Enrichment of Hazelnut Oil with Several Polyphenols: An Alternative Approach to A New Functional Food

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Abstract: Hazelnut oil has been examined according to its oxidative stability and antioxidant activity. The oil sample has been treated with gallic acid, ascorbic acid, catechin, vanillic acid, p-coumaric acid and rutin. Stability of the pure and treated oils against the oxidation has been assessed via Rancimat by detecting the protection factor. The quality parameters of the oil samples were compared depending on their antioxidant activity. D-Optimal design of Response Surface Method has been applied to optimize the enrichment conditions of hazelnut oil with several polyphenols. Principal component analysis has been applied to comprehend the relationship between the groups and their quality parameters. Depending on the analysis of variance test, the most important parameter (at $p < 0.0001$) affecting the relevant system has been found polyphenol type with respect to stability and antioxidant capacity. Gallic acid has enhanced the stability of hazelnut oil against oxidation ~3 times over that of pure sample. The maximum yields of protection factor, antioxidant activity and dissolved polyphenol level have been 2.738, 46.14% and 259.424 ppm under the optimum conditions (300 ppm gallic acid).

Key words: hazelnut oil, functional food, oxidative stability, D-optimal design, principal component analysis

1 Introduction

Antioxidants are the components that prevent the damages in food by preventing the oxidation by giving the hydrogen in their structures to the free radicals formed as a result of oxidation in the beginning and spreading stages of autoxidation that occurs with various effects in foods¹. Antioxidants added to edible oils increase the thermal stability of the products, and protect them from thermal degradation. Since the safety of synthetic antioxidants is controversial due to their possible toxic effects during long-term intake, it seems reasonable to try to replace them with natural antioxidants that are more compatible with human nature. Synthetic antioxidants such as BHA (butylated hydroxyanisole), BHT (butylated hydroxytoluene) and TBHQ (tertbutylhydroquinone) have started to be replaced by natural antioxidants³. As a result of this situation; researchers have begun intensive research on natural antioxidants. On the other hand, this fat-containing food products with high added-value compounds might be approved as functional foods due to the increase in their quality and nutritional value³. Treatment of vegetable oils with polyphenols, known as natural antioxidants has been gaining interest due to the concept of health benefit¹, 4–8.

The current study focuses on the improvement of the oxidative stability and antioxidant capacity of hazelnut oil. It has been selected as research material due to the fact that this oil has been attracting more and more attention. Because hazelnut oil has relatively superior nutritious properties. The most striking difference of the current oil from the other types is its tocopherol content. Additionally, it also contains fatty acids such as oleic and linoleic acid, which are special for human nutrition⁹. On the other hand, D-optimal design (DOD) through Response Surface Method (RSM) has been also applied to optimize the enrichment conditions (polyphenol type and addition amount of polyphenol) for the maximum oxidative stability and bioactive properties. DOD provides evaluation of categoric factors as well as numeric factors affecting a process. Additionally, this design of RSM is more practical when the time and cost means are restricted¹⁰. When the literature on statisti-
2 Experimental Section

2.1 Materials

Hazelnut oil sample was purchased from a local market. Ethanol (>99.5%), methanol (>99.8%) and hexane (>99%) were from Merck (Darmstadt, Germany), while ABTS (2,2′-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) diammmonium salt) (>98.0%), glacial acetic acid (>99.0%), acetonitrile (>99.9%), gallic acid (>98.0%), ascorbic acid (>99.0%), catechin (>98.0%), vanillic acid (>97.0%), p-coumaric acid (>98.0%) and rutin hydrate (>94.0%) were from Sigma-Aldrich (St. Louis, MO, USA).

2.2 Treatment of hazelnut oil with polyphenols

Ascorbic acid, catechin, vanillic acid, p-coumaric acid and rutin were selected as additives to enhance the stability of hazelnut oil against oxidation. 150, 225 and 300 ppm of polyphenols were applied into the selected oil (Table 1). The polyphenols were homogenized (IKA T25, ULTRATURRAX, Staufen, Germany) in the hazelnut oil under the optimum conditions, which were identified in our previous paper[4].

2.3 Chromatographic separation of polyphenols

The chromatograph was from Thermo Fisher Scientific Finnigan Surveyor HPLC system equipped with a Surveyor HPLC binary pump, a column thermostat, a Finnigan Surveyor photo-diode array detector, Symmetry C18 analytical column (4.6 mm x 250 mm, 5 μm), and a Hamilton 25 μL-syringe were used for chromatographic measurements. Data acquisition was accomplished using Thermo/ Dionex Chromelone 7. The analytical wavelengths of detection were 340 nm (flavonoids) and 285 nm (phenolic acids) for recording conventional.

To analyze phenolic compounds in hazelnut oil samples, the mobile phase consisted of two solvents, i.e., acetonitrile (A) and 1% of glacial acetic acid in bidistilled water (B). The polyphenolic antioxidants were analyzed using gradient elution: Vsample = 20 μL; flow rate = 1 mL/min; column temperature: 35°C; detection wavelength: 340 nm and 285 nm: 0. min 10% A – 90% B; 5. min 15% A – 85% B (curve 6.0); 11. min 20% A – 80% B (curve 2.0); 22. min 25% A – 75% B (curve 6.0); 30. min 60% A – 40% B (curve 6.0); 35. min 10% A – 90% B (curve 6.0). Using the above working mode, the calibration curves in the form of linear equations of peak area versus concentration were constructed for the antioxidants of interest.

2.4 Antioxidant capacity measurement

Antioxidant capacity of the treated and pure hazelnut oils were measured by the inhibition of the ABTS (2,2′-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) diammmonium salt) radical. 3 μL of diluted ABTS solution was added into 30 μL of sample, and left for incubation for 5 minutes. The absorbance value was measured at 734 nm. The ability to inhibit free radicals was calculated using the following equation[4]:

$$\text{Antioxidant activity (}\%\text{ inhibition)} = \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \times 100$$  \hspace{1cm} (1)

Acontrol represents the absorbance of the diluted ABTS solution, and A sample represents the absorbance of the sample.

2.5 Oxidative stability measurement

As an accelerated method, Rancimat test was used for the measurement of the oil’s stability against oxidation. The equipment (Rancimat 892, Metrohm, Herisau, Switzerland) was set to 140°C by providing the conditions under

Table 1 Operation parameters of the enrichment of the hazelnut oil with several polyphenols.

| Name            | Unit | Type    | Level | L[1] | L[2] | L[3] | L[4] | L[5] | L[6] |
|-----------------|------|---------|-------|------|------|------|------|------|------|
| Polyphenol      | ppm  | Continuous | 6     | 150  | 300  |      |      |      |      |

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20 L of air per hour to 3 g of oil sample\(^5\).

Induction time (IT) is utilized as an indicator for oxidative stability of the fat-containing foods. Protection factor (PF) was also exploited to evaluate the differences of the effects of applied polyphenols in the oil:

\[
P_F = \frac{\text{IT}_{\text{Treated}}}{\text{IT}_{\text{Untreated}}} \tag{2}
\]

\(\text{IT}_{\text{Treated}} = \) Induction time of treated oil with polyphenol
\(\text{IT}_{\text{Untreated}} = \) Induction time of untreated oil

2.6 Statistical experimental design

D-Optimal design of Response Surface Method was exploited to design the experimental study as well as modeling and optimization of the enrichment conditions (Table 1). Additionally, analysis of variance (ANOVA) test was used to statistically analyze the model fitting and the interaction between the parameters by means of Design-Expert\textsuperscript{®} version 11 (Stat-Ease, Minneapolis, MN, USA) software trial\textsuperscript{10}.

2.7 Principal component analysis

Principal component analysis (PCA) is a statistical technique, providing a better understanding and visualization of complex data sets. It also helps us to understand the relationship between groups and variables and between the variables themselves\textsuperscript{10}. In the present study, there are 7 groups with untreated hazelnut oil. Their antioxidant activity, phenolic content and oxidative stability were assessed by applying PCA method for the visualization the correlations of the data set. XLSTAT 2019 (trial version) was employed for the relevant statistical analysis.

2.8 Statistical analysis

ANOVA statistical test of Tukey-Kramer multiple comparisons test of InStat\textsuperscript{®} software (GraphPad, San Diego, CA, USA) was also operated to analyse the means of three replicates. Additionally, Pearson correlation coefficient (r) between PF and antioxidant activity of the oils was calculated via InStat\textsuperscript{®} software.

3 Results and Discussions

3.1 Quality parameters of the treated and untreated hazelnut oils

Table 2 represents the amount of stability, antioxidant activity and dissolved polyphenols of the treated oil samples under several conditions. Table 3 gives the conventional HPLC linear calibration equations, retention times and working ranges of polyphenols dissolved in the hazelnut oil.

Oxidative stability and antioxidant activity of the pure hazelnut oil is also given as control reasons (Table 2). PF is a kind of description of the oil’s resistance to oxidation reaction\textsuperscript{2}. Depending of this parameter, gallic acid increased the oil stability against oxidation remarkably (almost 3 times). This output was followed by catechin. Ascorbic acid, rutin and vanillic additions demonstrated statistically the same \((p > 0.05)\) increase in oxidative stability. However, \(p\)-coumaric acid decrease the stability of the oil as seen in Table 2. Findings on the stability factors are in good agreement with antioxidant capacity of the hazelnut oil. Actually, the correlation between the PF and antioxidant activity is already a sign of this result (Fig. 1). Pearson correlation coefficient (r) was found to be 0.9591 at 95% confidence interval \((0.9045 \text{ to } 0.9828)\), where number of point was 23. On the other hand, coefficient of determination \((r^2)\) was 0.9199. There was no correlation between the dissolved polyphenols and antioxidant activity/protection factor.

As already seen in Table 2, gallic acid has statistically different results, which surpassed the other polyphenols. The \(P\) value is <0.001. Similar effects of gallic acid were also observed by Artajo et al. (2006)\textsuperscript{16}, Kurtulbaş et al. (2018)\textsuperscript{17}, and Gülmez & Şahin (2019)\textsuperscript{18} for the extending the shelf-lives of olive, cottonseed and hazelnut oils, respectively. This success was explained by the ring structure of gallic acid, which has 3,4,5-trihydroxy groups. This structure let this polyphenol easily dislocate its hydrogen.

Catechin was also observed as a prosperous antioxidant in the extension of the stability of the oil against oxidation (Table 2). This result is expectable since it has been applied into the lipid containing food products to prevent lipid oxidation, and recommended as novel additive with at least 300 ppm concentration in the related products\textsuperscript{17}. It increased the antioxidant activity of the hazelnut oil 5 to 7 times depending on its concentration. McCarthy et al. showed its efficacy on pork meat as higher than those of Vitamin E and commercial antioxidants such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT)\textsuperscript{18, 19}. Addition of catechin into raw beef increased the oxidation stability of the product more comparing to Vitamin C as observed for hazelnut oil samples in the present study\textsuperscript{20}.

Ascorbic acid also known as Vitamin C was used for the extension of various food products (beef steaks, dark chicken meat, sunflower oil), by enhancing their antioxidant capacities with successful results as in the current study\textsuperscript{21-23}. Similar to our results, Zhang et al. (2018) also reported the same order \((\text{catechin} > \text{rutin} > \text{BHA} > \text{BHT})\) for the antioxidant activity enhancement of pecan oil\textsuperscript{24}. In another study, rutin was reported to be more effective antioxidant comparing to BHA and BHT in delaying of lipid oxidation of canola oil\textsuperscript{25}. Regarding vanillic acid as natural antioxidant additive, Vaisali et al. (2016)\textsuperscript{26} reported that it had a mild pro-oxidant effect on the pecan oil\textsuperscript{27}. However, it failed to increase the antioxidant activity of the sardine oil comparing to other phenolic acids such as gentisic acid and protochatechuic acid due to the hydroxyl group location for the substitution. Our output is in consistent with
Table 2  Effects of the factors on the oxidative stability, antioxidant activity and polyphenol enrichment (responses) of treated hazelnut oil*.

| Run | Factor 1 A: Addition (ppm) | Factor 2 B: Polyphenol | Response 1 PF | Response 2 Antioxidant activity (% inhibition) | Response 3 Polyphenol (ppm) |
|-----|--------------------------|------------------------|--------------|-----------------------------------------------|----------------------------|
|     | Control sample           | 1 ± 0.00               | 3.52 ± 0.00  | –                                             | –                          |
| 1   | 300                      | Ascorbic acid          | 1.088 ± 0.03 | 6.88 ± 0.00 *                                  | 128.81 ± 0.00              |
| 2   | 300                      | Catechin               | 1.614 ± 0.05 | 24.92 ± 0.01                                  | 285.77 ± 0.00              |
| 3   | 150                      | Gallic acid            | 1.894 ± 0.06 | 31.46 ± 0.02 b                                 | 136.10 ± 0.00              |
| 4   | 150                      | Ascorbic acid          | 1.047 ± 0.01 | 2.27 ± 0.00                                    | 66.20 ± 0.00               |
| 5   | 300                      | Ascorbic acid          | 1.088 ± 0.02 | 6.88 ± 0.00 *                                  | 127.00 ± 0.00              |
| 6   | 150                      | Coumaric acid          | 0.824 ± 0.00 | 3.70 ± 0.00                                    | 82.67 ± 0.00               |
| 7   | 225                      | Rutin                  | 1.111 ± 0.01 | 6.80 ± 0.00                                    | 184.97 ± 0.00              |
| 8   | 225                      | Ascorbic acid          | 1.053 ± 0.03 | 4.61 ± 0.00                                    | 102.53 ± 0.00              |
| 9   | 225                      | Coumaric acid          | 0.929 ± 0.00 | 2.66 ± 0.00                                    | 154.24 ± 0.00              |
| 10  | 150                      | Catechin               | 1.203 ± 0.02 | 18.46 ± 0.02                                  | 141.83 ± 0.00              |
| 11  | 300                      | Rutin                  | 1.065 ± 0.01 | 8.22 ± 0.00                                    | 233.56 ± 0.00              |
| 12  | 225                      | Gallic acid            | 2.094 ± 0.09 | 39.68 ± 0.03                                  | 153.04 ± 0.00              |
| 13  | 300                      | Coumaric acid          | 0.947 ± 0.01 | 3.10 ± 0.00                                    | 254.00 ± 0.00              |
| 14  | 300                      | Vanillic acid          | 1.052 ± 0.04 | 6.21 ± 0.00                                    | 217.12 ± 0.00              |
| 15  | 150                      | Vanillic acid          | 1.013 ± 0.00 | 5.20 ± 0.00                                    | 131.00 ± 0.00              |
| 16  | 150                      | Gallic acid            | 1.894 ± 0.05 | 31.46 ± 0.02 b                                 | 136.10 ± 0.00              |
| 17  | 300                      | Coumaric acid          | 0.947 ± 0.00 | 11.49 ± 0.00                                  | 254.91 ± 0.00              |
| 18  | 300                      | Catechin               | 1.614 ± 0.00 | 24.92 ± 0.00                                  | 287.83 ± 0.00              |
| 19  | 225                      | Vanillic acid          | 1.02 ± 0.02  | 5.37 ± 0.00                                    | 153.00 ± 0.00              |
| 20  | 225                      | Catechin               | 1.353 ± 0.04 | 22.4 ± 0.00                                    | 176.34 ± 0.00              |
| 21  | 300                      | Gallic acid            | 2.818 ± 0.09 | 45.76 ± 0.01                                  | 271.55 ± 0.00              |
| 22  | 150                      | Rutin                  | 1.052 ± 0.03 | 6.29 ± 0.00 *                                  | 109.81 ± 0.00              |
| 23  | 150                      | Rutin                  | 1.052 ± 0.01 | 6.30 ± 0.00 *                                  | 107.99 ± 0.00              |

* ± represents the standard deviation of three replicates. Lines for each column sharing the same letter shows statistical similar results at p > 0.05.

Table 3  Conventional HPLC linear calibration equations, retention times and working ranges of phenolic compound expected to be present in hazelnut oil samples to be analyzed.

| Polyphenol   | t<sub>r</sub> (min) | Working range (ppm) | Calibration equation* | Correlation coefficient (r) |
|--------------|---------------------|---------------------|------------------------|-----------------------------|
| Ascorbic acid| 2.63                | 17.61 – 176.10      | y = 17942.31 c + 16794 | 0.9999                      |
| Gallic acid  | 3.34                | 1.70 – 17.00        | y = 50552.55 c – 7870  | 0.9966                      |
| Catechin     | 6.20                | 11.61 – 145.13      | y = 13057.26 c + 4474  | 0.9997                      |
| Vanillic acid| 7.71                | 1.68 – 16.80        | y = 36874.03 c + 16034 | 0.9992                      |
| p-Coumaric acid | 11.49             | 1.64 – 16.40       | y = 118258.71 c + 11652 | 0.9999                      |
| Rutin        | 13.56               | 6.1 – 61.0          | y = 29180.33 c + 48900 | 0.9992                      |

* c = ppm
the concerned previous report. As seen in Table 2, vanillic acid increased the oxidative stability even though it was not so efficient to improve the antioxidant capacity of the oil (only ≈ 1.5 times).

3.2 D-Optimal design and modelling

As already mentioned before, Table 2 gives the protec-

The failure of p-coumaric acid might be attributed to methoxy substitution, which decreases the effect of free radical scavenging. Structural properties of hydroxycinnamic acids would normally dictate that one –OH bearing hydroxycinnamic acid (ferulic acid (TEAC<sub>CUPRAC</sub> = 1.2)) should exhibit higher TEAC (Trolox<sup>®</sup> equivalent antioxidant capacities) coefficient than one –OH bearing p-coumaric acid (TEAC<sub>CUPRAC</sub> = 0.6). Since, ferulic acid having an electron donating methoxy group (–OCH<sub>3</sub>) in ortho-position relative to the phenolic –OH, thereby allowing increased stabilization of the resulting aryloxyl radical through electron delocalization after H-atom donation by the –OH group, it should show a higher TEAC coefficient than p-coumaric acid which lacks such a group. Thus structural requirements dictate that p-coumaric acid has the lower TEAC value. Therefore, it demonstrated pro-oxidant activity in the hazelnut oil (Table 2).

**Table 4** Analysis of variance test for the D-optimal design for the enrichment of hazelnut oil with several polyphenols.

| Source     | Sum of Squares | df  | Mean Square | F-value | p-value |
|------------|----------------|-----|-------------|---------|---------|
| PF         | Model          | 5.07| 12          | 0.4221  | 87.44   | <0.0001 significant |
|            | A-Addition     | 0.2726| 1          | 0.2726  | 56.48   | <0.0001          |
|            | B-Polyphenols  | 4.61| 5           | 0.9218  | 190.96  | <0.0001          |
|            | AB             | 0.3937| 5          | 0.0787  | 16.31   | 0.0002           |
|            | A<sup>2</sup>  | 0.0075| 1          | 0.0075  | 1.56    | 0.2402           |
|            | Residual       | 0.0483| 10         | 0.0048  |         |                 |
|            | Pure Error     | 0.0000| 5          | 0.0000  |         |                 |
|            | Cor Total      | 5.11| 22          |         |         |                 |
| Antioxidant activity | Model | 3815.28| 12         | 317.94  | 1797.72 | <0.0001 significant |
|            | A-Addition     | 89.85| 1           | 89.85   | 508.01  | <0.0001          |
|            | B-Polyphenols  | 3715.81| 5         | 743.16  | 4202.04 | <0.0001          |
|            | AB             | 92.28| 5           | 18.46   | 104.35  | <0.0001          |
|            | A<sup>2</sup>  | 0.0054| 1          | 0.0054  | 0.0308  | 0.8643           |
|            | Residual       | 1.77| 10          | 0.1769  |         |                 |
|            | Pure Error     | 0.0000| 5          | 1.000E-05|         |                 |
|            | Cor Total      | 3817.05| 22        |         |         |                 |
| Polyphenol | Model          | 95559.97| 12        | 7963.33 | 35.77   | <0.0001 significant |
|            | A-Addition     | 59321.32| 1         | 59321.32| 266.49  | <0.0001          |
|            | B-Polyphenols  | 31897.21| 5         | 6379.44 | 28.66   | <0.0001          |
|            | AB             | 5741.14| 5         | 1148.23 | 5.16    | 0.0134           |
|            | A<sup>2</sup>  | 1308.36| 1         | 1308.36 | 5.88    | 0.0358           |
|            | Residual       | 2225.98| 10        | 222.60  |         |                 |
|            | Pure Error     | 5.83| 5           | 1.17    |         |                 |
|            | Cor Total      | 97785.95| 22        |         |         |                 |
tion factor stands for the oxidative stability, antioxidant activity and dissolved polyphenol after the enrichment under several conditions designed by DOD through RSM. Table 4 stands for the statistical findings of the related system depending on the ANOVA test of DOD of RSM. The first response is stabilization factor. Its second-order model in terms of coded factors is given by Eq.3:

\[
P_F = 1.26 + 0.1258A - 0.2176B(1) + 0.1029B(2) + 0.9941B(3) - 0.2595B(4) - 0.2269B(5) - 0.1108AB(1) + 0.0809AB(2) + 0.3161AB(3) - 0.1063AB(4) - 0.1078AB(5) + 0.0418A^2
\] (3)

\(p < 0.0001\) and \(F\)-value of 87.44 for the model (Eq.3) are sign of significance. Additionally, if \(p\)-value is less than 0.05 \((p<0.05)\), it means that the terms of the model are statistically important. Depending on this evaluation, polyphenol type was found the most effective parameter, followed by addition amount and interaction of these two as seen in Table 4 at \(p<0.0001\). Interaction between these two parameters was also statistically significant to affect the current system (Table 4).

Based on the ANOVA fit statistics, coefficient of variation \(R^2\) was calculated to be 0.9906, while adjusted \(R^2\) was 0.9792. \(R^2\) and adjusted \(R^2\) seems close to each other, showing a satisfactory relationship between the experimental and predicted data (Fig. 2)\(^{21}\). On the other hand, predicted \(R^2\) was 0.9021, which is in consistent with adjusted \(R^2\), since the difference between predicted and adjusted is \(<0.2\).

Regarding Response 2 (antioxidant activity), the final equation in terms of coded factors for is stated by Eq.4 as written below:

\[
Antioxidant \text{ activity } = 13.59 + 2.30A - 6.43B(1) + 8.32B(2) + 25.34B(3) - 7.97B(4) - 8.46B(5) - 1.39AB(1) + 0.8653AB(2) + 4.94AB(3) - 1.80AB(4) - 0.6483AB(5) - 0.0356A^2
\] (4)

Similarly, polyphenol type was found the most significant factor, followed by addition amount and interaction of these two as seen in Table 4 (at \(p<0.0001\)). Figure 3 shows the good agreement between the experimental and predicted data, where \(R^2\), adjusted \(R^2\) and predicted \(R^2\) are 0.9995, 0.9990 and 0.9950, respectively. Eq.5 has been produced for the Response 3 as shown below:

\[
Polyphenol = 154.02 + 60.27A + 8.28B(1) + 37.26B(2) + 23.27B(3) + 1.40B(4) - 68.16B(5) + 4.89AB(1) + 14.08AB(2) + 4.42AB(3) - 17.21AB(4) - 31.51AB(5) + 17.44A^2
\] (5)

The derived model from DOD was also found statistically influential to make predictions about the response for given levels of each factor (Table 4). Polyphenol quantity \(p<0.0001\) was followed by polyphenol type \(p<0.0001\), interaction and second power of quantity as the statistically important model terms \(p<0.05\). As already seen in Fig. 4, there is a sufficient relation between the actual and predicted results, where \(R^2\), adjusted \(R^2\) and predicted \(R^2\) are 0.9772, 0.9499 and 0.7658, respectively. Actually, predicted \(R^2\) of 0.7658 is acceptable, since the difference between

![Fig. 2](image-url) Diagnostics graphs for protection factor drawn by Design-Expert® Software.

![Fig. 3](image-url) Diagnostics graphs for antioxidant activity drawn by Design-Expert® Software.
predicted $R^2$ and adjusted $R^2$ is less than 0.2.

3.3 Optimization

The optimum conditions to make the maximum yields of PF (2.738), antioxidant activity (46.14%) and dissolved polyphenols (259.424 ppm) have been reached with 300 ppm gallic acid treatment. Depending on the validation study, there was a poor error (less than 2.5%) between the real and predicted values (2.75 PF, 45% inhibition of ABTS radical and 255.00 ppm gallic acid addition).

3.4 Principal component analysis

PCA was also applied to investigate the effects of PF and antioxidant activity of pure and treated hazelnut oils under various polyphenol levels (150, 225 and 300 ppm). It is possible to examine the structure of multidimensional different and complex data in graphical form via PCA. Figure 5 was created as a biplot graph.

As seen in Fig. 5a, the first component explained 88.77% of the total variability while the second component explained 11.23% of the remaining variability. Hazelnut oil treated with gallic acid is characterized by PF whereas the oil treated with coumaric acid is characterized by antioxidant activity. However, pure hazelnut oil, and hazelnut oil enriched with rutin, catechin, ascorbic acid and vanillic acid are not designated by protection factor and antioxidant activity.

When the addition of polyphenol was 225 ppm, the first component explains 85.95% of the total variability (Fig. 5b). Second component describes 14.05% of the remaining variability. In this PCA analysis, it is seen that hazelnut

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**Fig. 4** Diagnostics graphs for dissolved polyphenol drawn by Design-Expert® Software.

**Fig. 5** Principal component analysis of protection factor and antioxidant activities of treated and untreated hazelnut oils at 150 ppm (a), 225 ppm (b) and 300 ppm (c) of addition.
oil treated with gallic acid is characterized by only PF. Furthermore, none of the active observers can be explained by either PF or antioxidant activity. As for 300 ppm addition of polyphenols (Fig. 5c), the first component accounts for 77.49% of the total variability, and the second component describes 22.51% of the remaining variability. As seen in Fig. 5c, protection factor characterizes the hazelnut oil enriched by gallic acid, whilst antioxidant activity defines the oil treated with coumaric acid. However, none of the active observers can be explained by either protection factor or antioxidant activity.

4 Conclusions
The findings of this study indicate that addition of 300 mg of gallic acid into one liter of the hazelnut oil subscribes to oil stability against oxidation problems along with the remarkable improvement of its antioxidant activity. On the other hand, the proposed second-order models of D-optimal design has been decided to be convincing based on the other hand, the proposed second-order models of D-optimal design has been decided to be convincing based on the statistical indicators such as $p<0.0001$, $R^2$ and adjusted $R^2 >0.94$. Furthermore, there was a convincing correlation between the oxidative stability and ABTS scavenging activity in the oil depending on the Pearson correlation ($r = 0.9591$). PCA shows that hazelnut oil enriched by gallic acid is characterized by protection factor, while hazelnut oil treated with coumaric acid is described by antioxidant activity.

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Conflict of Interest
The authors declare that there is no conflict of interest in writing upon submission of the manuscript.

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