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Modeling of Microwave Extraction of Total Phenols and Antioxidant Activity of Rosemary (*Rosmarinus Officinalis L.*)

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Abstract: The aim of this work was to optimize the parameters of microwave extraction and to find the most suitable conditions for the application of extraction for Rosemary (*Rosmarinus officinalis L.*). The efficiency of the optimized method was compared with conventional solvent extraction. The results also shed light on the influence of geographical location, the micro-location and plant habitat for seven rosemary samples. Spectrophotometric methods (Folin-Ciocalte for total phenols and FRAP for antioxidant activity) were used to characterize the rosemary extracts. The data analysis showed a strong dependence of the total phenolic content on the specific micro-location. Proximity to the sea and the exposure to sun play a key role in antioxidant activity (sample Makarska 1858.4 μM Fe^{2+}) and total phenolic content (sample Milna 947.8 mg GAE L^{-1}). This is not surprising as the name rosemary (Rosmarinus) is derived from the Latin words *ros* (dew) and *marinus* (sea born).

Keywords: rosemary, microwave extraction, phenols, antioxidant activity.

INTRODUCTION

THE ROSEMARY (*Rosmarinus officinalis L.*) is a dense, evergreen, hardy, perennial aromatic herb that originates from the Mediterranean region. It is known for its characteristic needle-like leaves and strong scent and is an extremely rich source of phenolic compounds responsible for its high bioactivity. Rosemary is an extremely rich source of phenolic compounds, which are responsible for its high bioactivity. In recent years, this plant has been extensively studied as a source of natural bioactive compounds due to its strong antioxidant and anti-inflammatory effects, antibacterial and antimutagenic properties and chemopreventive action. Insufficient intake of antioxidants has consequences for the human body. The phenomenon of the so-called "Oxygen Paradox" explains the danger posed by reactive oxygen species to living organisms.

In Dalmatia, which has long been rich in autochthonous medicinal plants, rosemary is widespread as a cultivated or wild plant. Rosemary has a long history of use in Dalmatia, which is known for its sunny days and mild climate that favours the growth of this plant. Rosemary is often used in cooking because of its rich taste and smell. Its health benefits have great potential for the cosmetics and pharmaceutical industries. In recent years, this plant has been extensively studied as a source of natural bioactive compounds. Potent antioxidant, antiviral, antifungal, anti-inflammatory and antiproliferative effects on cancer cells have been pharmacologically confirmed in several studies on antibacterial and antimutagenic properties.[1–7]

A review of the literature also revealed and confirmed the ability to produce thrombin time, antidepressant/anxiolytic properties and anticholinesterase activity[8–11] while consumption of rosemary water or inhalation of the scent of rosemary essential oil has positive cognitive effects.[12] Rosemary extracts have been used since ancient times to treat diseases due to their hepatoprotective potential, their therapeutic potential in Alzheimer's disease and their anti-angiogenic effects.[13] On the other hand, they are also used to preserve food, as they protect against oxidation and microbial contamination. However, the most important property of rosemary

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is its antioxidant property, which plays an important role in the formation of free oxygen radicals (ROS) and accordingly reduces the oxidative stress of cells. Rosemary essential oil has been studied in many different region.[14] The main constituents 1,8-cineole (~ 25 %), camphor (~ 25 %), α-pinene (~ 20 %) and verbenol are phenolic diterpenes, which are responsible for the antioxidant properties of rosemary. Other important constituents of rosemary essential oil that can be obtained by distillation are rosmarinic acid (one of the greatest natural antioxidants), borneol, bornyl acetate, p-cymene, geraniol, carnosic acid, carnosol, rosmanol, epirosmanol, geraniol, but many constituents are slightly soluble in water (0.3 – 3.5 mg mL^{-1}). Rosmanol, carnosic acid are very slightly soluble in water < 0.1 mg mL^{-1}.[13]

The European Food Safety Authority (EFSA, Parma, Italy) has also approved rosemary extract as a food additive (E392). The safety assessment for the use of rosemary extracts as a food supplement is 100 – 300 mg kg^{-1} body weight per day for infants and 200 – 600 mg kg^{-1} body weight per day for adults.[15,16]

Implementing new extraction methods with greater utilization of energy and raw materials and the use of "green" solvents make a significant contribution to environmental protection and energy efficiency. Water as the main solvent in combination with microwaves offers numerous advantages to achieve these goals. Today, economic aspects determine global development in all areas of technology and science. In this sense, the optimization of all aspects of production and constant improvement of production efficiency is the focus of numerous research projects. The high demand for certain plant species requires an increased commitment to improving existing methods and optimizing extraction conditions for bioactive species and minimising waste must also be a key factor in achieving high efficiency.

Time-consuming traditional methods have become economically unviable. Modern extraction methods overcome all economic and environmental disadvantages, such as short time, low solvent volume, use of environmentally friendly solvents and less waste. Assisted extractions are faster, more efficient, allow working at higher temperatures and pressures and are far ahead of traditional methods in terms of safety. These include ultrasound, microwaves, supercritical fluid extraction and accelerated solvent extraction, which have been researched for two decades. When reviewing the literature for rosemary, different extraction methods were compared and optimized.[14,17,18]

From the ecological aspect[19] aim of this work was optimization of the microwave extraction parameters for extraction of rosemary. Characterization of the obtained extracts was performed by spectrophotometric methods: concentration of the total phenols by Folin-Ciocalte and FRAP method for antioxidant activity. Based on the obtained results from 7 different locations in Dalmatia, influence of the geographical location, micro-location and habitat of the plant on the mentioned properties, were discussed.

EXPERIMENTAL

Reagents and Solutions

All solutions were prepared from analytical grade chemicals by dissolving the appropriate mass of pure substance in distilled or ultrapure water. The Folin-Ciocalte reagent from VWR Chemicals and gallic acid and TPTZ (2,4,6-tripiridil-s-triazin) reagent from Thermo Scientific Chemicals were freshly prepared before measurement due to their instability.

Optimisation of Microwave Extraction (MWE) Parameters

The Milestone flexiWAVE device was used for microwave extraction (MWE). The device is equipped with two 950 W magnetrons, which makes a total power of 1900 W and ranks it among the most powerful devices currently available for MWE and synthesis. Modelling the MWE and designing the experiment is certainly the most difficult part of any analysis. Since the MWE is influenced by many parameters and these parameters are closely interrelated, it is necessary to apply a strategy of statistical optimization of the working conditions. Several examples of different strategies for optimising MWE parameters can be found in the literature. As a result of a series of experiments and statistical processing of factors, optimal results can be obtained using appropriate software. Some of the statistical programmes used for optimization purposes are STATGRAHICS, MINITAB, STATGRAPHICS PLUS and DOE software.[17,20,21]

Solvent – The volume of the solvent must be enough to ensure completely immersed plant matrix in the solvent during extraction. The volume ratio of solvent to solid matrix can vary greatly and can be between 1 : 10 and 1 : 100 (g dry substance / mL solvent). There is no exclusive rule, but this is a parameter that must be optimized for each type of analytes. According to a review of the literature, water as "green and eco-friendly" solvent is the optimal choice as a solvent, since the extracts obtained are to be used as additives in the pharmaceutical industry, in the food industry and as food supplements for skin care, but also to evaluate their content in daily consumption by cooking food or tea.[19,21] Even when dried, tiny microscopic traces of moisture remain in the plant cells, which are the "target" of microwave heating. Under the influence of the

microwaves, the water inside the cell creates pressure on the cell walls, causing them to stretch and eventually tear. The solvent can penetrate through the pores of the cell and wash out bioactive substances from the matrix. Due to the higher temperature achieved by the microwave radiation, the ether bonds in the cellulose, which is main component of the cell walls, can be hydrolyzed within 1 to 2 minutes and converted into soluble fractions. At the same time, the mechanical strength decreases and makes it easier for the solvent to access the compounds inside the cell. Preferably, the solvent should have a high selectivity towards the analytes of interest. To improve and optimise the properties, solvents are often mixed to achieve ideal properties: conventional solvents, water, ethanol, methanol, hexane, etc. The effect of microwaves strongly depends on the dielectric constant of the solvent, on the solid plant matrix and the solvent ability to absorb microwave energy. It is also important to know properties of the bioactive species we are targeting, thermostability and solubility.[22–25]

The ability of the solvent to absorb microwave energy and convert it into heat depends on the dissipation factor (tan δ) given by Kingston and Jassie.[26] The dissipation factor is a measure of the amount of heat accumulated in a solvent or matrix:

$$\tan\delta = \varepsilon'' / \varepsilon' \quad (1)$$

where ε' is the dielectric constant of the solvent, which expresses the ability of the molecule to be polarized by an electric field, and ε'' is the dielectric loss factor, which expresses the efficiency of the transformation of electromagnetic energy into heat. Polar solvents such as water have high dielectric losses (9.889), good microwave absorption properties (ε' = 80.4) but they also exhibit significant energy dissipation due to these losses. Ethanol is a relatively good absorber of microwave energy (ε' = 25.7), but it is not an ideal solvent for extraction purposes. Hexane as a non-polar solvent, for example, has a very low absorption capacity for microwaves; we say that it is "transparent" to microwaves, low loss (0.038), good extraction properties, but it is not a good absorber of microwave energy (ε' = 2.0).

Extraction time – With increasing extraction time, the amount of extracted bioactive substances increases up to the solubility threshold, but with the risk of degradation. Solvents such as water, ethanol and methanol can become very hot during prolonged exposure, which affects the stability of the ingredients. The extraction time can also depend on the type and size of the matrix.[19,27]

Microwave power – The combination of low or medium microwave power with longer extraction time is the optimal choice when dealing with compounds that are susceptible to degradation. High power with longer extraction time always carries the risk of thermal degradation. In general, the efficiency of extraction is improved by increasing the microwave power. The power of a conventional household microwave oven is lower (30 – 270 W) than that of modern microwave reactors (30 – 1250 W). Apart from the short exposure time, the power used also depends on the solvent itself, not only on the plant species. In the sensitive parts of the plant (petals or whole flower, the cell wall ruptures rapidly at higher temperatures, causing undesirable components to end up in the extract along with desirable ones. At low power, the rupture of the cell wall is gradual, allowing selective extraction.[19,27]

Particle sizes – Generally they are in the range of 100 µm to 5 mm. A fine powder can improve extraction due to a larger surface area that allows better contact between the plant matrix and the solvent. Finer particles allow more efficient microwave action, i.e. deeper penetration. On the other hand, ground samples yield cloudy extracts after extraction. Separation of the fine powdered matrix from the solvent after microwave irradiation is difficult, the extracts are not suitable for spectrophotometric analyses, and any further purification of the extract may influence the results.[19]

Temperature – Temperature and power are interrelated and require special attention. In a closed system, the temperature can reach values far above the boiling point of the solvent. These higher temperature, actually leads to improved extraction efficiency as desorption of analytes from the active sites in the matrix increases, sample wetting is improved and the solvent penetrates the matrix. In closed systems, this is associated with an increase in pressure, which can shorten extraction time but increase operational risk. A higher temperature can also affect and reduce the dipole character of the solvent, so that supercritical water (water at very high temperature) has the properties of a non-polar solvent.[27,28]

Spectrophotometric Measurement

The content of the total phenols and their antioxidant activity were determined with an Agilent Cary 4000 UV-VIS spectrophotometer.

Determination of total phenols – Total phenols are determined by the Folin-Ciocalte spectrophotometric method. This method is based on the oxidation of phenolic groups by the addition of Folin-Ciocalte reagent which form coloured product. Staining intensity is measured by determining the absorbance at 765 nm relative to the blank.[29]

Using the calibration obtained curve, from the concentration–absorbance ratio, the total phenols in the extracts are estimated in relation to the gallic acid standard, according to the equation:

$$A = 9.027 \times 10^{-4} \times Y + 0.068 \quad (2)$$

where Y represents the mass concentration in mg L^{-1} of the

extract, and the result is expressed as the equivalent, mg GAE L^{-1}. After 2 hours, real sample absorbance at 765 nm is measured. Samples were measured in triplicates.

Determination of antioxidant activity by FRAP method – FRAP (Ferric reducing / antioxidant power) is a simple, one of the most widely used methods to measure the antioxidant activity (reducing power) of the samples, based on the ability of the sample to reduce Fe^{3+} to Fe^{2+} ions. At low pH, in the presence of TPTZ, ferric-tripyridyltriazine (Fe^{3+}–TPTZ) complex is reduced to the intense blue ferrous-tripyridyltriazine (Fe^{2+}–TPTZ). The results of reducing activity of the samples were monitored at 593 nm at different time intervals (4 and 10 minute) and expressed in μM of Trolox equivalents (μM TE g^{-1} of sample).[29] Absorbance at 593 is continuously monitored over time and recorded after 4 and after 10 minutes. Using the calibration obtained curve, from the concentration-absorbance ratio, the antioxidant activity in the extracts is estimated according to the equation:

$$A = 0.993657 \times c + 0.00203 \quad (3)$$

where c represents the concentration of antioxidants in the extract, and the result is expressed as the equivalent, μM Fe^{2+}. Samples were measured in triplicates.

Preparation and Characterization of Real Samples

A total of 7 samples were collected from different locations in Dalmatia: Makarska, Zadar, Sinj, Milna (Island of Brač), Imotski, Split (Campus) and Kaštel Kambelovac. The specific microclimatic features and growing conditions for rosemary are provided in Table 1 (Data taken from the Croatian Meteorological and Hydrological Service).

All collected and analyzed rosemary samples belonging to the same species, *Rosmarinus officinalis L.*, were harvested in winter and dried in a shaded, closed room at a temperature of about 20 °C. The phenolic content and antioxidant activity were determined from the leaves. The dried rosemary was crushed before extraction. Real samples were prepared by extracting 1 g of plant material, rosemary, in 200 mL of redistilled water. The samples prepared in this way were transferred to a flask for MWE. The extraction in a closed microreactor with water cooling is pre-optimised. The loss of solvent is prevented. To compare the results, the antioxidant activity and total phenols were determined by conventional heating of 1 g of the sample in 200 mL of water at 100 °C without the use of microwaves for 5 minutes (similar to the preparation of tea or short-term boiling). The selected extraction parameters include a preheating time of 2 minutes, a microwave power of 600 W and extraction times of 2, 5 and 10 minutes.

RESULTS AND DISCUSSION

Results of Optimization of the Microwave Extraction Parameters

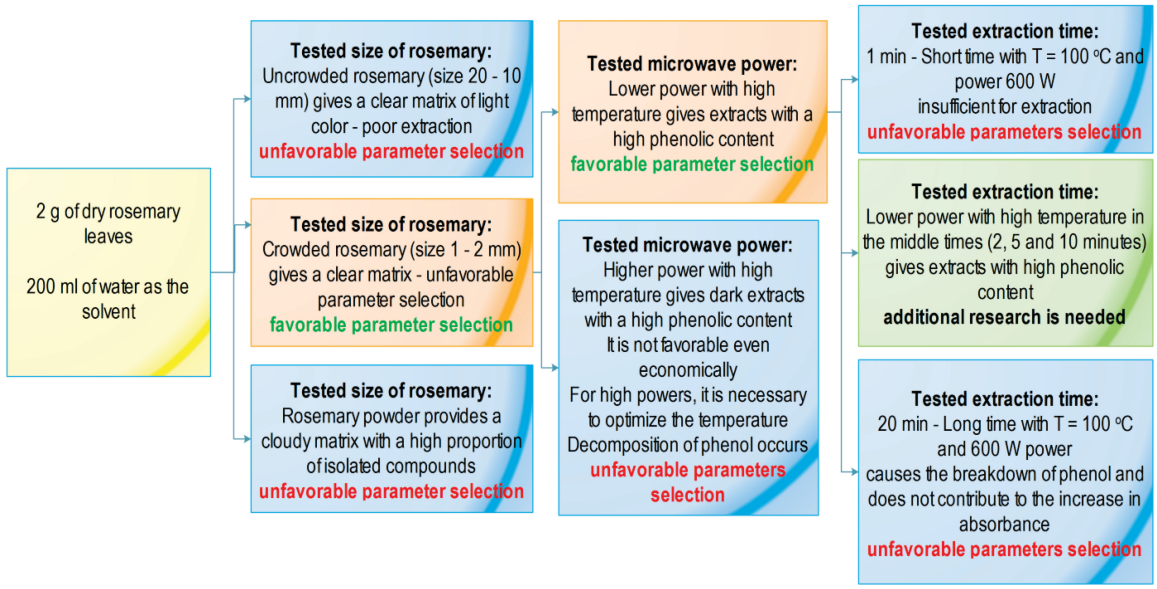
The scheme for optimization of the parameters is shown in Scheme 1. At the beginning, some parameters were defined for the optimization. A solvent volume of 200 mL was chosen for the reactor and the mass of dry rosemary leaves for the optimization is 2 g. The first step is the optimization of size, followed by the optimization of microwave power and the final optimization of time. Blue squares show unfavourable while orange squares show favourable selected parameters. The parameters in the green square require additional investigation. All steps and conclusions are described below and shown graphically in Figure 1 as dependence of the absorbance signal on the different MWE parameters.

First step – The dependence of absorbance upon **temperature** for three different particle size is shown in Figure 1A. In order to optimize the properties of the matrix,

Table 1. Location and growing conditions for rosemary.

Location	Insolation	R_{sol} / mJ m^{-2}	ϕ_{av} / %	T_{av} / °C	R / mm	Altitude / m	Traffic	Watering	Proximity to the sea
Makarska	All day	148	69	10.4	2.2	< 30	Medium	Yes	By the sea
Zadar	No sun	131	71	8.6	3.2	< 30	Intesive	No	1 – 2 km
Sinj	All day	128	73	8.0	3.8	326	Medium	No	> 30 km
Milna (Brač)	All day	155	71	10.5	2.0	< 30	No traffic	Yes	0.5 km
Imotski	All day	143	73	5.7	4.2	395	Medium	Yes	> 30 km
Split (Campus)	Partially	155	71	11.3	2.2	< 30	Intesive	Yes	1 – 2 km
Kaštel Kambelovac	Partially	155	71	11.3	2.2	< 30	Medium	No	0.5 km

*R_{sol} Average solar radiation; φ_{av} Mean daily relative humidity; T_{av} mean daily air temperature; R_{mm} average daily precipitation



Scheme 1. Steps for optimization of MWE parameters and conclusion.

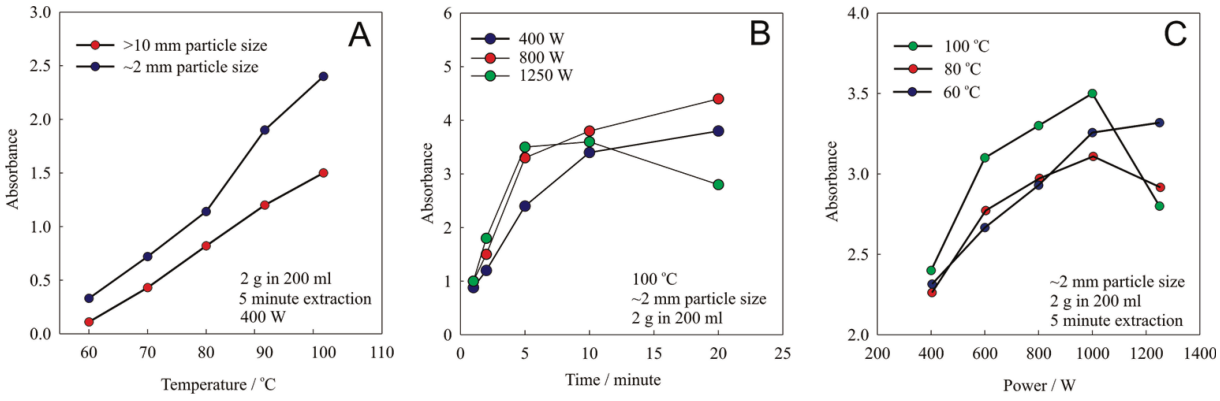


Figure 1. Dependence of the absorbance signal on the different MWE parameters: A – temperature with matrix size; B – time with extraction power; C – extraction time with temperature.

particle size, three samples were selected: uncrushed (> 10mm), crushed rosemary (~ 2 mm particle size) and grounded, powder rosemary. The results obtained with rosemary powder yielded cloudy extracts that were unsuitable for spectrophotometric measurements (not shown). The extraction yield increased with the decrease in particle size, uncrushed rosemary gives slightly higher absorbance after 5 minutes, by providing larger surface area, better contact between the leaves and solvent, and allows enhanced penetration of the microwave.

Second step – The dependence of absorbance upon **time** for three different microwave powers is shown in Figure 1B. Absorbance decreases with longer extraction times and higher power. High power combined with high longer time was unfavourable for rosemary as it leads to the breaking of bonds in bioactive compounds and/or thermal degradation of the sample. The greatest increase in the dependence of absorbance on time for low and middle power was found between 1 and 5 minutes, and up to 10 minutes with a small deviation from linearity.

Third step – The dependence of absorbance upon **microwave power** for three different temperatures is shown in Figure 1C. It can be seen that a high power, even in such a short time, leads to a significant degradation of the matrix at all three temperatures, while a low power has no major impact on the rosemary sample, regardless of the temperature. The highest absorption was achieved at 100 °C. The application of 600 W eliminates any possibility of degradation, while the absorption value is high enough even at the temperature of boiling water.

According to the results, dried rosemary is crushed before extraction at particle size ~ 2mm. The selected parameters for extraction are: preheating 2 minutes, microwave power 600 W, and the selected time is 2, 5 and 10 minutes. Although the modelling of all parameters was optimized for a dry/solvent ratio of 1 : 100, a ratio of 1 : 200 (1 g dry rosemary in 200 mL of redistilled water) was chosen for the analysis of a real sample due to high absorbance values.

Results of Spectrophotometric Measurements

FOLIN-CIOCALTEU METHOD – DETERMINATION OF TOTAL PHENOLIC COMPOUNDS

The obtained plant extracts were added instead of the gallic acid standard to the flask and after two hours the absorbance was measured at 765 nm relative to the blank. Based on three measurements, the mean absorbance value was found. The concentration of phenol in the sample was calculated or read from the calibration obtained curve for standard gallic acid, that is, according to the equation for the line equation, according to the equation given in Experimental part. The obtained results are expressed as gallic acid equivalents (mg GAE L^{-1}), since it was used as a standard.

DETERMINATION OF ANTIOXIDANT ACTIVITY BY FRAP METHOD

Absorbance at 593 nm is continuously monitored over time and recorded after 4 and after 10 minutes. Based on three measurements, the mean absorbance value was found. Antioxidant activity was calculated from calibration obtained curve, from the concentration Fe^{2+} – absorbance ratio, according to equation given in Experimental part.

The results for total phenolic compounds and antioxidant activity for conventional solvent extraction are presented Table 2 and with microwave-assisted extraction in Table 3. Multiple bar graphs at the Figure 2 present total phenols results and Figure 3 antioxidant activity after 10 minutes. The efficiency and performance of the MWE can be assessed on the basis of all results. It should be noted that conventional solvent extraction at 100 °C consumes much more energy, as the cooking power is about 1500 W, and a much lower efficiency is achieved. By using microwaves with a lower temperature and lower power, a higher efficiency was achieved. The MWE process offers faster heating, better energy performance (no loss of solvent and heat), shorter heating time, more precise control of the process and a safer process, so we can conclude that it is also economically more favourable.[30,31]

The obtained experimental values suggest a significant reduction in extraction time as well as an improved extraction yield in comparison to conventional extraction. The content of total phenols of the samples during conventional heating at 100 °C for 5 minutes is two to three times lower than the content of total phenols during MWE with the same time. The antioxidant activity follows the same trend.

Looking at the overall results for MWE, an increase in total phenols and antioxidant activity can be observed with increasing MWE time. The results obtained with the Folin-Ciocalteu method show that the rosemary sample from Milna (island of Brač) has the highest content of total phenols (947.8 mg GAE L^{-1}). The Makarska sample showed the highest antioxidant activity (1576.6 and 1858.4 μM Fe^{2+}). As the total phenolic content increases, the antioxidant activity also increases, indicating that in the Milna sample the phenols are the largest contribution to the antioxidant activity, while in the other samples other non-phenolic antioxidants have a dominant influence on the antioxidant activity.

The results of the extraction of total phenols F-C with the optimized MWE method show the trend that the concentration of total phenols increases with increasing extraction time, which means that a longer extraction time

Table 2. Content of total phenols and antioxidant activity of samples obtained by spectrophotometric methods extracted without microwaves; conventional solvent extraction at 100 °C during 5 minutes.

Samples 100 °C 5 min	Total phenolic compounds Folin-Ciocalteu reagents	Antioxidant activity after 4 minutes FRAP method	Antioxidant activity after 10 minutes FRAP method
	mg GAE L^{-1}	μM Fe^{2+}	μM Fe^{2+}
Makarska	39.5	686.9	972.7
Zadar	25.8	465.6	651.7
Sinj	9.0	366.2	450.7
Milna (Brač)	292.5	516.1	720.2
Imotski	33.2	431.8	601.2
Split (Campus)	7.5	407.3	552.9
Kaštel Kambelovac	5.2	403.1	518.7

Table 3. Content of total phenols and antioxidant activity of samples obtained by spectrophotometric method with microwave assisted extraction.

Samples 100 °C 5 min	Total phenolic compounds Folin-Ciocalteu reagents	Antioxidant activity after 4 minute FRAP method	Antioxidant activity after 10 minute FRAP method
	mg GAE L^{-1}	μM Fe^{2+}	μM Fe^{2+}
Makarska			
2 min	53.4	301.9	365.6
5 min	191.5	1080.5	1278.3
10 min	289.1	1576.6	1858.4
Zadar			
2 min	45.4	268.9	319.2
5 min	132.6	763.6	893.8
10 min	216.5	1254.6	1454.6
Sinj			
2 min	36.1	218.3	260.6
5 min	98.1	508.6	594.3
10 min	196.9	1025.7	1233.4
Milna (Brač)			
2 min	368.6	273.3	336.8
5 min	775.0	713.9	1041.9
10 min	947.8	1088.6	1703.3
Imotski			
2 min	48.4	290.1	339.4
5 min	134.5	716.2	831.3
10 min	257.6	1263.4	1442.7
Split (Campus)			
2 min	54.5	331.0	385.6
5 min	117.5	649.6	733.8
10 min	198.9	986.8	1122.6
Kaštel Kambelovac			
2 min	30.7	198.6	229.7
5 min	103.4	532.9	669.8
10 min	207.0	916.9	1066.1

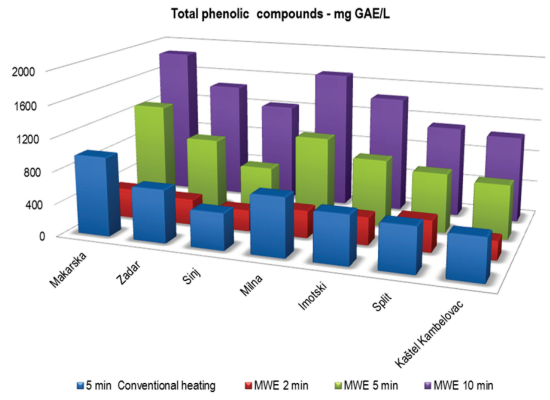


Figure 2. Comparison of the obtained results for total phenols for all extracts.

allows a greater extraction of phenolic compounds from the plant material. For example, the samples with the longest extraction time, such as Milna (Island of Brač) and Makarska, have the highest concentrations of total phenols, namely 947.8 and 289.1 mg GAE L^{-1}, respectively.

Despite the small number of analytes taken into account, it was found that the samples from Makarska and Milna (Island of Brač) have overall highest antioxidant contribution, compared to the samples from other areas. The reason for this could be the geographical and microclimatic characteristics of the terrain and the conditions under which this rosemary was grown. The samples from Split (Campus) and Sinj show slightly lower values of total phenols. The close proximity to the sea and several hours of sunlight most likely contribute to an increase in the

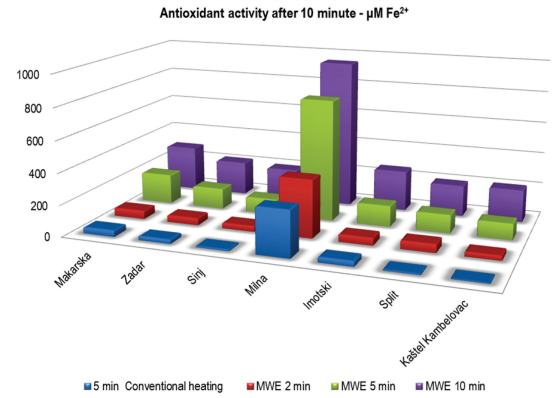


Figure 3. Comparison of the obtained results for antioxidant activity after 10 minutes for all extracts.

proportion of bioactive species in rosemary. It can therefore be concluded that proximity to the sea and sunlight are the most important factors for the high antioxidant activity of rosemary. Plants from urban areas harvested near traffic have negatively correlated antioxidant parameters, suggesting that rosemary has great potential as a tool for determining the extent of traffic-related pollution in urban areas.

CONCLUSION

Microwave extraction – The advantages of MWE lie in the faster, more efficient, more precise, safer and more economical method, compared to conventional methods. In optimizing the parameters of microwave extraction of rosemary, the following conclusions were drawn. By optimizing the extraction power and temperature, it was found that the highest absorbance was achieved at 100 °C and a power of 600 W with eliminated possibility of degradation. A lower power combined with a high temperature is an optimal combination that yields extracts with an extremely high phenolic content.

Phenolic content – The samples from Milna (Island of Brač) and Makarska have the highest concentrations of total phenols, namely 947.8 and 289.1 mg GAE L^{-1} respectively, while the samples from Kaštel Kambelovac and Split–Campus have lower values of total phenols, namely 207.0 and 198.9 mg GAE L^{-1} respectively. Thus, the key parameter for the concentration of total phenols in rosemary is the time of sun exposure. With the exception of the sample from Milna (Island of Brač), all samples grew in urban areas. The influence of environmental pollution is also confirmed by the content of total phenols, because it is known that phenolic compounds are the key to protection against oxidative stress and pollutants to which the plant was exposed.

Antioxidant activity – The Makarska sample shows the highest antioxidant activity (1858.4 μM Fe^{2+} after 10 minutes FRAP method) and it is also the sample grown closest to the sea. So the key parameter for the antioxidant activity of rosemary is the sea exposure.

The ideal combination of nature is justified not only by the obtained results, but also by the origin of the name rosemary, Rosmarinus, which is derived from the Latin words ros (dew) and marinus (sea born). Rosemary is a treasure trove of antioxidants and biologically active compounds and can be considered a gift to the Dalmatian climate, as it is widely used in medicine, cosmetics, aromatherapy and cooking.

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