16
14.64	Carvacrol	499-75-2	236	2.66	2.79	2.43	2.25	2.26
15.36	Thymol acetate	528-79-0	241	0.21	0.08	0.14	0.10	0.08
16.75	β -(E)-	87-44-5	254	2.78	2.93	2.47	2.38	2.17

	Caryophyllene							
17.16	α -Curcumene	644-30-4	275	0.11	0.12	0.11	0.11	0.10
17.34	Germacrene D	23986-74-5	236	0.06	0.05	0.08	0.07	0.06
17.46	β -Curcumene	-	273	1.51	0.63	0.45	0.68	0.98
17.64	γ -Cadinene	39029-41-9	271	1.35	0.18	0.45	0.22	0.44
17.80	δ -Cadinene	523-47-7	279	0.44	0.19	0.16	0.21	0.40
17.86	Caryophyllene oxide	1139-30-6	279	0.01	0.08	0.12	0.08	-
18.56	γ -Eudesmol	1209-71-8	301	0.07	0.08	-	0.09	0.08
	Total main components			74.17	78.57	75.69	74.88	76.29

For experimental conditions, see table IV.3.

• *Rosemary Essential Oil Results*

Table IV.7. Chemical composition of rosemary EO extracted by MHG, MHD, and CHD. Single GC analysis was performed (Calinescu et al. 2017a).

RT	Compound	CAS	Boiling point, [°C]	Composition, [%]				
				Exp.				
				6	7	8	9	10
9.23	α -Pinene	80-56-8	155	5.56	3.96	9.01	9.45	8.29
9.49	Camphene	79-92-5	159	3.57	2.33	4.84	6.77	5.60
9.78	β -Pinene	127-91-3	165	2.47	2.79	2.65	2.33	2.47
9.96	α -Phellandrene	99-83-2	171	1.76	1.15	2.05	2.27	2.55
10.81	Eucalyptol	470-82-6	176	21.39	20.49	21.02	21.56	21.33
11.25	γ -Terpinene	99-85-4	183	1.03	0.86	0.79	0.82	1.11
11.36	trans-Sabinene hydrate	17699-16-0	201	0.20	0.15	0.15	0.13	0.18
11.66	α -Terpinolene	586-62-9	187	0.07	0.08	0.08	0.08	0.07
11.77	Linalool	78-70-6	198	3.55	3.71	2.79	2.77	2.81
12.51	Camphor	76-22-2	204	37.41	40.79	33.90	29.93	31.81
12.93	Borneol	507-70-0	213	1.87	2.28	1.53	2.00	1.61
13.09	Terpinen-4-ol	562-74-3	209	0.72	0.87	0.66	0.68	0.71
13.24	α -Terpineol	98-55-5	219	1.74	2.13	1.76	1.75	1.77
13.43	Verbenone	80-57-9	227	3.21	3.62	6.36	5.49	5.92
14.64	Bornyl acetate	76-49-3	228	1.25	1.04	0.68	0.79	0.90
15.92	α -Copaene	3856-25-5	246	0.16	0.20	0.09	0.10	0.13
16.15	Methyleugenol	93-15-2	254	0.20	0.25	0.18	0.11	0.09
16.75	β -(E)-Caryophyllene	87-44-5	254	2.65	3.06	2.21	1.28	1.13
17.15	Humulene	6753-98-6	166	0.48	0.59	0.42	0.26	0.23
17.64	γ -Cadinene	39029-41-9	271	0.04	0.05	-	0.16	-
17.80	δ -Cadinene	483-76-1	279	0.14	0.19	0.13	0.13	0.09
17.86	Caryophyllene oxide	1139-30-6	279	0.34	0.47	0.32	0.14	0.13
18.56	γ -Eudesmol	1209-71-8	301	0.04	0.06	0.04	0.06	0.04
	Total main constituents			71.15	71.18	75.13	73.19	72.96

For experimental conditions, see table IV.4.

For all methods, 23 components were identified by GC-MS analysis. The results are shown in table IV.7. The main components of rosemary EO are camphor, eucalyptol, α -pinene, verbenone, and camphene. These five constituents represent approximately 72% of the total amount of resulted EO. As shown in table IV.7, there is only a small difference between MHG, MHD, and CHD samples. These results show a similar behavior as in the case of thyme. The highest percentage of the main components was achieved for the extraction in the presence of water and low pressure (75.13%) (Calinescu et al. 2017a).

IV.3.2. Polyphenols extraction from MHG pretreated leaves

The second part of this study is dedicated to polyphenols extraction from plant material. Thus, the dried plant residue resulted from MHG extraction of EO is further used for polyphenols extraction by MAE. Besides the advantage of extracting EO in a very short time, the MHG can be considered a pretreatment method of vegetable materials.

In order to determine the efficiency of MHG pretreatment of rosemary and thyme leaves before polyphenols extraction, the MAE of untreated vegetable material was performed. The results are presented in tables IV.8 and IV.9. It can be noticed that rosemary and thyme leaves and stems contain a significant amount of polyphenols. The polyphenol concentrations of extract obtained from pretreated vegetal material are higher than those obtained from untreated plants (approximately 40% higher for thyme and approximately 36% for rosemary). The microwave pretreatment of vegetable material before MAE causes the degradation of the cell wall, thus releasing more easily the polyphenolic compounds (Calinescu et al. 2017a).

Table IV.8. Extraction conditions and TPC results for thyme (Calinescu et al. 2017a).

Exp.	Pretreatment method	Added water [g]	Pretreatment pressure [at]	Pretreatment time [s]	TPC [mgGAE/g DM]
1	MHG	-	1	612	38.28 \pm 0.6
2	MHG	25.3	1	686	36.21 \pm 0.0
3	MHG	24.5	0.7	768	32.36 \pm 0.0
5	No	-	1	-	23.12 \pm 0.9

Table IV.9. Extraction conditions and TPC results for rosemary (Calinescu et al. 2017a).

Exp.	Pretreatment method	Added water [g]	Pretreatment pressure [at]	Pretreatment time [s]	TPC [mgGAE/g DM]
6	MHG	-	1	579	55.5 \pm 2.0
7	MHG	23.8	1	518	43.39 \pm 0.5
8	MHG	23.4	0.7	781	48.43 \pm 6.1
5	No	-	1	-	35.29 \pm 1.1

Since the solubility of polyphenols in water is low, their concentration in the residual water resulted from MHG extraction of EO is small (approximately 1.2 mg GAE/g DM for thyme and only 0.04 mg GAE/g DM for rosemary). However, in order to increase the efficiency of polyphenols extraction, it is recommended to use as extraction solvent a solution of ethanol and residual water from MHG (Calinescu et al. 2017a).

IV.4. Ginger essential oil extraction by microwave hydrodiffusion and gravity method – energy considerations

IV.4.1. Determination of extraction efficiency and energy consumption

In order to highlight the advantage of the MHG process compared with MHD, several parameters were calculated (specific microwave energy supplied by the magnetron, specific microwave energy absorbed by the sample, total efficiency of EO obtained from pressed pulp ginger, and separation efficiency of the EO in distillate).

The actual absorbed microwave power, established by water calorimetry, was determined depending on the actual microwave power supplied by the magnetron, for different volumes of water. The equations used to calculate these parameters were the following:

$$E_{magn_sp} = \frac{E_{magn}}{V_{EO_d}} \quad (IV.12)$$

Where: E_{magn} – microwave energy supplied by the magnetron (kJ), E_{magn_sp} – specific microwave energy supplied by the magnetron related to the EO obtained from 100 g feedstock (kJ/mL), V_{EO_d} – EO volume in distillate obtained from 100 g feedstock (mL/100g).

$$E_{ab_sp} = \frac{E_{ab}}{V_{EO_d}} \quad (IV.14)$$

Where: E_{ab} – microwave energy absorbed by the sample (kJ), E_{ab_sp} – specific microwave energy absorbed by the sample related to the EO obtained from 100 g feedstock (kJ/mL).

$$Ef_1 = \frac{EO_{MHG_d} + EO_{MHG_p}}{EO_{MHD}} \quad (IV.15)$$

$$Ef_2 = \frac{EO_{MHG_d}}{EO_{MHG_d} + EO_{MHG_p}} \quad (IV.16)$$

Where: Ef_1 – total extraction efficiency of the EO obtained from pressed pulp ginger (%), EO_{MHG_d} – EO volume in distillate obtained by MHG (mL), EO_{MHG_p} – EO volume remained in the ginger treated pulp (mL), EO_{MHD} – EO volume obtained by MHD method (mL), Ef_2 – separation efficiency of the EO in distillate (%).

a) Extraction of the EO from ginger by MHD

The total amount of the EO found in shredded ginger rhizomes or pressed pulp was determined by MHD extraction (see table IV. 10). This approach was used as reference method to compare the amount of the EO resulted after MHG extraction. Thus, the extraction efficiencies for the total amount of the EO from shredded ginger rhizomes or pressed pulp was assumed to be 100%.

Table IV.10. Experimental conditions and results for MHD extraction of EO from shredded and pressed pulp ginger. Single GC analysis was performed.

Exp.	Ginger type	P* [W]	SAR** [W/kg]	E _{magn_sp} [kJ/mL UE]	E _{ab_sp} [kJ/mL UE]	V _{EO_d} [mL/100g]
A.	Pressed pulp	380	0.8-0.5 x10 ³	2284	2284	0.599
B.	Shredded ginger	380	0.5-0.3 x10 ³	6080	6080	0.225

*P – supplied microwave power during the extraction;

**SAR – specific absorption rate related to feedstock

b) *Extraction of the EO from ginger by MHG*

In this part of the study, the influence of SAR and the addition of saturated steam into the system on the extraction efficiency of ginger EO by MHG were followed. The experimental conditions and results are shown in table IV.11. The evolution of SAR values are shown in fig. IV.5.

Table IV.11. Experimental conditions and results for MHG extraction of EO from shredded and pressed pulp ginger. Single GC analysis was performed.

Exp.	Ginger type	P [W]	SAR $\times 10^3$ [W/kg]	E _{magn_sp} [kJ/mL UE]	E _{ab_sp} [kJ/mL UE]	V _{EO_d} ^{***} [mL/100g]	V _{EO_p} [mL/100g]
1.	Shredded ginger	678	3.41-5.5	2487.5	1216.7	0.21	0.054
2.	Shredded ginger	678-366	3.46-3.94	2421.3	1059.9	0.191	0.103
3.	Pressed pulp	460	2.3-3.5	1268	923.1	0.447	0.283
4.	Pressed pulp	432-205	2.16-1.45	650.4	494.2	0.64	0.394
5.	Pressed pulp	615-423	3.07-2.55	1370	904.1	0.376	0.521
6.	Pressed pulp	460	2.3-3.2	960.3	759.9	0.619	0.417
7.	Pressed pulp	435-209	2.34-3.29	699.5	533.6	0.648	0.359
8.	Pressed pulp	615-441	3.07-3.42	1064.5	826.4	0.504	0.291

***V_{EO_p} – EO volume remained in the treated ginger related to 100 g feedstock.

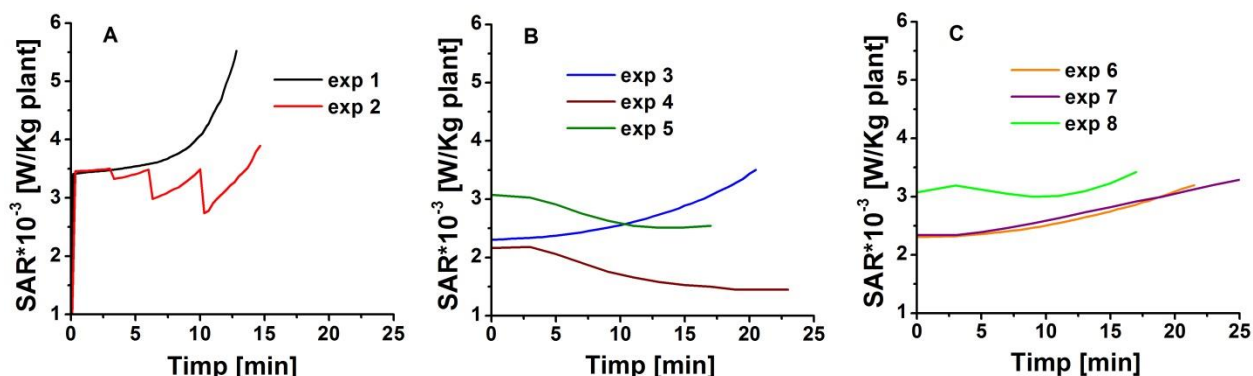


Fig. IV.5. SAR evolution during MHG (A – experiments with shredded ginger; B – experiments with pressed pulp without saturated steam; C – experiments with pressed pulp and addition of saturated steam into the system).

The change of magnetron power during the extraction process is determined by a dramatic decrease of the mass sample (from 200 g to approximately 60 g for pressed pulp). This decrease in mass sample causes a significant rise of SAR when the microwave power is maintained constant (see fig. IV.5 exp. 1). Analyzing these data reveal that the experiments performed at variable SAR with limited values lead to better results in terms of the efficiency of EO extraction (see fig. IV.6).

The total efficiency of the EO obtained from pressed pulp ginger and separation efficiency of the EO in distillate determined for all the experiments are shown in fig. IV.6. For the best extraction conditions (exp. 4 and 7), the energy consumption is approximately 3 times lower than the classical extraction by MHD or than the experiments performed by MHG at constant power.

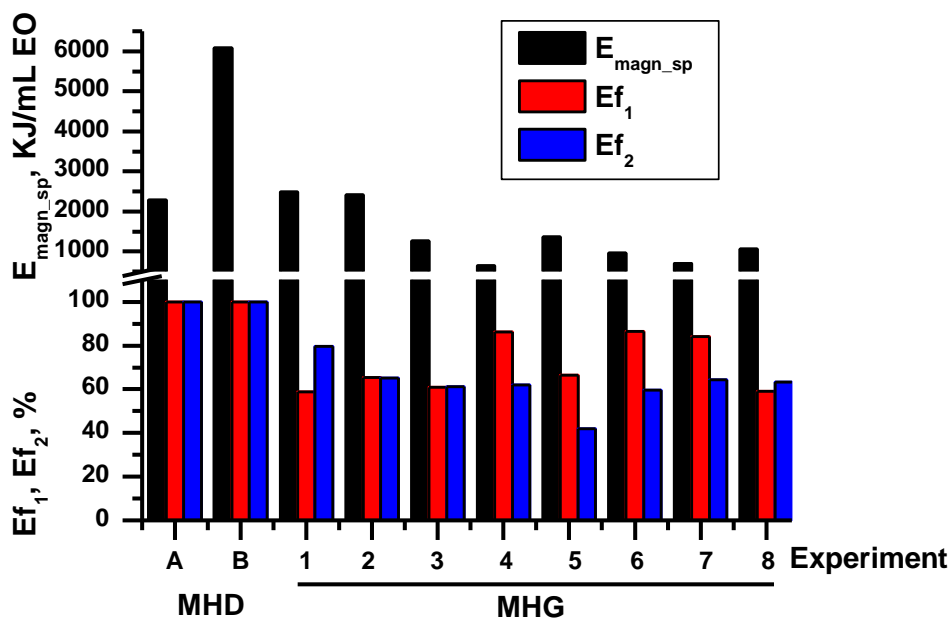


Fig. IV.6. Extraction efficiencies and microwave specific energy for MHG compared with MHD
IV.4.2. SEM analysis of ginger pulp

Fig. IV.7 shows the SEM images of untreated ginger pulp and pulp after MHG treatment. The essential oil is biosynthesized in specialized cells, such as glandular trichomes, osmophores, or ducts and cavities (Rehman et al. 2016). As shown in fig. IV.7A the essential oil can be found in those small spherical vacuoles contained by the glandular trichome cells. After MHG treatment the vacuoles are no longer presented (see fig. IV.7B).

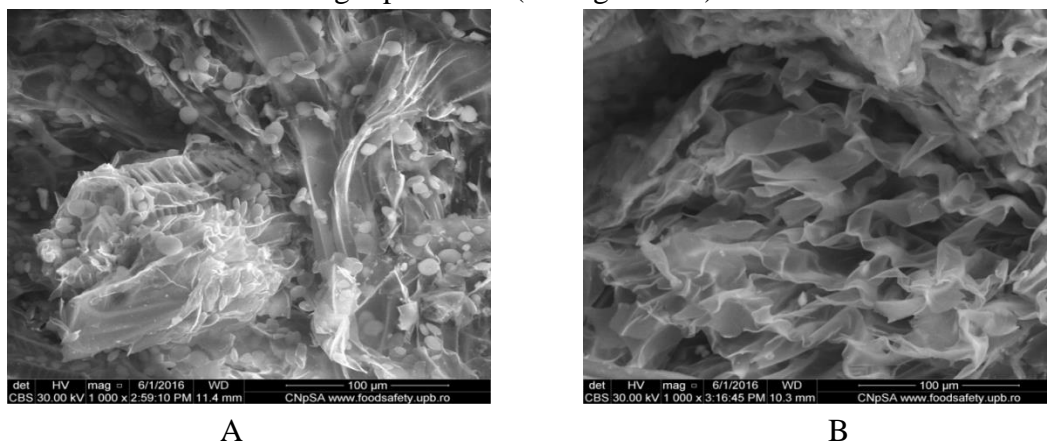


Fig. IV.7. SEM images of ginger pulp (A – untreated pulp, B – after MHG treatment)

GENERAL CONCLUSIONS

The aim of this research was to develop new strategies for an efficient extraction of active principles from plants (polyphenolic compounds and essential oils).

The main objectives of this study were the following:

- Microwave assisted extraction of polyphenols from sea buckthorn leaves,
- Microwave assisted extraction of essential oils from aromatic herbs (rosemary, thyme and ginger).

Thus, considering the developed strategies and the experimental data obtained, it was concluded that:

- The optimum parameters of batch extraction of polyphenols from sea buckthorn leaves were: a stirring rate of 900 rpm, a concentration of ethanol in water of 50%, an extraction time of 450 s and a temperature of 90°C, for MAE, using the modified reactor which allowed an efficient stirring of the extraction mixture.
- The semicontinuous extraction of polyphenols from sea buckthorn leaves was also more efficient in the case of MAE and the optimum parameters were: a stirring rate of 900 rpm, a solvent flow rate of 6 mL/min with a residence time of 50 s and the solvent pre-heating, using the modified reactor which allowed an efficient stirring of the extraction mixture.
- The extraction process has been intensified by the combined use of a coaxial antenna microwave applicator and an efficient cooling and stirring systems; thus, a high specific absorption rate was achieved, maintaining in the same time the average temperature at low values; in addition, the uneven heating that occurred in such equipment, allowed the efficient extraction of polyphenols in the area around the antenna (where the microwave field was very intense) and protected them against thermal degradation in the area where the microwave field was very weak.
- The extraction process of rosemary and thyme essential oils has been intensified with the help of innovative equipment which is based on the microwave hydrodiffusion and gravity principle; this equipment consists of an efficient stirring system which allowed a uniform irradiation of vegetal material.
- The yield of rosemary and thyme essential oil has been increased by pulverizing the vegetal material with water and working at sub-atmospheric pressure.
- The residue obtained from the extraction of rosemary and thyme essential oil was used further to extract polyphenols; thus MHG can be considered an efficient strategy of vegetal material pretreatment.
- MHG extraction of ginger essential oil was more efficient when the magnetron power was modified during the experiment (thus avoiding excessive SAR values) and when saturated steam was introduced into the system to entrain the vapors rapidly (thus avoiding losses outside the system).

- Considering the shorter extraction time and the lowest energy consumption, the extraction efficiencies for ginger essential oil by MHG were good enough compared with classical methods.

PERSPECTIVES

Part of the doctoral thesis has been done within PN-II-PT-PCCA-2013 no. 172/2014, “Green extraction processes of valuable compounds from herbs” – ECOVALUEPLANT project with intern no. UPB CH391406/2014. The partners of this project, SC HOFIGAL SA, provided the raw materials and, INFLPR Măgurele, built and designed the extraction equipment (IEI-1 and IEI-2 presented in chapters III and IV).

Considering the latter, the perspectives of this thesis refer to scaling up the equipment and to use at industrial scale the processes developed with the project partners.

ORIGINAL CONTRIBUTION

The elements of originality that are distinguished by this research are:

- The significant impact of efficient extraction medium stirring by changing the reactor geometry,
- Establishing the optimum temperature value for batch extraction process, regarding the energy consumption,
- Performing, for the first time, a semicontinuous extraction of polyphenols from sea buckthorn leaves and establishing the influence of different parameters on the extraction in order to achieve a high concentration in fewer collected fractions,
- The use, for the first time, of a coaxial antenna to extract polyphenols from sea buckthorn leaves and intensifying the extraction process by the combined use of the coaxial antenna and an efficient cooling and stirring system,
- The use of an innovative equipment, based on the microwave hydrodiffusion and gravity principle, provided with an efficient stirring system which allows a uniform irradiation of raw material,
- Developing an integrated process of rosemary and thyme essential oil extraction by MHG simultaneously with the raw material pretreatment, before polyphenols extraction, in order to enhance the extraction yield,
- Establishing the energy considerations of ginger essential oil extraction by MHG compared with classical methods.

RESULTS DISSEMINATION

Published papers

1. **I. Asofiei**, I. Calinescu, A. Trifan, I. G. David, A. I. Gavrilă, 2016, Microwave Assisted Batch Extraction of Polyphenols from Sea Buckthorn Leaves, *Chemical Engineering Communications*, 203 (12), 1547-1553. (IF – 1.29) – ISI
2. I. Calinescu, V. Lavric, **I. Asofiei**, A. I. Gavrilă, A. Trifan, D. Ighigeanu, D. Martin, C. Matei, 2017, Microwave Assisted Extraction of Polyphenols using a coaxial antenna and a cooling system, *Chemical Engineering & Processing: Process Intensification*, <http://dx.doi.org/10.1016/j.cep.2017.02.003>. (IF – 2.23) – ISI
3. I. Calinescu, **I. Asofiei**, A. I. Gavrilă, A. Trifan, D. Ighigeanu, D. Martin, C. Matei, M. Buleandra, 2017, Integrating microwave assisted extraction of essential oils and polyphenols from rosemary and thyme leaves, *Chemical Engineering Communications*, 204 (8), 965-973. (IF – 1.29) – ISI
4. **I. Asofiei**, I. Calinescu, A. I. Gavrilă, D. Ighigeanu, D. Martin, C. Matei, 2017, Microwave hydrodiffusion and gravity, a green method for the essential oil extraction from ginger – energy considerations, *Buletinul UPB*, paper accepted - ISI
5. A. I. Gavrilă, **I. Asofiei**, A. Trifan, P. Chipurici, E. Rusen, 2017, Microwave assisted synthesis of 5-hydroxymethylfurfural using mild reaction conditions, *Revista de Chimie*, 68 (3), 435-438. (IF – 1.23) – ISI
6. A. I. Gavrilă, **I. Asofiei**, P. Chipurici, 2017, Factorial Design for Optimization of Microwave Assisted Synthesis of 5-Hydroxymethylfurfural, *Revista de Chimie*, 68 (4), 639-641. (IF – 1.23) – ISI

International conferences

1. **AMPERE 2015**, Krakow, Poland - I. Calinescu, A. M. Popescu, **I. Asofiei**, A. Gavrilă, A. Trifan, D. Ighigeanu, D. Martin, C. Matei, Microwave Assisted Extraction of Polyphenols Using an Open-end Coaxial Applicator and a Cooling System.
2. **RICCCE 2015**, Sibiu, Romania - **I. Asofiei**, I. Calinescu, A. Trifan, A.I. Gavrilă, D. Ighigeanu, D. Martin, Microwave Assisted Extraction of Polyphenols from Sea Buckthorn Leaves.
3. **RICCCE 2015**, Sibiu, Romania - I. Calinescu, **I. Asofiei**, A. Trifan, A.I. Gavrilă, D. Ighigeanu, D. Martin, A Semi-Continuous Process for Polyphenols Extraction from Sea Buckthorn Leaves.
4. **IUPAC 2016**, Venice, Italy - I. Calinescu, V.Lavric, **I. Asofiei**, A.I. Gavrilă, D. Ighigeanu, D. Martin, C. Matei, Microwave Assisted Extraction Using an Open-end Coaxial Antenna and a Cooling System.
5. **IUPAC 2016**, Venice, Italy - D. Ighigeanu, I. Calinescu, **I. Asofiei**, A.I. Gavrilă, D. Martin, C. Matei, Extraction of Essential Oil from Ginger Using Microwave Hydro-diffusion and Gravity Method – Energy Considerations.

6. RICCCE 2017, Poiana Braşov, Romania - **I. Asofiei**, I. Calinescu, A.I. Gavrila, D. Ighigeanu, D. Martin, Microwave pretreatment of plant materials to improve the maceration process.

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