Mathematical Modeling and Optimization of the Microwave Assisted Extraction of the Solid Waste of the Pomegranate Juice Industry

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Abstract: Pomegranate pomace is the solid waste of the pomegranate juice industry which accounts for approximately 50% of the quantity of the fruits, which is processed into juice and is a good raw material for production of high added value products with diverse uses. Pomegranate pomace is rich in polyphenols and flavonoids which could substitute the potentially hazardous synthetic antioxidants/antimicrobials used in agro-food and cosmetics sectors. In this work, eco-friendly aqueous microwave assisted extraction of pomegranate pomace was investigated and optimized in order to produce effectively novel natural antioxidant/antimicrobial extracts. A three-factorial response surface optimization methodology with centered Box & Behnken experimental design was used to obtain the predictive models and the maximum values of total polyphenols, total flavonoids and total antioxidant capacity (TAC). The three optimization factors involved were: (a) water/solid ratio; (b) extraction temperature; (c) extraction time and the effectiveness and robustness of the three models were statistically verified by ANOVA.

Key words: Pomegranate pomace, microwave assisted extraction, response surface optimization, natural antioxidants, polyphenols, flavonoids.

1. Introduction

Pomegranate fruit (Punica granatum L.) production is a fast growing agricultural activity as the fruit is globally recognized as a “super-food”, due to its nutritious characteristics. The global pomegranate market was valued at 8.2 billion USD in 2018 and is expected to reach 23.14 billion USD by year 2026 [1], while the global pomegranate fruit production runs into 3,000,000 MT [2]. According to Damian [3], pomegranate is an important fruit of tropical and subtropical regions, which originated in the Middle East and India and has been used for centuries in ancient cultures for its medicinal properties. It is also widely reported that pomegranate exhibits antiviral, antioxidant, anticancer, and anti-proliferative activities [4-6]. Pomegranate can be consumed fresh or in processed form as juice, wine, flavor, and extract. Compared to other fruit juices, red wine, and green tea, commercial pomegranate juice has the highest antioxidant activity and is currently a product of high value in the agricultural market [7].

Pomegranate fruit contains valuable antioxidants and according to Li et al. [8] the polyphenolic content of pomegranate juice is higher when this is produced by the whole fruit instead of the arils only. This indicates that there is a considerable phenolic content in the pomegranate peel, as well as in the solid pomegranate pomace, which is a by-product of the pomegranate juice industry and represents about 50% of the total processed fruits.
As cited by Li et al. [8], Fischer et al. [9] and Saad et al. [10], the profile of polyphenolic content of the pomegranate pomace contains polyphenols, flavonoids, proanthocyanidins, hydrolysable tannins (like ellagic acid, pedunculagin, punicalin and gallic acids) in substantial amounts, ranging from 27 g/kg to 172 g/kg of dry pomace, expressed as gallic acid equivalents. Furthermore, in a research paper published by Elfalleh et al. [11] the total polyphenols content of pomegranate pomace (expressed as gallic acid) was found to be 85.60 ± 4.87 mg/g. According to Farag et al. [12] and Dimou [13] the primary polyphenols contained in pomegranate pomace are gallic acid, proto-catechuic acid, chlorogenic acid, vanillic acid, cumarin, caffeic acid, oleuropein, ferulic acid, quercetin and caffeine.

Nowadays, natural antioxidants and antimicrobials have become very popular for novel food/nutraceuticals, cosmetics and phyto-protection applications and are preferred by consumers to synthetic antioxidants, such as butylhydroxyanisole (BHA) and dibutylhydroxytoluene (BHT) or propyl gallate (PG) [14-17] or synthetic preservatives like sorbate salts and chemical pesticides in agricultural applications. Besides avoiding the undesirable health effect of some synthetic chemicals, the use of natural alternatives of antimicrobials and antioxidants from pomegranate can have beneficial health effects. For example, enrichment of ice cream with pomegranate by-products resulted in increased phenolic content of ice creams, which caused an improvement in antioxidant and anti-diabetic activities, mainly due to the functional properties of punicalagins in pomegranate peel, and punicic acid in pomegranate seed oil [18]. Furthermore, addition of pomegranate to popular chicken meat products enhanced its shelf life by 2-3 weeks during chilled storage [19]. In addition, the enhanced antioxidant activity of pomegranate peel extract was found to inhibit lipid oxidation in cooked chicken patties [20].

A recent literature review [21] cited that several studies have reported the *in vitro* bioactivity of pomegranate peel extracts including antioxidant, antitumor, anti-inflammatory, and anti-proliferative properties. Kanatt et al. [19] investigated the antioxidant and antimicrobial potential of pomegranate peel extract (PPE) and concluded that the efficacy of PPE in scavenging hydroxyl and superoxide anion radical was very high. In addition, the extract had good reducing power and iron chelation capacity and showed good antimicrobial activity against *Staphylococcus aureus* and *Bacillus cereus*, having a minimum inhibitory concentration of only 0.01%. *Pseudomonas* species could be also inhibited at a higher concentration of 0.1%, while PPE was ineffective against *Escherichia coli* and *Salmonella typhimurium*. Thus, PPE could potentially be included in several industrial products (e.g. as ingredient in functional foods), due to its versatile functional properties. After addition of PPE at a concentration of 800-850 ppm in sunflower oil [22] and 200-1,000 ppm in fish oil [23] high stabilization efficiency was exhibited, which was comparable to that achieved by conventional synthetic antioxidants (i.e. BHT used at its maximum allowed concentration). Similarly, Kumudavally et al. [24] and Devatkal et al. [25] reported that PPE significantly increases the stability of beef and goat meat products against lipid peroxidation. Furthermore, addition of PPE to jams [26], juices and wines [27] increased their phenolic, flavonoid, and thiol concentration with a significant improvement of the free radical scavenging and product stability features. In addition, Kaderides et al. [28], incorporated pomegranate peel extract in hazelnut paste and reported an inhibition of lipid oxidation with reduced formation of peroxides.

Many more references in the literature point out the potential of pomegranate pomace or peel extract to substitute synthetic antioxidants and antimicrobials. Its exceptional bioactivity is largely attributed to the presence of punicalagin, one of the main polyphenols of pomegranate peel [29-32].

The production of high quality bioactive natural
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extracts depends on the extraction method and on the conditions that maximize the concentration of the bioactive compounds in the final extract. For this reason, new eco-green extraction methods have been used and optimized in order to produce effective, natural extracts from organic agro-food byproducts like pomegranate pomace [3, 21, 33]. The main “green” extraction technologies which are nowadays available in the market at reasonable price are microwave assisted extraction and ultrasound assisted extraction, which have certain advantages, compared with conventional extraction methods, such as: less quantity of solvent, better retention of the bioactivity of the extracted polyphenols, lower operation temperatures and less energy consumption. These two technologies can also involve operation under vacuum, which is preferable for preserving the bioactivity of the polyphenols and prevents their oxidative degradation during the extraction process, thus yielding an extract of high quality. Kaderides et al. [21] suggested that, between these two “green” extraction technologies microwave technology is more advantageous compared to ultrasound technology, since it can provide 1.7 times higher polyphenol concentration in the extract in about half the time needed for ultrasound assisted extraction.

In the context of the present work, raw (not dried) pomegranate pomace was extracted with water (as solvent) using a lab scale microwave extractor and the effectiveness of the extraction was mathematically modeled and optimized by response surface methodology and centered Box & Behnken experimental design with three experimental factors, namely: (1) extraction temperature (2) extraction time and (3) water/solid ratio, using three optimization responses (quality indicators) of pomegranate pomace extract, namely (a) the total polyphenols (expressed as gallic acid equivalents) in mg/L of pomegranate extract, (b) the total flavonoids expressed as quercetin equivalents in mg/L of pomegranate extract; and (c) the total antioxidant activity, expressed as the IC50 value of the DPPH test in mg/L of pomegranate extract.

The target of the present work was to obtain the optimum conditions in terms of extraction temperature, extraction time and water to pomegranate pomace ratio in order to get maximum amount of polyphenols and/or flavonoids and/or maximum total antioxidant activity. This information can then be used in the scale up and industrial production of high added value bioactive aqueous pomegranate extracts in an economically viable manner, by utilizing a widely available agricultural by-product, which would otherwise become food waste. The produced aqueous pomegranate pomace extracts could be used either as natural antioxidants (e.g. the extracts with minimized IC50-DPPH values), or as natural antimicrobials (e.g. the extracts with maximum polyphenols and flavonoids content) targeting food, cosmetic and phyto-protection applications (e.g. green/organic antifungals). This reuse and valorization of organic waste would also improve the carbon footprint of the pomegranate juice production industry.

2. Materials and Methods

2.1 Pomegranate Pomace

The pomegranate pomace was derived from the pomegranate fruit variety “Wonderful” and was kindly supplied by the Greek pomegranate juice producer, ALBERTA S.A. (Argos, Peloponnese-Greece). Before use, the obtained pomace was grinded to 3 mm size using a commercial meat mincer (model CANDY COMET supplied by D. Tomporis Co, 92 Cyprus str, Larisa, Greece) in order to be finely comminuted and then it was kept in properly sealed vacuum polypropylene plastic bag (2 kg/bag) at -25 °C until it was used for extraction. Drying was not applied to the pomegranate pomace in order to avoid oxidative degradation of the bioactive compounds.

2.2 Description of the Microwave Extractor and of the Extraction Methodology
The extraction of the pomegranate pomace samples was conducted by using the Lab Scale microwave extractor NEOS-GR/Milestone Technologies which is established in the premises of PELLAS NATURE Co (Edessa, Greece) and it is illustrated in Fig. 1.

The extraction trials of the pomegranate pomace samples were conducted following the procedure described below. The frozen pomegranate samples were first thawed at ambient temperature and 100 g of each sample was then collected and used as the extraction sample. The 100 g pomegranate pomace sample was mixed with distilled water in the PYREX glass beaker (of either 2 L or 5 L capacity, depending on the quantity of the total mixture). The quantity of the water used in each trial was according to the water/solid ratio suggested by the experimental plan (shown below). The loaded beaker was then adjusted on the TEFLOW base in the extractor, and the desired values of the extraction temperature and time were set via the electronic panel of the extractor, according to the experimental plan, and the lab scale extractor was set in automatic operation. The progress of the extraction was monitored by using a camera and agitation was applied by using the relevant facility of the extractor. After the end of each extraction trial the extract was collected, filtered in plain filter paper and the filtrate was filled in plastic bottles and coded accordingly in order to distinguish different samples. The collected samples were kept frozen at -25 °C in the Laboratory of Food and Biosystems Engineering (University of Thessaly) for a short period until the selected bioactivity parameters were analyzed.

2.3 Total Polyphenols Determination Method

For the determination of the total polyphenols as GAE (gallic acid equivalents) of the obtained pomegranate extracts, a slightly modified version of the method of Singleton et al. [34] and Waterhouse [35] was used. According to this method, initially a gallic acid solution was prepared by dissolving 0.5 g gallic acid in 10 mL pure ethanol and the solution was then transferred in a 100 mL volumetric flask and the rest of the volume was filled by distilled water (preparation of a gallic acid stock solution of 5,000 ppm). In addition, in a 1 L glass beaker, 200 g of anhydrous sodium carbonate was dissolved in 800 mL distilled water and the solution was boiled until the salt was fully dissolved. The solution was then cooled and kept at 24 h in dark, which resulted in the formation of crystals of anhydrous sodium carbonate, which were removed by filtration the next day. The

Fig. 1 The setup of the Lab Scale microwave extractor NEOS-GR/Milestone Technologies.
clear filtrate was finally dissolved in a total volume of 1 L by adding the remaining distilled water in a 1 L volumetric flask. Consequently, a set of standards of gallic acid was prepared by diluting 0 mL, 1 mL, 2 mL, 3 mL, 5 mL, 10 mL, and 20 mL of the gallic acid stock solution in six volumetric flasks of 100 mL each and filled with distilled water up to 100 mL volume in order to prepare standard solutions of 0, 50, 100, 150, 250, 500 and 1,000 ppm gallic acid. From each standard solution a quantity of 20 μL was mixed with 1.58 μL distilled water and 100 μL Folin Ciocalteu reagent in a glass tube and within 8 min a quantity of 100 μL sodium carbonate solution was added and the tubes were incubated for 2 h at 20 °C, after which their absorbance was measured by a UV-Vis photometer (model EVOLUTION™ 201 supplied by Thermo-Scientific Co, Shanghai, China) against the blind solution (0 ppm gallic acid concentration). The standard curve depicting gallic acid concentration vs. absorbance was constructed using the Microsoft Excel software and its $R^2$ value was 0.9982. Calculation of the total polyphenols of extracts of pomegranate pomace was carried out following the same procedure and using the following equation of the standard curve:

$$\text{Total polyphenol concentration of extract in ppm of GAE} = \frac{\text{Absorbance at 765 nm}}{0.001} \quad \text{Eq. (1)}$$

Before each respective measurement the relevant pomegranate extract had diluted to 1:30 dilution in order to have expressed the extracted polyphenols to the same final volume and thus the obtained values to be directly comparable and to mark the achieved extraction of polyphenols from the pomace matrix. Each measurement concerning total polyphenols was carried out in triplicate and the result was the average of the three obtained values.

### 2.4 Total Flavonoids Determination Method

The total flavonoids content expressed as mg of QE L$^{-1}$ of the obtained pomegranate pomace extracts was determined by using the colorimetric method of AlCl$_3$, as described by Chandra et al. [36]. The method is based on the principle that AlCl$_3$ reacts with the hydroxyls of the flavonoids and produces a colored complex which has maximum absorbance at 420 nm. The total flavonoids content was expressed as quercetin equivalents (QE) per L of extract. The determination method for the total flavonoids was carried out as below: 1.0 mL of the pomegranate pomace extract or standard solution (used for the construction of the calibration curve) was added in a glass test tube to which 3 mL methanol, 200 μL of aqueous solution of 10% w/v AlCl$_3$, 200 μL 1 M potassium acetate solution and 5.6 mL distilled water were added. The tube was then agitated by vortex and incubated for 30 min at ambient temperature for the completion of the chemical reaction. The absorbance of each sample was measured at 420 nm against a blind solution which contained all the reagents except for the pomegranate pomace extract which was replaced by distilled water.

For the construction of the calibration curve, a quercetin stock solution of 1,000 ppm was prepared as well as a series of standard solutions of 50, 100, 200, 500 and 1,000 ppm by serial dilutions of the stock. The absorbance of standard solutions was measured and plotted against their concentration and the linear equation obtained by Excel was used for the determination of the concentration of the total flavonoids of the pomegranate pomace extracts. The $R^2$ value of the obtained linear correlation was 0.9834.

Calculation of the total flavonoids of extracts of pomegranate pomace was carried out following the same procedure and using the following equation of the standard curve:

$$\text{Total flavonoids concentration mg QE/L of extract} = \frac{\text{Absorbance at 420 nm}}{0.0055} \quad \text{Eq. (2)}$$

Each measurement concerning total flavonoids was carried out in triplicate and the result was the average of the three obtained values.

### 2.5 Total Antioxidant Capacity (TAC) Determination Method
The colorimetric method of DPPH was used for the determination of the total antioxidant capacity of the pomegranate pomace extracts, as described by Brand-Williams et al. [37].

According to the method, the free-radical scavenging capacity (RSC) of both the standards and the pomegranate extracts was evaluated by DPPH radical assay [37].

Briefly, a 1.0 mL of freshly prepared methanolic solution of DPPH radical (100 mM) was mixed with the tested samples at various concentrations (0-30 mg/mL). The contents were vigorously mixed, incubated at room temperature in the dark for 20 min, and the absorbance was measured at 517 nm. The measurement was conducted on a Thermo-Scientific spectrophotometer (Model Evolution 201). In each experiment, the solution of the tested sample in methanol (without DPPH) was used as blank and a DPPH solution in methanol as control.

The percentage RSC of the tested extracts was calculated using the following equation:

\[
\text{RSC} \, (\%) = \left( \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \right) \times 100 \quad \text{Eq. (3)}
\]

where, \( A_{\text{control}} \) and \( A_{\text{sample}} \) are the absorbance values of the control and the tested sample, respectively.

Moreover, in order to compare the radical scavenging efficiency of the extracts and effectively the total antioxidant capacity, the IC50 value was calculated (from the graph of % RSC vs. extract concentration), which represented the concentration needed for 50% scavenging of the DPPH radical. All experiments were carried out in triplicate to ameliorate possible measurement errors and the average values were taken.

2.6 Chemicals Used for Antioxidant Tests

All the chemicals used for the above mentioned antioxidant tests were purchased by Sigma Aldrich and supplied by Life Sciences Chemilab (Thessaloniki, Greece).

2.7 Modeling and Optimization Methodology

The methodology used for optimization of the pomegranate pomace extraction had the following aims:

- Optimization of the total polyphenol content;
- Optimization of the total flavonoids content;
- Optimization of the total antioxidant capacity (TAC);
- Simultaneous optimization of total polyphenols and total flavonoids and total antioxidant capacity (TAC) of pomegranate extracts.

A central composite Box & Behnken experimental design was used to select the experimental points along with response surface methodology (RSM) to obtain the 3rd order mathematical models for total polyphenols, total flavonoids and total antioxidant capacity of the pomegranate extracts and predict the optimum values. Three factors were used as optimization factors and in particular: (a) the \( w/w \) ratio of extraction water to pomegranate solid in a range from 5 to 30; (b) the extraction temperature in a range of 40 °C to 80 °C; and (c) the extraction time in a range of 30 min to 90 min, as well as four responses (quality parameters): (a) total polyphenol content; (b) total flavonoids content; (c) total antioxidant activity; and (d) total polyphenols and total flavonoids simultaneously. The Design Expert 7.0.0 statistical software was used to perform process optimization. Third order (cubic) polynomial mathematical models were adopted and a forward regression technique, which proved effective in order to obtain reliable models. The reliability of the obtained cubic models was validated by Statistical analysis (ANOVA), which (in all cases) proved the soundness and robustness of this type of models.

3. Results

3.1 Modeling and Optimization of the Total Polyphenol Content of the Pomegranate Extracts

The results of the total polyphenol content of the pomegranate extracts are presented in Table 1. In particular, three respective microwave extraction
experiments were carried out for each one of the seventeen (17) sets of Box & Behnken Design experimental conditions (51 experiments in total) and the total polyphenol contents of each one of the obtained three extracts were determined. The average values of these are listed in Table 1.

By using the Design Expert software Version 7.0.0 the data presented in Table 1 for total polyphenols were analyzed by RSM (Surface Response Methodology) and the mathematical modeling yielded the following total pomegranate polyphenols model equation:

\[
\text{Total pomegranate polyphenols (mg/L)} =
-2,500.39700 + 51.51013 \times \text{ratio} + 104.70958 \times \text{temperature} + 29.96111 \times \text{time}0.53867 \times \text{ratio} \\
\times \text{temperature} + 0.12756 \times \text{ratio} \times \text{time}0.30736 \\
\times \text{temperature} \times \text{time} - 0.46715 \times \text{ratio}^2 - 0.7139 \times \text{temperature}^2 + 0.090472 \times \text{time}^2 + (9.97917E–003) \times \text{temperature}^2 \times \text{time} \\
\text{Eq. (4)}
\]

Furthermore, by ANOVA statistical analysis, it was disclosed that the developed model is significant while its lack of fitness is not significant which implies that the developed model is a good tool for prediction of total pomegranate polyphenols in the extracts as a function of the three experimental factors involved.

In addition, according to ANOVA analysis, the $R^2$ value = 0.9797 is very high and very close to the adjusted $R^2$ = 0.9458, which means that there is very good prediction by the developed model equation. The effectiveness of the model prediction is also supported by the data presented in Fig. 2, where the predicted values of the central linear plot are very close to the actual values of total polyphenols concentration.

Furthermore, in Figs. 3-5 the plots illustrate the paired interactions of the factors A = water/solid ratio, B = extraction temperature and C = extraction time on the total polyphenols extraction yield.

| A/A Water to solid ratio | Temperature (°C) | Extraction time (min) | *Concentration of total pomegranate polyphenols, expressed as gallic acid equivalents (mg/L) | *Concentration of Total flavonoids in the extract expressed as quercetin equivalent (mg/L) | *IC50-DPPH Total Antioxidant activity of pomegranate extract (ppm) |
|-------------------------|-----------------|-----------------------|---------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------|---------------------------------------------------------------|
| 1 5.00 80 60.00         | 1,067.333 ± 19.009 | 727.273 ± 4.340 | 137.333 ± 2.517                                                                 |
| 2 17.50 60 60.00        | 1,104.333 ± 6.028  | 576.364 ± 6.345  | 103.000 ± 15.620 Adamite                                                     |
| 3 5.00 60 90.00         | 941.333 ± 24.194  | 641.818 ± 7.456  | 139.333 ± 4.041                                                                 |
| 4 5.00 60 30.00         | 1,005.000 ± 35.341 | 821.818 ± 10.345 | 153.667 ± 9.452                                                                 |
| 5 30.00 60 90.00        | 1,329.667 ± 57.274 | 558.182 ± 3.000  | 75.667 ± 12.014                                                                 |
| 6 17.50 40 90.00        | 1,284.000 ± 56.321 | 774.545 ± 3.678  | 101.000 ± 5.568                                                                 |
| 7 17.50 60 60.00        | 1,115.000 ± 13.229 | 687.273 ± 6.034  | 100.667 ± 12.220                                                                |
| 8 17.50 80 90.00        | 1,280.333 ± 44.501 | 710.909 ± 7.943  | 83.333 ± 4.726                                                                 |
| 9 17.50 80 30.00        | 1,140.667 ± 4.041  | 598.182 ± 2.560  | 91.333 ± 19.140                                                                 |
| 10 30.00 80 60.00       | 1,030.000 ± 2.000  | 507.273 ± 3.004  | 118.000 ± 1.000                                                                 |
| 11 30.00 60 30.00       | 1,202.000 ± 16.000 | 589.091 ± 4.502  | 93.667 ± 16.921                                                                 |
| 12 5.00 40 60.00        | 684.667 ± 25.070   | 852.727 ± 5.006  | 166.000 ± 1.000                                                                 |
| 13 30.00 40 60.00       | 1,186.000 ± 4.000  | 710.909 ± 6.123  | 76.000 ± 5.292                                                                 |
| 14 17.50 40 30.00       | 880.667 ± 85.143   | 587.273 ± 5.967  | 153.000 ± 28.054                                                                |
| 15 17.50 60 60.00       | 1,150.333 ± 85.212 | 600.000 ± 1.234  | 107.667 ± 8.737                                                                 |
| 16 17.50 60 60.00       | 1,048.000 ± 91.329 | 603.636 ± 2.367  | 111.667 ± 13.796                                                                |
| 17 17.50 60 60.00       | 1,137.667 ± 22.234 | 541.818 ± 2.376  | 101.333 ± 9.018                                                                 |

* The values were determined after dilution of all extracts at the same 30/1 water to solids ratio and they were the average of triplicate determination.
Fig. 2 Correlation of predicted vs. actual values of total pomegranate polyphenols.
Fig. 3  Response surface plot of total pomegranate polyphenols.

(A × B interaction): A (water/solid ratio) × B (extraction temperature).
Fig. 4  Response surface plot of total pomegranate polyphenols (A × C interaction).

(AC): A (water/solid ratio) × C (extraction time (min)).
Using the developed RSM cubic model and the Expert Design 7.0.0 statistical package, the maximum value of the pomegranate total polyphenols was achieved at the following conditions:

- Water to solid pomegranate pomace ratio = 29.95
- Extraction Temperature: 40 °C
- Extraction Time: 90 min

The maximum total pomegranate polyphenols concentration achieved at the above optimized conditions was 1,526.87 mg/L (or 1.527 g/L).

3.2 Modeling and Optimization of the Total Flavonoids Content of the Pomegranate Extracts

The results of the total flavonoids content of the pomegranate extracts are presented in Table 1. In particular, three respective microwave extraction experiments were carried out for each one of the seventeen (17) sets of Box & Behnken Design experimental conditions (51 experiments in total) and the total flavonoids contents of each one of the obtained three extracts were determined and the average values of them were listed in Table 1.

By multiplying 1,000 times the values of the total pomegranate flavonoids concentrations presented in Table 1 and calculating the inverse values of the results and by applying mathematical modeling by RSM methodology using the Design Expert Statistical software, a reliable mathematical model was constructed to simulate the response $1/1,000 \times$ total pomegranate flavonoids as a function of the three
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The model equation (Eq. (5)) has the following form:

\[
\frac{1}{(1,000 \times \text{total pomegranate flavonoids concentration in mg QE L}^{-1} \text{ of extract})} = (+4.47549E–006) + (1.24715E–007) \times \text{ratio} - (1.22747E–007) \times \text{temperature} - (8.34419E–008) \times \text{time} - (1.54848E–009) \times \text{ratio} \times \text{temperature} + (2.84121E–009) \times \text{temperature} \times \text{time} - (3.74325E–009) \times \text{ratio}^2 + (1.08763 –009) \times \text{temperature}^2 + (5.45961E–011) \times \text{ratio}^2 \times \text{temperature} - (2.31676E-011) \times \text{temperature}^2 \times \text{time}
\]

Furthermore, by ANOVA statistical analysis, it was disclosed that the developed model is significant while its lack of fitness is not significant which implies that it is a good tool for prediction of total pomegranate flavonoids in the extracts as a function of the three experimental factors involved.

The \( R^2 \) value = 0.8767 is very high and reasonably close to the adjusted \( R^2 \) = 0.7182 which means there is very good prediction by the developed model equation. The robustness of model prediction is additionally supported by the data presented in Fig. 6 where the predicted vs. actual values of total pomegranate flavonoids are correlated and all the individual points of actual measurement fit very well with the predicted central linear plot.

Furthermore, in Figs. 7 and 8 the plots illustrate the paired interactions of the factors A = water/solid ratio, B = extraction temperature, C = extraction time on the total flavonoids extraction yield of the pomegranate pomace. The optimized extraction conditions appear to be:

- Water to solid pomegranate pomace ratio = 5.06
- Extraction temperature: 40 °C
- Extraction time: 89.09 min

The minimum inverse (1,000 \times total pomegranate flavonoids concentration) achieved at the above optimized conditions is: 9.8088 \times 10^{-7} mg/L, which corresponds to a total pomegranate flavonoids value of 1,020 mg/L (or 1.02 g/L).

3.3 Modeling and Optimization of the IC50-DPPH Total Antioxidant Capacity of the Pomegranate Extracts

The results of the total antioxidant capacity of the pomegranate extracts expressed as IC50 value are presented in Table 1. In particular, three respective microwave extraction experiments were carried out per each one of the seventeen (17) sets of Box & Behnken Design experimental conditions (51 experiments in total) and the IC50-DPPH total antioxidant capacity of each one of the obtained three extracts was determined and the average value was listed in Table 1.

By using the average IC50 values presented in Table 1 and applying mathematical modeling with RSM methodology using the Design Expert Statistical software, a reliable mathematical model was constructed to correlate the model response of IC50-DPPH total antioxidant capacity of the pomegranate extracts to the three model factors: (a) water to solid ratio; (b) extraction temperature (°C); and (c) extraction time (min).

The model equation has the following form:

Total antioxidant capacity (DPPH/IC50) of pomegranate extract (ppm) =

\[+618.31233 - 6.44760 \times \text{ratio} - 8.46083 \times \text{temperature} - 9.94200 \times \text{time} + 0.070667 \times \text{ratio} \times \text{temperature} - 0.054089 \times \text{ratio} \times \text{time} + 0.17278 \times \text{temperature} \times \text{time} + (6.93333E-004) \times \text{ratio}^2 + 0.013812 \times \text{temperature}^2 + 0.073639 \times \text{TIME}^2 + (1.47556E-003) \times \text{ratio}^2 \times \text{time} - (1.28704E-003) \times \text{temperature} \times \text{time}^2\]

Eq. (6)

Furthermore, by ANOVA statistical analysis, it was disclosed that the developed model is significant while its lack of fitness is not significant, which implies that it can serve as useful tool for the prediction of Total Antioxidant Capacity (IC50-DPPH parameter) of pomegranate extracts as a function of the three experimental factors involved.
Fig. 6  Correlation of predicted vs. actual values of total pomegranate flavonoids.
Fig. 7  Response surface plot of total pomegranate flavonoids (A × B interaction).
A (water/solid ratio) × B (extraction temperature (°C)).
The $R^2$ value = 0.9909 is very high and very close to the adjusted $R^2 = 0.9708$, which means that there is very good prediction by the developed model equation. The effectiveness of model prediction is additionally supported by the data presented in Fig. 9 where the predicted vs. actual values of total antioxidant activity (IC50/DPPH) of the pomegranate extracts are correlated and most of the individual actual measurements overlap to a great extent with the central linear plot of predicted values.

Furthermore, in Figs. 10-12, the plots illustrate the paired interactions of the factors $A =$ water/solid ratio, $B =$ extraction temperature, $C =$ extraction time with the total antioxidant capacity of the pomegranate pomace extract expressed as IC50-DPPH.

Furthermore, based on the developed RSM cubic model and the Expert Design 7.0.0 statistical package, the minimum value of the IC50-DPPH parameter, which corresponds to the maximum value of the total antioxidant capacity of the pomegranate extracts, was obtained at the following extraction condition:

- Water to solid ratio = 29.98
- Extraction temperature: 41 °C
- Extraction time: 78.94 min
Fig. 9  Correlation of predicted vs. actual values of total antioxidant capacity of pomegranate extracts.
Fig. 10  Response surface plot of the total antioxidant activity of pomegranate extracts. (A × B interaction): A (ratio of water to solids) × B (extraction temperature (°C)).
Fig. 11  Response surface plot of the total antioxidant activity of pomegranate extracts. 
(A × C interaction): A (ratio of water to solids) × C (extraction time (min)).
3.4 Simultaneous Optimization of Total Polyphenols Content and IC50/DPPH of Pomegranate Extracts

According to the Expert Design 7.0.0 statistical analysis, the simultaneous maximum point of total polyphenol content and maximum value of Total Antioxidant Capacity (minimum IC50 value) of the pomegranate extracts occurred at the following extraction conditions:

- Water to solid pomegranate pomace ratio = 30
- Extraction temperature: 40 °C
- Extraction time: 89.04 min

In this case of simultaneous optimization of two quality parameters (polyphenol content and antioxidant activity), the maximum value of total polyphenols content of pomegranate extract reached 1,515.3 mg/L (1.5 g/L), while the minimum value of IC50-DPPH was 73.539 ppm (mg/L).

3.5 Simultaneous Optimization of Total Polyphenols Content and Total Flavonoids Content of Pomegranate Extracts

In a similar manner, using the Design Expert 7.0.0 statistical program, the simultaneous maximum point of total polyphenol content and total flavonoids content of the pomegranate extracts were investigated. The methodology which was used in order to obtain
the desired common optimum of both parameters was based on the capability of the Design Expert to perform optimization with double targets and in particular to find out the set of optimal extraction parameters which converges as much as possible on the total polyphenols maximum (1,526.27 mg/L) and the total flavonoids maximum (1,020 mg/L). Following statistical analysis (using Design Expert 7.0.0) of the data presented in Table 1 for total polyphenols and total flavonoids, the two parameters were maximized simultaneously and the following optimum extraction conditions were obtained:

- Water to solid pomegranate pomace ratio = 30
- Extraction temperature: 40 °C
- Extraction time: 88.19 min

After adopting the above extraction conditions, the maximum value of the total polyphenols content of pomegranate extract was 1,503.8 mg/L (98.6% of the independent maximum of total polyphenols reached in all processes), while the maximum value of the total flavonoids was 847 ppm (mg/L) (83% of the independent maximum of total flavonoids of all processes).

3.6 Simultaneous Optimization of Total Flavonoids Content and Total Antioxidant Capacity IC50-DPPH of Pomegranate Extracts

In addition, the mutual optimization of total flavonoids content and total antioxidant capacity (IC50-DPPH) of pomegranate extracts were examined via the Design Expert software, so that total flavonoids will converge on the optimum obtained value of total flavonoids (1,020 mg/L of quercetin equivalents) and total antioxidant capacity IC50-DPPH will be close to the minimum obtained value of 70.124 ppm (mg/L). Statistical processing of the data presented in Table 1 for total flavonoids and IC50-DPPH allowed the simultaneous optimization of both total flavonoids and total antioxidant capacity, which was achieved in the following extraction conditions:

- Water to solid pomegranate pomace ratio = 30
- Extraction temperature: 40 °C
- Extraction time: 88.19 min

In this case the actual maximum value of total flavonoids content of pomegranate extract reached 847.1 mg/L (83.1% of the independent maximum of total flavonoids), while a minimum value of 73 ppm (mg/L) was obtained in relation to the IC50-DPPH value (which corresponds to 97.3% of the independent maximum of IC50-DPPH total antioxidant capacity).

3.7 Simultaneous Optimization of Total Polyphenols Content, Total Flavonoids Content and Total Antioxidant Capacity IC50-DPPH of Pomegranate Extracts

Furthermore, the mutual optimization of the total polyphenols content, total flavonoids content and total antioxidant capacity IC50-DPPH of pomegranate extracts were carried out using the Design Expert software for the above three quality parameters. The aim was that total polyphenols content, total flavonoids and total antioxidant capacity will all converge on the optimum values of total polyphenols (1,526.27 mg/L), total flavonoids (1,020 mg/L) and total antioxidant capacity (IC50-DPPH =70.124 ppm), respectively. Statistical processing of the data of Table 1 for total polyphenols, total flavonoids and IC50-DPPH total antioxidant capacity revealed that the above parameters could all be maximized simultaneously under the following extraction conditions:

- Water to solid pomegranate pomace ratio = 30
- Extraction temperature: 40 °C
- Extraction time: 88.19 min

In these extraction conditions, the maximum value of total polyphenols content reached 1,503.8 mg/L (98.61% of the independent maximum of total polyphenols), total flavonoids content was 847.1 mg/L (83.1% of the independent maximum of total flavonoids) and total antioxidant capacity of pomegranate extract corresponded to IC50-DPPH value of 73 ppm (mg/L) (97.3% of the independent maximum of IC50-DPPH...
Table 2  Optimum values of antioxidant parameters as a function of extraction parameters.

| Optimization target | Optimized extraction conditions | Optimized antioxidant parameters |
|---------------------|--------------------------------|----------------------------------|
|                     | Water/pomegranate pomace ratio | Extraction temperature (°C) | Extraction time (min) | Maximum total poly-phenols content (ppm) | Maximum total flavonoids content (ppm) | Optimum maximum antioxidant capacity IC50-DPPH (ppm) |
| Total polyphenols   | 29.95                          | 40                              | 90                   | 1,526.87                                    |                                      |                                      |
| Total flavonoids    | 5.06                           | 40                              | 89.09                | 1,020                                        |                                      |                                      |
| Total antioxidant capacity IC50-DPPH | 29.98          | 41                              | 78.94                | 70.124                                       |                                      |                                      |
| Total polyphenols + total flavonoids | 30                        | 40                              | 88.19                | 1,503.8                                      | 847.0                                 | 73.54                                 |
| Total polyphenols + total antioxidant Capacity IC50/DPPH | 30                        | 40                              | 89.04                | 1,515.3                                      | 73                                    |                                      |
| Total flavonoids + total antioxidant capacity IC50/DPPH | 30                        | 40                              | 88.19                | 1,503.8                                      | 847.1                                 | 73                                    |
| Total polyphenols + flavonoids + TAC IC50/DPPH | 30                        | 40                              | 88.19                | 1,503.8                                      | 847.1                                 | 73                                    |

Table 2 summarizes the recommended optimum extraction conditions and the predicted optimum values of response (antioxidant capacity values) independently, or as a combination of two or three responses.

3.8 Validation of the Mathematical Models Developed to Predict the Total Polyphenols Content, Total Flavonoids Content and Total Antioxidant Capacity IC50-DPPH of Pomegranate Extracts

For the validation of the developed mathematical models, five respective sets of values of the experimental conditions evenly spread within the experimental grid of points and different values that have been used in order to develop the models, were selected. Consequently, for each one of them, microwave assisted extraction was carried out and the total polyphenols concentrations, the total flavonoids concentration as well as the total antioxidant capacity IC50-DPPH of the obtained pomegranate extracts were measured and corrected to the same dilution water/solids 30:1 while the developed models were used to predict the same values.

The experimentally obtained values as well as the predicted values of the three above mentioned antioxidant parameters of pomegranate extracts are listed in Table 3 along with the calculated % mean error of prediction.

Based on the calculated values of % mean error of prediction for the three antioxidant parameters of pomegranate extracts (3.85% for the total polyphenols, 4.14% for the total flavonoids and 6.99% for the total antioxidant capacity expressed as IC50/DPPH), it is concluded that this error appears relatively small and this confirms the effectiveness of the produced mathematical models.

In addition, a statistical paired $t$-test was performed between the measured and the predicted values of the three respective antioxidant properties of the pomegranate pomace extracts listed in Table 3. According to the $t$-test results, which are summarized in Table 4, there was found no statistically significant difference between the measured and predicted values of the antioxidant parameters, which is an additional proof of the precision of the produced predictive models.
Table 3  Validation data for the developed mathematical models.

| A/A | Water/solid ratio | Extraction temp. (°C) | Extraction time (min) | PM (1) | PP (2) | Error (%) | FM (3) | FP (4) | Error (%) | MIC50 (5) | PIC50 (6) | Error (%) |
|-----|-------------------|-----------------------|-----------------------|--------|--------|-----------|--------|--------|-----------|-----------|-----------|-----------|
| 1   | 10                | 50                    | 45                    | 936.3  | 919.3  | 1.82      | 610.3  | 684.8  | 12.21     | 131.4     | 143.2     | 8.98      |
| 2   | 10                | 70                    | 45                    | 1,048.6| 1,093.6| 4.29      | 687.5  | 680.2  | 1.06      | 119.2     | 124.6     | 4.53      |
| 3   | 10                | 70                    | 75                    | 1,081.2| 1,077.8| 0.31      | 631.7  | 654.6  | 3.63      | 111.5     | 118.2     | 6.01      |
| 4   | 25                | 50                    | 45                    | 1,014.3| 1,128.8| 11.29     | 597.2  | 610.7  | 2.26      | 106.7     | 98.2      | 7.97      |
| 5   | 25                | 70                    | 45                    | 1,124.2| 1,141.5| 1.54      | 578.4  | 569.4  | 1.56      | 93.8      | 100.8     | 7.46      |

Mean error of the prediction (%): 3.85, 4.14, 6.99

(1) PM: Measured concentration of total polyphenols of pomegranate pomace;
(2) PP: Predicted concentration of total polyphenols of pomegranate pomace (Eq. (4));
(3) FM: Measured concentration of total flavonoids of pomegranate pomace;
(4) FP: Predicted concentration of total flavonoids of pomegranate pomace (Eq. (5));
(5) MIC50: Measured total antioxidant capacity IC50/DPPH of pomegranate extracts;
(6) PIC50: Predicted total antioxidant capacity IC50/DPPH of pomegranate extracts (Eq. (6));

% Mean error = absolute value of (predicted value-measured value) × 100 / measured value.

Table 4  Results of the statistical t-test analysis applied between predicted and measured concentration values of total polyphenols, total flavonoids as well as total antioxidant capacity (IC50-DPPH).

| A/A | Pair | t     | df   | Statistical significance (2-tailed) |
|-----|------|-------|------|-----------------------------------|
| 1   | Pair 1 PM - PP | -0.6126 | 4 | \( p = 0.55715 > 0.005 \) (not significant) |
| 2   | Pair 2 FM - FP | -0.65453 | 4 | \( p = 0.531134 > 0.005 \) (not significant) |
| 3   | Pair 3 MIC50-PIC50 | -0.4323 | 4 | \( p = 0.676943 > 0.005 \) (not significant) |

(1) PM: Measured concentration of total polyphenols of pomegranate pomace;
(2) PP: Predicted concentration of total polyphenols of pomegranate pomace (Eq. (4));
(3) FM: Measured concentration of total flavonoids of pomegranate pomace;
(4) FP: Predicted concentration of total flavonoids of pomegranate pomace (Eq. (5));
(5) MIC50: Measured total antioxidant capacity IC50/DPPH of pomegranate extracts;
(6) PIC50: Predicted total antioxidant capacity IC50/DPPH of pomegranate extracts (Eq. (6)).

4. Discussion

According to the results of the present study the maximum amount of the polyphenols extracted by microwave assisted extraction is 1,526.87 mg/L of obtained pomegranate extract. Furthermore, as all the measured extracts, are 1:30 diluted and the pomegranate pomace amount is 100 g the total volume of the extract is 3 L and this means that the quantity of the total extracted polyphenols is 3 L × 1,526.87 mg/L × 10 = 45,788 mg (or 45.8 g) of gallic acid equivalents (GAE) per kg of fresh pomegranate pomace. In the same way, the quantity of the total flavonoids extracted is: 3 L × 1,020 mg/L × 10 = 30,600 mg (or 30.6 g) of quercetin equivalents per kg of raw pomegranate pomace. Furthermore, the determination of total polyphenols and total flavonoids of the raw material (pomegranate pomace) used in the present study yielded 47,123 mg of gallic acid equivalents (GAE) per kg of fresh pomegranate pomace and 32,456 mg of quercetin equivalents per kg of fresh pomegranate pomace. These values correspond to extraction yields of 97.2% for the total polyphenols and 94.3% for total flavonoids which is an additional proof of the effectiveness of the optimization applied in the present work.

Zheng et al. [38] studied the aqueous microwave-assisted extraction and antioxidant activity of total phenolic compounds from pomegranate peel and found out that the optimum extraction yield for total polyphenols was 214,000 mg GAE kg\(^{-1}\) of pomegranate peel and the optimum value of total...
antioxidant activity of pomegranate extracts was found to be 14.52 mg/L of pomegranate extract.

In addition, Wang et al. [3] studied the aqueous pomegranate pomace extraction and concluded that the optimized yield of the total polyphenols extracted was 140,000 mg GAE g\(^{-1}\) of pomegranate pomace, while the yield of extracted total pomegranate flavonoids was 10,000 mg of quercetin equivalents (QE) kg\(^{-1}\) of pomegranate peel. Elfalleh et al. [11] experimented with the aqueous extraction of pomegranate peel and reported a yield of 53,650 mg GAE kg\(^{-1}\) total polyphenols from pomegranate peel extract as well as a yield of 21,030 mg of flavonoids as rutin equivalents (RE) per kg of pomegranate peel. Amyrgialaki et al. [39] achieved a total polyphenol extraction yield of 324,900 mg GAE kg\(^{-1}\) of pomegranate solid waste, by using water/ethanol extraction of pomegranate solid waste. In addition, Huang et al. [40] used water/ethanol mixtures as solvent in order to study the total flavonoids of microwave assisted extraction of pomegranate peel. They observed that the maximum yield of flavonoids reached 42,600 mg GAE kg\(^{-1}\) of pomegranate peel, while the optimum DPPH-IC50 value of the extracts was 187 mg/L.

Kim et al. [13] performed aqueous extraction of pomegranate pomace and found out that the optimum yield of the extracted total polyphenols was 53,650 mg GAE kg\(^{-1}\) of pomegranate pomace, while the optimum yield of the extracted total flavonoids was 21,030 mg of quercetin equivalents (QE)/kg of pomegranate peel.

Dimou et al. [13] performed aqueous extraction of pomegranate pomace and found that the optimum yield of the extracted total polyphenols was 53,650 mg GAE kg\(^{-1}\) of pomegranate pomace, while the optimum yield of the extracted total flavonoids was 21,030 mg of quercetin equivalents (QE)/kg of pomegranate peel.

Kaderides et al. [21] found out that only 4 min in aqueous ethanol solution is needed to recover polyphenols from pomegranate peel with a high yield (199,400 mg GAE kg\(^{-1}\) dry peel), whereas conventional extraction procedures needed much longer extraction times (up to 60 times).

Furthermore, Kennas et al. [42] conducted research on the extraction of pomegranate peel antioxidants with various solvents and according to their results, in the case of water solvent, the optimum yield of extracted total polyphenols was as high as 242,050 mg GAE kg\(^{-1}\) of pomegranate peel, while the yield of extracted total flavonoids was 11,500 mg of quercetin equivalents (QE) kg\(^{-1}\) of pomegranate peel. On the other hand, in the course of the same study the optimum of IC50-DPPH of the obtained extracts was found to be 184 mg/L.

From the above literature it is concluded that with regards to the optimum yield of the polyphenols content for aqueous extraction the results are in the range of 53,650-242,050 mg of GAE kg\(^{-1}\) of pomegranate pomace and therefore the optimum results obtained at the present study (45,806 mg of GAE kg\(^{-1}\) of raw pomegranate pomace) are close to the lower limit of this range. This can be attributed to the fact that in all these studies the raw material was dry powder with large contact surface and not slurry material like in the case of the present study. Despite the lower yield in our case we must consider the fact that the drying of pomace is a costly process, not only because of high investment costs, but also because of high operation and maintenance cost. Furthermore, regarding the optimum aqueous extraction yield of the total flavonoids of the pomegranate pomace, it is obvious that in the case of the present study the obtained yield of 30,600 mg of quercetin equivalents kg\(^{-1}\) of pomegranate extract is by far higher than the 10,000-21,030 mg of quercetin equivalents kg\(^{-1}\) of pomegranate pomace reported previously probably due to the fact that we did not employ drying which destroys easily the flavonoids.
Finally, the optimum total antioxidant activity (IC50 values of DPPH scavenging) of the pomegranate extract in the present study was found to be 70.124 mg/L, which is considerably higher than the 14.52 mg/L obtained by Zheng et al. [38] and Juhar et al. [41] and much lower than the 184 mg/L reported by Kennas et al. [42]. In the first case, the higher antioxidant activity (lower value of IC50) can be justified by the considerably higher polyphenol yield (214,000 mg GAE kg⁻¹ of pomegranate extract), while in the second case it appears that the higher optimum antioxidant activity obtained in the present study is not related to the higher polyphenol content, but it can be attributed instead to the much higher yield of extracted flavonoids. Also, another possible reason for this difference in the above studies could be the difference in the varieties of the used pomegranate and the quality of the used solid waste of the pomegranate juice industry. Shahidi & Naczk [43] have pointed out that the quality characteristics of pomegranate solid waste extracts are dependent on the origin of the raw materials which affect the polyphenol and flavonoid content of the obtained extracts. Yet another potential factor that could affect the yield of total pomegranate pomace polyphenols and flavonoids, and accordingly the total antioxidant activity of the extracts, is the kind of the extraction equipment and the mode of mass transfer introduced by a particular extractor in order to obtain the extracts. For this reason as an extension of the present work a comprehensive study will be carried out by using an industrial size microwave extractor operating under vacuum in the near future.

5. Conclusions

In the present work, for the first time, the production of bioactive pomegranate pomace extracts from raw fresh pomace (instead of dry pomace or dry peel powder) was investigated and optimized. This can improve the economics of the pomegranate extracts production because it omits the drying and milling steps required for industrial production when dry pomegranate pomace or peel is used as raw material. According to the results obtained in the present study, antioxidant extracts can be produced by a “green” aqueous microwave extraction process from raw pomegranate pomace at specific conditions which were optimized by response surface methodology and Box & Behnken experimental design. The optimum extraction yield for total polyphenols was found to be 45,788 mg of GAE kg⁻¹ of fresh pomace, while for total flavonoids the optimum extraction yield reached 30,600 mg of quercetin equivalents kg⁻¹ of fresh pomegranate pomace. The corresponding optimum values of the extraction parameters for the above mentioned maximum polyphenol and flavonoids extraction yields were: (a) extraction temperature = 40 °C, water/solid ratio= 1:29.95, and extraction time = 90 min for maximum total polyphenols; and (b) extraction temperature = 40 °C, water/solid ratio= 1:5.06, and extraction time = 89.09 min for maximum total flavonoids. Practically, a reduction of ratio of water to solids improves the yield of flavonoids instead of polyphenols. Furthermore, the optimum of total antioxidant activity (70.124 mg/L of pomegranate extract) was achieved at extraction temperature = 41 °C, water/solid ratio = 1:29.98, and extraction time = 78.94 min.

In addition, the mathematical models which were developed to predict the extraction yields of total polyphenols, total flavonoids and total antioxidant capacity of the obtained pomegranate pomace extracts were found to be of cubic form and further they were validated for their effectiveness to predict correctly the antioxidant properties of the pomegranate pomace extracts demonstrating non-significant prediction errors.

It is also worth noticing that the set of extraction parameters of extraction temperature = 40 °C, water/solid ratio = 1:30, and extraction time = 89.19 min could be an effective compromise in order to apply simultaneously optimized conditions for the total polyphenols content, total flavonoids content and
total antioxidant capacity (IC50-DPPH) of the pomegranate pomace extract. Yet another important conclusion, based on the literature review and on the results of the present study, is the large variability of the extraction yield of total phenolics and flavonoids as well as total antioxidant capacity of pomegranate extracts. This can be attributed to the varying composition and pre-extraction processing of the raw material, as well as to differences in the extraction technology. This, in turn, makes it necessary to optimize extraction conditions at industrial scale, using the specific raw materials available in each industry and following the successful statistical optimization methodology described in detail in the present work.

Author Contributions

The individual contribution of the authors are: (a) Konstantinos Petrotos, supervision and modeling participation in the writing; (b) Ioannis Giavasis, participation in the writing and data analysis; (c) Konstantinos Gerasopoulos, measurement of antioxidant parameters; (d) Chrysanthe Mitsagga, measurement of antioxidant parameters; (e) Chryssoula Papaioannou, experimental work microwave assisted extraction; (d) Paschalis Goutsidis, experimental work microwave assisted extraction.

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Conflicts of Interest

The authors declare no conflict of interest.

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