




Communication

# Is the Household Microwave Recommended to Obtain Antioxidant-Rich Extracts from *Lycium barbarum* Leaves?

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**Abstract:** Nowadays, much interest is devoted to the extraction of plant materials, considering also their waste and by-products, to obtain antioxidant-rich products. The effect of household microwave-assisted extraction (MAE) on the phenolic content and antioxidant activity of *Lycium barbarum* leaf extracts was investigated. An experimental design approach was adopted considering solid/liquid ratio (1, 3, and 5 g of leaves in 150 mL water), irradiation time (1, 3, and 5 min), and microwave power (300, 400, and 500 W) as independent variables. These three factors and their interactions were studied to evaluate the effect of MAE conditions on the responses of total phenolic content, antioxidant activity, and chlorogenic acid content. The results showed that the analytical parameters were positively influenced by the solid/liquid ratio and time. On the contrary, microwave power was inversely correlated with the investigated responses. This research revealed that microwave extraction conditions should be carefully monitored to obtain bioactive-rich aqueous extracts with high antioxidant activity. A comparison with household traditional methods showed an unexpected lower phenolic content and antioxidant activity for MAE extract in respect to the decoction and infusion. In fact, it was found that *L. barbarum* leaf infusion had the best functional properties, regarding the investigated characteristics. The outcome of this study has implications for raising awareness that household preparation conditions strongly affect the health properties of herbal extracts.

**Keywords:** *Lycium barbarum* leaves; aqueous infusion; chlorogenic acid; optimization; microwave-assisted extraction



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## 1. Introduction

Functional herbal-based beverages, the most consumed drink category in the world, play an important role in daily life and contribute to nutritional well-being. The daily use of functional food can be considered a complementary approach for the prevention of some systemic diseases. Their daily intake has been associated with health benefits due to the presence of important bioactive molecules such as vitamins, minerals, and phenols [1]. Moreover, nowadays there is a great deal of interest in the use and preparation of plant-based aqueous beverages using waste and by-products, interesting sources of bioactives. As an example, numerous papers report on leaf infusions [2,3] and decoctions [4,5]. *Lycium* spp. leaves are interesting wastes of the goji berry production for their traditional use as herbal drugs and the actual potentiality as functional tea and dietary supplements [6]. In fact, even if *Lycium* leaves are less studied in respect to most used berries [7,8], some authors reported valuable biological activities, including antioxidant, antimicrobial, enzyme inhibitory, and antiinflammatory activities [9–13]. These activities were

associated with the presence of numerous bioactive compounds, including lipopolysaccharides and phenolic compounds, mainly represented by flavonols and phenolic acids. It was reported that chlorogenic acid (CA) was the main phenolic acid in *Lycium* leaves in alcoholic and hydroalcoholic extracts [9,11,12].

Extraction is the first essential process for obtaining herbal-based products [14]. Recently, in the food and nutraceutical industries, new techniques have been developed and applied in the extraction of various phytochemicals [15,16]. For example, supercritical fluid extraction, ultrasound, and microwave-assisted extraction (MAE) are replacing the conventional extraction techniques (i.e., maceration and Soxhlet).

Regarding functional herbal-based beverages, to the best of our knowledge, no study was carried out on the use of *Lycium* leaves to prepare a drink with potential health properties. For the first time, the effect of household MAE on the antioxidant properties and CA content of *L. barbarum* leaf extracts was investigated. The objectives of the present study are (i) to investigate, using experimental design, the effect of MAE extraction conditions on phenolic content and antioxidant properties of *L. barbarum* leaf extracts; (ii) to make a comparison with traditional home beverages (decoction and infusion).

## 2. Materials and Methods

### 2.1. Materials

*L. barbarum* dried leaves were collected in 2019 in Umbria (Perugia, Italy). Diammonium 2,2'-azino bis(3-ethylbenzothiazoline-6-sulphonate) (ABTS;  $\geq 98\%$ ), Folin-Ciocalteu's phenol reagent, gallic acid (97.5–102.5%), ( $\pm$ )-6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox; 97%), and chlorogenic acid (CA;  $\geq 95\%$ ) were purchased from Sigma-Aldrich (Milan, Italy). High Performance Liquid Chromatography (HPLC) grade, acetonitrile, formic acid, and ultrapure water, were from Carlo Erba (Milan, Italy).

### 2.2. Household Microwave Extraction of *L. barbarum* Leaves

#### 2.2.1. Sample Treatment

The extractions were carried out in a household microwave oven (1350 W max power; 2450 MHz frequency; Samsung-G633C, London, UK). Dried whole *L. barbarum* leaves were placed in 150 mL room-temperature oligomineral water (fixed residue: 276 mg/L) in a 300 mL glass beaker and located in the oven in different conditions, reported in the following section (Section 2.2.2). Then, the aqueous extracts were placed on ice to cool to room temperature, and filtered through paper filters (MN 615, Macherey-Nagel, Düren, Germany). Finally, the final volume was checked and, if evaporation occurred, water was added to have the same initial volume (150 mL). The filtered samples were collected in an amber glass bottle and promptly subjected to analysis.

#### 2.2.2. Experimental Design

An experimental design software (UMETRICS, Umeå, Sweden), named Statistical Design Package MODDE 5.0<sup>TM</sup>, was employed to evaluate the influence of three independent microwave parameters (solid/liquid ratio, time, microwave power) on the phenolic content and antioxidant activity of MAE extracts from *L. barbarum* leaves. Table 1 shows the considered factors affecting the extraction efficiency; the independent variables were ratio ( $X_1$ ), time ( $X_2$ ), and microwave power ( $X_3$ ).

**Table 1.** Independent variables, unit and values of experimental design.

Independent Variables	Unit	Values
Ratio ( $X_1$ ) <sup>1</sup>	g/150 mL	1; 3; 5
Time ( $X_2$ )	Min	1; 3; 5
Microwave Power ( $X_3$ )	Watt	1; 3; 5

<sup>1</sup> Solid/liquid ratio, i.e., dry leaves/water, expressed as leaf weight in grams of dry matter/liquid volume (g DM/mL).

As dependent variables, the following responses were selected:

- Total phenolic content (TPC), reported as milligrams of gallic acid equivalent per gram of dry matter (mg GAE/g DM);
- Free radical-scavenging activity by ABTS assay, reported as milligrams of Trolox equivalents per gram of dry matter (mg TE/g DM);
- Ferric reducing antioxidant power (FRAP) assay, reported as micromoles of Fe<sup>+2</sup> per gram of dry matter (μmol Fe<sup>+2</sup>/g DM);
- CA content, reported as micrograms per gram of dry matter (μg/g DM).

A total of 15 experiments, including three replicated center points, were obtained setting the screening objective and a D-optimal design. Table 2 shows the worksheet with the experimental conditions of the extractions, which were carried out in random order. Multiple linear regression analysis was used to fit the model.

**Table 2.** Worksheet and responses of the experimental design.

Experimental Number	Ratio	Time	Power
1	1	1	300
2	5	1	300
3	1	3	300
4	1	5	300
5	3	5	300
6	5	5	300
7	3	3	400
8	1	5	400
9	1	1	500
10	5	1	500
11	1	5	500
12	5	5	500
13	5	5	500
14	5	5	500
15	5	5	500

### 2.3. Infusion and Decoction Preparations

The infusion and decoction were obtained by using 2 g of *L. barbarum* dried whole leaves (corresponding to the weight of herbal material in a teabag) and 150 mL of room temperature oligomineral water (corresponding to the volume of a tea cup) in a 300 mL glass beaker.

The infusion was prepared by adding the herbal material in boiling water and allowing it to repose for 5 min. The decoction was prepared by adding cold water on herbal material and boiling for 5 min.

Both infusion and decoction herbal beverages were placed on ice to cool to room temperature and filtered through paper filters. Finally, the final volume was checked and, if evaporation occurred, water was added up to the initial volume (150 mL). The filtered samples were collected in an amber glass bottle and promptly subjected to analysis.

### 2.4. Determination of Total Phenolic Content (TPC)

The TPC was determined according to the spectrophotometric method reported by Pagano et al. (2017) [17]. The assay involved the reduction of Folin and Ciocalteu's reagent, and the absorbance was measured at 765 nm. The TPC was reported as mg GAE/g DM.

## 2.5. In Vitro Antioxidant Activities

### 2.5.1. ABTS Assay

ABTS assay was carried out following the procedure described by Rocchetti et al. (2020) [18]. Sample was added to ABTS<sup>+</sup> reagent and, after 10 min, the absorbance was measured at 734 nm. The antioxidant capacity of each sample was reported as mg TE/g DM.

### 2.5.2. FRAP Assay

The reducing capacity of the extracts was determined using the FRAP assay [19]. The sample was added to FRAP reagent and, after 30 min, the absorbance was measured at 593 nm. The calibration curve was obtained using aqueous solutions of known Fe<sup>+2</sup> concentrations (2–5 Mm). The reducing capacity was expressed as  $\mu\text{mol Fe}^{+2}/\text{g DM}$ .

## 2.6. HPLC-DAD Analysis of Phenolic Compounds

The HPLC analysis of leaf extracts was carried out according to a previous paper [9]. A pump Thermo Spectraseries, a diode array detector Spectra System UV6000LP (Thermo Separation Products, Waltham, MA, USA), and a Hypersil GOLD column (3  $\mu\text{m}$ , 150  $\times$  4.6 mm) were used. The mobile phase consisted of 0.1% (*v/v*) formic acid in water (A) and 0.1% (*v/v*) formic acid in acetonitrile (B), with the following gradient: phase B increased from 5% to 20% in 30 min, and then to 95% in 5 min. The flow rate was 1.0 mL/min. Xcalibur software version 1.2 (Finnigan Corporation 1998–2000, San Jose, CA, USA) was used for chromatogram and data acquisition.

## 2.7. Statistical Analysis

All the analytical procedures were carried out in triplicate, and the results were expressed as mean  $\pm$  standard deviation on dry leaves. Microsoft Excel 2016 (Microsoft Corporation, Redmond, WA, USA) was used for data analysis.

One-way ANOVA (Analysis of Variance) was used to assess the differences among the means of the investigated groups performing the post-hoc Tukey's HSD (Honest Significant Difference) methodology (with a confidence level of 95%). The statistical analyses were performed using Originpro 2021 software (OriginLab Corporation, Northampton, MA, USA).

## 3. Results and Discussion

### 3.1. MAE Extraction of *L. barbarum* Leaves

Nowadays, microwave ovens are ubiquitous in homes and workplaces because irradiation is a time-saving and energy-efficient cooking method, useful to prepare herbal beverages. In the present study, the optimization of the conditions of household microwave extraction on some bioactive properties of *L. barbarum* leaf extracts was studied.

Table 2 shows the worksheet with the extraction conditions of the experiments, designed around a mathematical model with the highest G-efficiency using the software MODDE<sup>TM</sup> (MKS Umetrics, Umeå, Sweden). Among the considered responses, in addition to TPC, two complementary antioxidant assays were used: ABTS to evaluate the radical scavenging ability of the MAE extracts and FRAP to evaluate their reducing capacity. CA was chosen because, on the basis of previous results [9,11,20], it was the main phenolic acid detected in *L. barbarum* leaf extracts. Table 3 shows the experimental values obtained for TPC, FRAP, ABTS, and CA. In order to estimate the coefficients of the terms in the model, representing the relationship between the response (*Y*) and the factors ( $X_1, X_2, \dots$ ), multiple linear regression analysis was used.

**Table 3.** Experimental values of TPC, ABTS, FRAP, and CA of MAE extracts.

Experimental Number	TPC	ABTS	FRAP	CA
	mg GAE/g	mg TE/g	$\mu\text{mol Fe}^{+2}/\text{g}$	$\mu\text{g/g}$
1	3.4	8.4	34.7	1.5
2	4.4	8.4	43.3	13.2
3	4.8	12.4	57.7	7.5
4	5.0	13.2	61.4	43.5
5	6.2	16.3	71.9	72.0
6	5.5	11.9	60.4	195.3
7	5.9	13.5	69.0	7.0
8	5.3	13.9	62.5	48.0
9	2.8	9.5	29.2	1.5
10	4.6	16.1	43.3	0.3
11	5.7	13.0	63.0	6.0
12	5.9	11.3	72.6	144.9
13	5.5	11.4	66.5	130.5
14	5.8	8.3	64.4	120.0
15	5.8	10.9	73.3	115.8

TPC, total phenolic content; ABTS, diammonium 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonate); FRAP, ferric reducing antioxidant power; CA, chlorogenic acid; GAE, gallic acid equivalents; TE, Trolox equivalents. The values are expressed as dry weight.

The factor interactions were entered for a better comprehensive understanding of the factor dynamics. In the following, the obtained Equations (1)–(4) for TPC, ABTS, FRAP, and CA are shown:

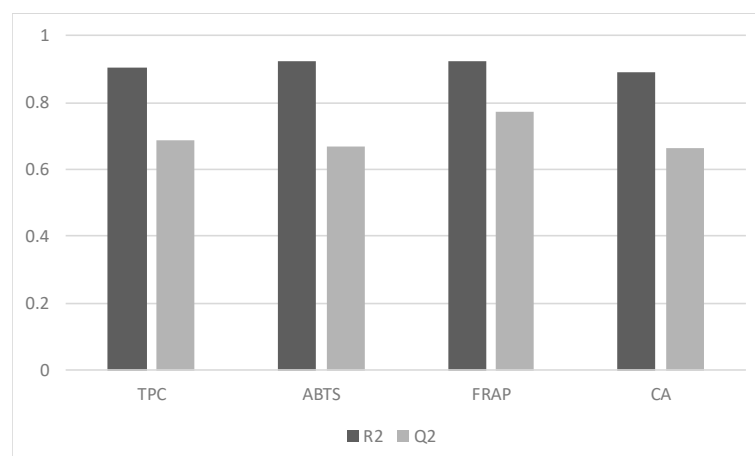
$$\text{TPC} = 4.81127 + 0.385671X_1 + 0.864694X_2 - 0.0420571X_3 - 0.267204X_1X_2 + 0.0510864X_1X_3 \quad (1)$$

$$\text{ABTS} = 13.5331 + 2.1559X_1 - 1.00171X_2 - 0.17909X_3 - 3.15727X_1X_2 - 0.15509X_1X_3 \quad (2)$$

$$\text{FRAP} = 53.3367 + 3.17606X_1 + 13.1511X_2 - 0.61149X_3 - 1.87349X_1X_2 + 2.14145X_1X_3 \quad (3)$$

$$\text{CA} = 424.573 + 326.841X_1 + 436.109X_2 - 114.033X_3 - 177.292.127X_1X_2 - 690.515X_1X_3 \quad (4)$$

The best summary of fit of the model is given by the R<sup>2</sup> and Q<sup>2</sup> values (Figure 1).



**Figure 1.** Statistic of the model—R<sup>2</sup> and Q<sup>2</sup> values of the four responses (TPC, total phenolic content; ABTS, diammonium 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonate); FRAP, ferric reducing antioxidant power; CA, chlorogenic acid).

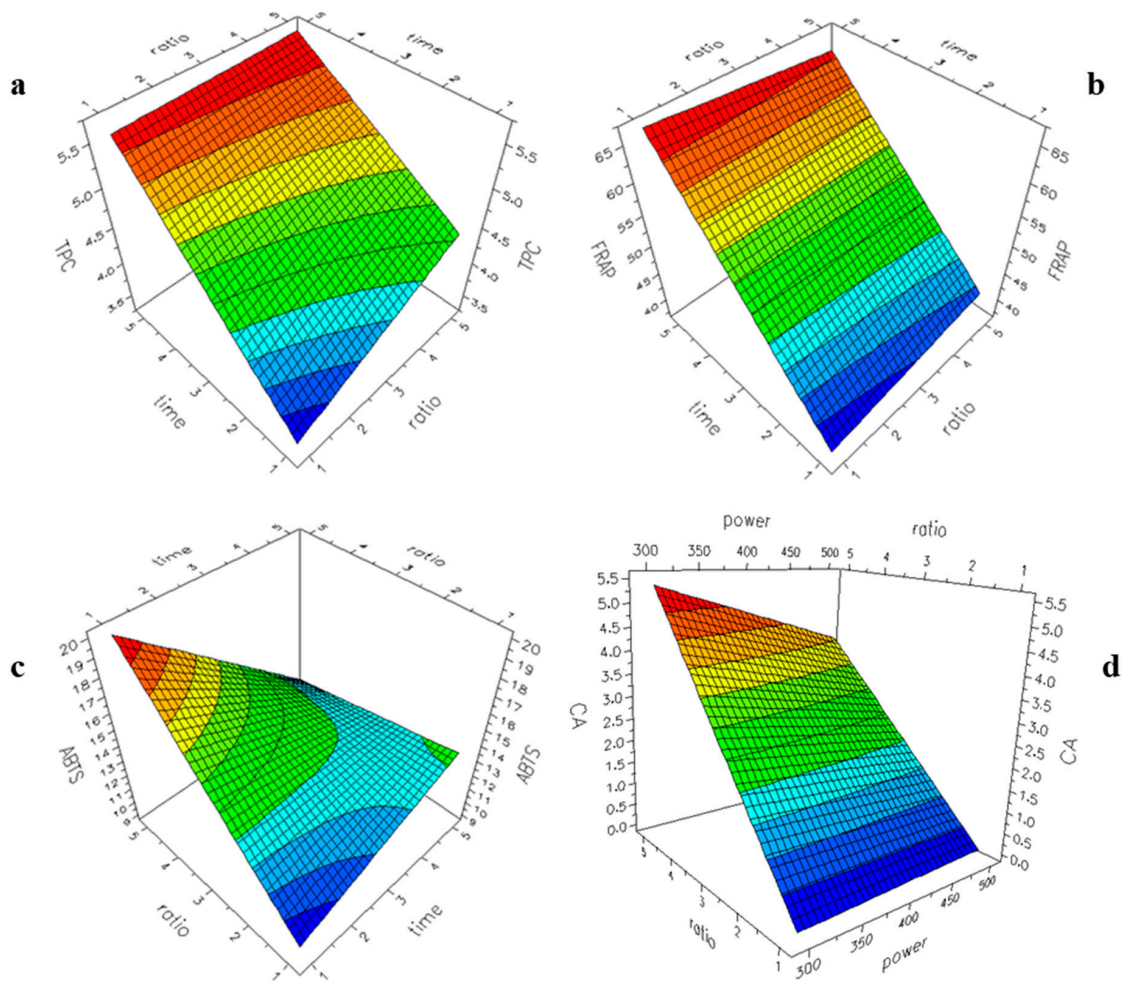
The R<sup>2</sup> values ranged from 0.9057 for TPC to 0.9234 for FRAP, meaning that the model fitted the data very closely. The Q<sup>2</sup> values ranged between 0.6670 for ABTS and 0.7739 for FRAP, indicating a high predictive accuracy.



As regards the influence of the different factors, it can be observed from the obtained Equations (1)–(4) that all responses are positively influenced by the  $X_1$  factor (weight of herbal material/150 mL of water), while an inverse correlation was found for the  $X_3$  factor (microwave power). The  $X_2$  factor (time) showed a high positive correlation with TPC, FRAP, and CA. Interesting interactions are represented by ratio  $\times$  time (inverse correlations except for CA) and L/S ratio  $\times$  power (positive for TPC and FRAP, negative for ABTS and CA).

As shown in Table 3, the values of the TPC of MAE extracts ranged from 2.8 mg GAE/g (of the sample obtained with ratio 1, irradiation time 1 min, microwave power 500 W) to 6.2 mg GAE/g of the sample, obtained with a lower microwave power (300 W), but with a higher ratio (3) and irradiation time (5 min). It was also observed that high irradiation time corresponded to high FRAP value and that TPC values were well correlated to FRAP values ( $R^2 = 0.9192$ ). As regards CA, the main phenolic acid in *L. barbarum* leaves, it was observed that its content was higher (195.3  $\mu\text{g/g DM}$ ) when the power of the household microwave was lower (300 Watt), the extraction time was longer (5 min), and the ratio was higher (5 g/150 mL). The results obtained for CA confirm those reported for TPC, and it can be deduced that microwave power could lead to the modification of phenolic structure.

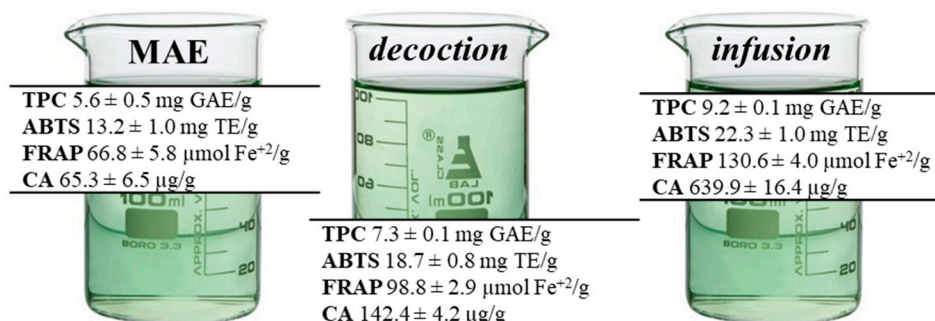
Figure 2 shows the surface plots of the responses TPC (a), FRAP (b), and ABTS (c) as a function of time and ratio factors, maintaining the power at 300 Watt, and of CA response (d) as a function of ratio and power factors, maintaining the time at 5 min. It is possible to observe that high TPC and FRAP values were relative to a long time, while a short time and high ratio positively influenced ABTS and CA responses.



**Figure 2.** Surface plot showing the responses: (a) TPC (300 Watt), (b) FRAP (300 Watt), (c) ABTS (300 Watt), (d) CA (5 min).

To make a comparison of the phenolic content and in vitro activities of the MAE extract with classic home preparations, infusions and decoctions were prepared and analyzed. Since two grams of *L. barbarum* leaves were used to prepare infusions and decoctions (150 mL), the prediction function of the software provided the values of TPC, FRAP, ABTS, and CA for a MAE extract from two grams of leaves in 150 mL, irradiated for 5 min at 300 W. Figure 3 shows the values of TPC, FRAP, ABTS, and CA content of *L. barbarum* leaf extracts obtained by MAE, decoction, and infusion.

The results of post-hoc Tukey's HSD test showed that the difference of the means was significant at the 0.05 level for all the analytical assays among MAE, decoction, and infusion treatments. Unexpectedly, lower values were obtained for the MAE extract compared to both infusion and decoction for all parameters studied; in particular, the CA content was strongly reduced when the MAE treatment was performed. Finally, *L. barbarum* leaf infusion had the highest values for the analytical assays, thus preserving bioactive compounds of the extract and its related properties.



**Figure 3.** TPC, ABTS, FRAP, and CA content of *L. barbarum* leaf MAE, decoction, and infusion extracts.

### 3.2. Comparison with Literature Data

In a previous paper, unconventional extraction techniques such as ultrasound-assisted extraction and MAE have been used to extract *L. barbarum* leaves [9]. The TPC and ABTS values of MAE extracts were similar with the highest values obtained in this paper; however, it should be observed that alcoholic and hydroalcoholic solvents were used. In fact, to the best of our knowledge, this is the first study on household microwave extraction for obtaining *L. barbarum* leaf extract, so it is difficult to make a comparison with the literature data. Other authors studied the microwave assisted extraction of different herbal materials, in particular, green tea leaves [16,21–23]. Taşkın and Özbek have investigated the microwave conditions for the extraction of bioactives from green tea and found that the TPC value increased with an increase in microwave power up to 440 W, while it showed a steady decrease when the power was higher, up to 600 W [16]. It was concluded that the use of the right combination of some extraction variables (moderate microwave power-long irradiation time) is recommended for obtaining bioactives from waste without thermal deterioration. Vuong et al. (2012) investigated the effect of household microwave on catechins, caffeine, and theanine extraction from commercial teabag [21]. They found that the extracted amounts were lower than those obtained using the manufacturer's instructions (classic decoction), a result similar to what observed in this work. Upadhyay, Ramalakshmi, and Rao (2012) have instead studied the microwave extraction of green coffee bean to obtain chlorogenic acids and reported that the yields of MAE under optimum conditions (water, 800 W, 5 min, and 50 °C) were higher than those from the conventional aqueous extraction at 5 min and 50 °C [23].

Finally, on the basis of the good results obtained in this paper for traditional infusion, a comparison can be made with other plant-based infusions. Among these, Li et al. (2013) studied the properties of 233 medicinal plants, including powdered *L. barbarum* berries [24] and found similar TPC value to those reported in this paper. Higher TPC values were instead reported for dried goji berry infusions [8].

#### 4. Conclusions

In this research, for the first time the investigation of MAE parameters on extraction efficiency of bioactives from *L. barbarum* leaves was carried out. The results showed that ratio and time had a positive influence on the functional properties of leaf extract, while the increase in power corresponded to lower phenolic content and antioxidant properties. The combination of moderate microwave power with long time and high ratio provided higher CA content in MAE extract. Unexpected results were obtained taking into consideration classic household preparations; in fact, the investigated analytical parameters provided lower values for MAE extracts in respect to decoction and infusion. The traditional infusion has proved to be the best procedure for extracting bioactives from *L. barbarum* leaves and obtaining a functional beverage. Further studies are underway to deepen the knowledge on the effect of microwave extraction on the chemical stability of phenolic compounds in aqueous solutions, with particular regard to the modification of chlorogenic acid due to irradiation power. The control of chemical stability of bioactive compounds from plant materials is mandatory for pilot and large-scale extraction processes.

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