NÖHÜ Müh. Bilim. Derg. / NOHU J. Eng. Sci., 2022; 11(4), 945-951

Niğde Ömer Halisdemir Üniversitesi Mühendislik Bilimleri Dergisi
Niğde Ömer Halisdemir University Journal of Engineering Sciences

Araştırma makalesi / Research article

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Bioactive component extraction from broccoli (*Brassica oleracea* L. var. *italica*) and optimization by Taguchi method

Brokoliden (*Brassica oleracea* L. var. *italica*) biyoaktif bileşen ekstraksiyonu ve Taguchi yöntemi ile optimizasyonu

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Abstract

In the study, bioactive component extraction was performed using the classical extraction method from broccoli. In the classical extraction method, the highest total phenolic substance (TPS), antioxidant activity (AOX) value and ascorbic acid content (AAC) determined were 690.94 mg GAE/kg dry matter (20°C/15min), 5.08 mg/ml (20°C/15 min) and 66.74 mg/100g dry matter (20°C/15 min), respectively. Extraction conditions were determined by using the Taguchi method. Accordingly, the best process conditions with the highest total phenolic substance, antioxidant activity and ascorbic acid value in the extraction were proved by the Taguchi method as 20°C/15min. When the colour values were examined, it was determined that the temperature had a significant effect on the colour values obtained in broccoli extract.

Keywords: Antioxidant activity, ascorbic acid, broccoli, extraction, phenolic compound

Öz

Yapılan çalışmada brokoliden klasik ekstraksiyon metodu kullanılarak biyoaktif bileşen ekstraksiyonu yapılmıştır. Klasik ekstraksiyon yönteminde, belirlenen en yüksek toplam fenolik madde, antioksidan aktivite değeri ve askorbik asit miktarı, sırasıyla 690.94 mg GAE/kg kuru madde (20°C/15dk), 5.08 mg/ml (20°C/15 dk) ve 66.74 mg/100g (20°C/15 dk) olarak tespit edilmiştir. Yapılan ekstraksiyonda Taguchi yöntemi kullanılarak ekstraksiyon koşulları belirlenmiştir. Buna göre ekstraksiyonda toplam fenolik madde, antioksidan aktivite ve askorbik asit değerinin en iyi olduğu proses koşulları 20°C/15dk olarak Taguchi metodu ile de kanıtlanmıştır. Renk değerleri incelendiğinde brokoli ekstrakstında elde edilen renk değerleri üzerinde sıcaklığın önemli bir etkisinin olduğu tespit edilmiştir.

Anahtar kelimeler: Antioksidan aktivite, askorbik asit, brokoli, ekstraksiyon, fenolik madde

1 Inroduction

Broccoli (*Brassica oleracea* L. var *italica*) is a vegetable belonging to the cabbage family (Brassicaceae) that is consumed raw or boiled [1, 5]. Broccoli, which has many positive effects in terms of a healthy life, can be consumed raw or boiled [6]. Broccoli contains vitamins, flavonoids, and antioxidant compounds as well as phytochemicals such as indole, sulforaphane isothiocyanate, and glucosinolates [7]. Broccoli is rich in minerals such as Ca, P and K, and vitamins A and C, as well as folic acid [8, 9]. Selenium-containing broccoli contains antioxidants and protects red blood cells from damage caused by oxidation and strengthens the immune system [10]. While broccoli is grown in a continental climate in summer, it is grown in autumn in a Mediterranean climate [11].

Phenolic compounds are a characteristic compound for plants. It has been observed that, thanks to its regular consumption, diseases such as cancer [12,13], high cholesterol [14], coronary heart [15], cataracts, diabetes [16] and aging can be prevented [17]. In addition to these, phenolic compounds have positive effects such as lowering blood pressure and regulating the permeability of the capillary circulation system [18].

Extraction is the process of taking the components in the solid or liquid phase into the liquid phase by utilizing the solubility properties of a substance at the appropriate pressure and temperature [19]. A desired extraction process must be efficient, fast, reliable and environmentally friendly. In the extraction process where high efficiency is desired, there are many variable parameters such as solvent type, solvent ratio (water/solvent), pressure, temperature, time, solid-liquid ratios [20,21]. The most commonly used solvents are methanol, ethanol, acetone, and hexane to extract bioactive compounds from solid material [19].

In classical extraction studies, testing all alternatives requires high time and cost [22]. In the studies carried out, it is aimed to reach the lowest cost and the highest efficiency in the shortest time. For this purpose, optimization techniques have been developed. One of these optimization techniques is the Taguchi method [23,24]. In this study, the classical extraction method was applied to dried broccoli samples to extract bioactive compounds and the independent variables, temperature and time parameters, were optimized with the Taguchi technique.

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Geliş / Recieved: 24.05.2022 Kabul / Accepted: 06.07.2022 Yayımlanma / Published: 14.10.2022
doi: 10.28948/ngmuh.1120580

2 Material and method

Broccoli plant was obtained from the local market in Niğde during its season. They were first washed, cleaned, cut into pieces, then dried with a freeze dryer (Scanvac Coolsafe 95-15 Pro, Denmark) at -100°C at 0.01 mbar pressure and stored at -18°C in moisture-proof glass containers (Arçelik, Turkey). Dry broccoli was used in the extractions.

2.1 Classical extraction

For the dried samples, 80% methanol (containing 1% hydrochloric acid) was used as a solvent for the extraction of phenolic substances. After weighing 4 g of the dried broccoli samples on a precision scale, it was put into a 200 ml flask. 40 ml of 80% methanol mixture was added on it. The extraction process was carried out on a magnetic stirrer at 20, 40 and 60°C for 15, 30 and 45 minutes. The extracts obtained after filtration with coarse filter paper were stored in an airtight bottle at -18°C in the refrigerator until the analysis [25].

2.2 Total phenolic substance (TPS)

TPS determination was performed according to the Folin-Ciocaltaeu method. 100 µl of the sample was completed with 0.75 ml of Folin-Ciocaltaeu solution (10% in water) and kept at room temperature for 5 minutes. 0.75 ml of Na_2CO_3 (in water, 75g/L) was added and mixed rapidly. They were kept in the dark for 1.5 hours at room temperature and then the absorbance values of the samples at 725 nm were read in the spectrophotometer. Gallic acid was used as a standard, the same procedures was repeated and it was prepared for the calibration curve and applied to different concentrations of gallic acid solutions. TPS concentration calculated on dry matter basis as gallic acid equivalent value (mg/kg GAE) [25].

2.3 Colour

Colour determination equipment (Konica Minolta CR400, Japan) was used to determine the colour properties of broccoli extracts. Liquid samples were placed in a cuvette and L*, a* and b* values were measured [25].

2.4 Antioxidant activities (AOX)

The determination of free radical scavenging efficiency was made using the 1.1-diphenyl-2-picrylhydrazil radical according to the method of Blois and by going through the steps applied by Brand-Williams et al. [26,27]. In this study, the DPPH radical removal effect of broccoli extracts was expressed by calculating EC_{50} values.

2.5 Ascorbic acid content (AAC)

High performance liquid chromatography (Shimadzu, LC-20A/ Prominence, Columbia, USA) was used for ascobic acid analysis. A reversed phase C-18 column (5µm particle size, 4.6 mm diameter, 250 mm length) was used for the analyses. A mixture of methanol and water (1 mL/min) prepared at a rate of 10:30 (v/v) was used as the mobile phase and was kept in an ultrasonic bath to remove air bubbles before using a mobile phase. The standard calibration curve was obtained using L-ascorbic acid (Sigma, Germany) at concentrations of 10, 20, 40, 60 and 80 ppm. 5 g samples were transferred to test tubes, 5 ml of 25% phosphoric acid was added and the mixture was centrifuged for 5 minutes under the effect of 9000g gravity (Nüve brand NR 800R model, Turkey). 0.5 ml was taken from the clear upper part, completed to 10 ml with 25% phosphoric acid, and after filtering through a 0.45 µm filter, 20 µL sample was injected into the HPLC device [28].

2.6 Statistical analysis

The data were analyzed with a 95% confidence interval using a computer program (Minitab 17, USA) and one-way ANOVA was used in the analysis of the data. Tukey's multiple comparison test was used to determine the differences between the applications. Each experiment was repeated at least three times. Orthogonal array design was used in the Taguchi technique. Factor number 2 was chosen as run 9. L27 single-level design was selected and each factor has 3 levels. Temperature and time were chosen as factor a and b, respectively.

3 Results and discussion

3.1 Total phenolic substance (TPS)

The study started with the analysis of phenolic compounds, which are determinative in terms of bioactive component. The temperature and time interval, which are the independent variables used for classical extraction, and the TPS values used as the dependent variable were examined. This method is simple, reproducible and reliable [29]. It is also widely used in antioxidant studies. Gallic acid was used as a standard in the method, and the TPS concentration was calculated as GAE/kg dry matter, according to the calibration curve drawn for the absorbance of the extracts. TPS of extracts and extraction conditions made by the classical extraction method are given in Figure 1.

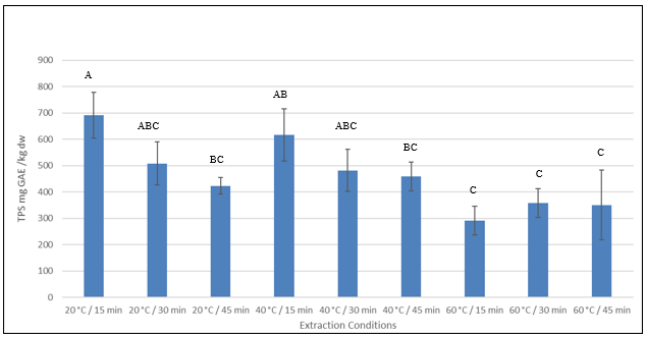

Figure 1. Classical extraction TPS values. Differences in letters show that temperature, time, and interaction of temperature and time are statistically effective on TPS. ($p \leq 0.05$).

According to the data, the effects of temperature, application time and the interaction of these two on TPS values were statistically significant ($p \leq 0.05$). In the extraction process at 20°C and 40°C, the amount of TPS extracted decreased with increasing time. Here, time has a negative effect on TPS. In this study, although it decreased at 20 and 40°C, the TPS increased at 60°C. As a result of the evaluation to be made between the extraction temperatures,

it is seen that the temperature has an effect on the extraction and this effect is statistically significant ($p \leq 0.05$). In a study on the determination of AOX and TPS compounds of broccoli, TPS amounts were reached in the range of 19.60–41.40 mg/100 g dry matter [29,30]. In the study of Lopez et al. [31], it decreased to 72.1 mg GAE/100 g with a 57.3% decrease at the end of the cooking process in broccoli samples analyzed fresh and cooked. When the TPS content was examined, an increase was observed in the phenolic substance content with the effect of high temperature and time. The reason for this is explained as the formation of phenolic compounds at high temperatures is the formation of precursors of phenolic molecules through non-enzymatic interconversion between phenolic molecules [32]. It is known that the bioactive component decreases with boiling in broccoli, and therefore, using it by drying and pulverizing reveals the possibility of using it for different purposes at any time.

The main effect graph corresponding to the S/N ratio for the extraction time in optimization using Taguchi is shown in Figure 2. In this plot, the slopes of each processing parameter are used to evaluate their importance and optimal processing combinations are determined. The best process parameter combination is the one that gives the highest S/N ratio value. Accordingly, the temperature-time combination, which gives the high amount of TPS for the temperature and time parameters, was determined as 20°C - 15 minutes (Figure 2).

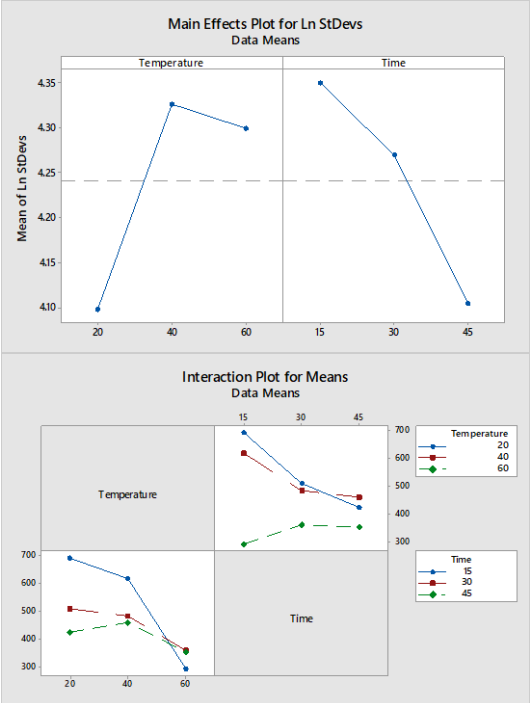

Figure 2. Parameters and main effect graphs evaluated in the TPS optimization experiment with classical extraction

3.2 Antioxidant activity (AOX)

Free radical scavenging is one of the methods by which antioxidants inhibit lipid oxidation. DPPH (2.2-diphenyl-1-picrylhydrazil) free radical scavenging method has been used to measure the AOX of some compounds or extracts in a short time [33,34]. The graph of the AOX amounts in the classical extraction performed at different temperatures and times is given in Figure 3. It was observed that the application time had a statistically insignificant effect on the AOX ($p > 0.05$). On the other hand, it was observed that the difference in temperature was significant at 60°C ($p \leq 0.05$). The extraction process at 60°C seems to have higher EC_{50} values. This means that a decrease in AOX value was observed with high temperature, and the negative effect of temperature was observed here as well as in TPS values. Similarly, a slight decrease in EC_{50} value was observed with the increase in the extraction process performed only at 60°C, which is explained by the behavior of the degradation products with the effect of temperature-time combination [35].

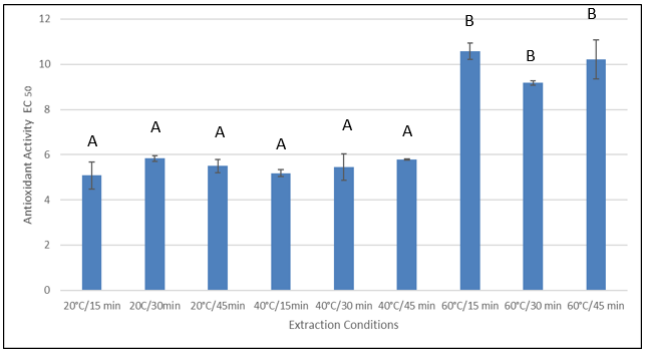

Figure 3. Results of AOX determination by conventional extraction (given as EC_{50} value). Differences in letters show that temperature, time, and interaction of temperature and time are statistically effective on AOX ($p \leq 0.05$).

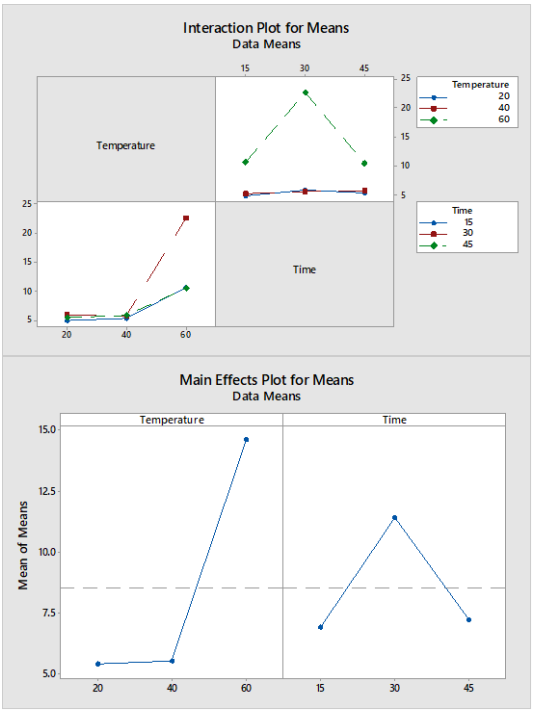

Figure 4. Parameters and main effect graphs evaluated in the antioxidant activity (EC_{50}) optimization experiment with classical extraction

The best process parameter combination for optimizing the effect of temperature and time of extraction on the antioxidant activity of the extract is given in Figure 4. Accordingly, the combination of the independent variables giving the highest AOX was determined as 20°C extraction temperature and 15 min.

3.3 Ascorbic acid content (AAC)

Ascorbic acid contains a dienol group, which has both reducing power and acidic properties. Likewise, vitamin C is a powerful antioxidant as it has strong reducing activity. It reacts easily with superoxide and hydroxyl radical and plays a role in inactivating them [36]. The results of AAC in the extracts obtained by the extraction are given in Figure 4. In the study, the amount of AAC decreased by 44% with the effect of temperature, while the highest value was determined as 66.74 mg/100g at 20°C. A negative effect of temperature increase was observed on the amount of AAC and this effect was statistically significant ($p \leq 0.05$). In addition, it is observed that the effect of time is significant on the AAC values ($p \leq 0.05$). Studies have shown a decrease in the amount of AAC between 34-66% for a maximum of 5 minutes, according to the temperatures applied in cooking methods. Water-soluble ascorbic acid can be easily degraded by heat treatment. When the ascorbic acid values in raw broccoli were examined, different values such as 84.6 mg/100g and 2.92 mg/g DW were reported [31, 37]. The reason for these differences is stated as growing conditions and climate.

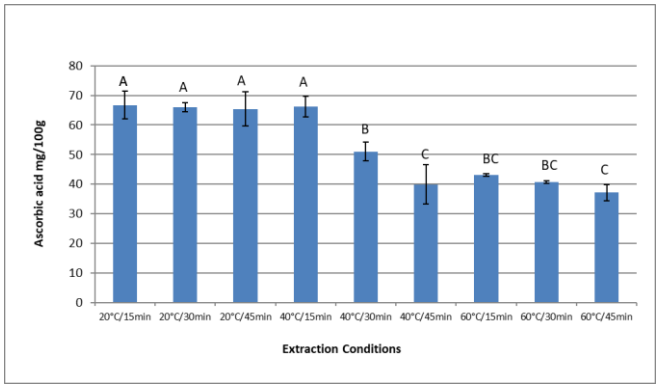

Figure 4. AAC activities of extracts**.** Differences in letters show that temperature, time, and interaction of temperature and time are statistically effective on AAC ($p \leq 0.05$).

In Figure 4, the highest vitamin C amount is 66.743 mg / 100 g at 20°C / 15min, while the lowest vitamin C amount is 37.137 mg / 100 g at 60°C / 45 min condition.

In the calculation made using the Taguchi method of the effect of temperature and time in the extraction on the ascorbic acid level of the extract, the best process parameter combination was determined as 20 °C and the time as 15 min.

3.4 Colour

The L* value gives the brightness value and can take values ranging from 0-100 according to the measured color. The color measured in the a* positive value range is red, and the color measured in the negative value range is green. Likewise, the color measured in the b* positive value range is yellow, and in the negative value range it is blue [38,39].

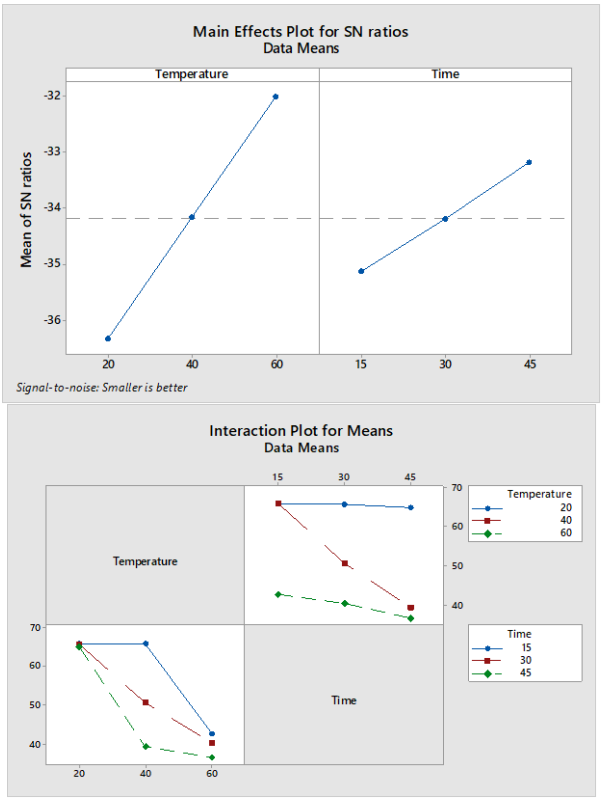

Figure 5. Parameters and main effect graphs evaluated in the ascorbic acid experiment with classical extraction

As can be seen from Table 1, differences were observed between the L*, a*, b* values of broccoli extracts at different times and temperatures. While the highest L* value was observed at 25.23 at 20°C/15 min condition, the lowest L* value was determined at 16.66 at 60°C/15 min condition. The highest value of a* value was determined at 7.73 at 20°C/30 min condition, while the lowest a* value was determined at 3.61 at 60°C/30 min condition. The highest value of b* was determined at 16.72 at 20°C/15 min condition, while the lowest b* value was determined at 2.67 at 60°C/15 min condition. As a result of these evaluations, the values closest to the green color were obtained in the extraction performed at 60°C. A positive effect is seen between the temperature and the transition of the green color to the extraction solvent. The decrease in a* value with time is explained by filling the intercellular spaces with extraction solvent. During the classical extraction, the temperature and time had a significant effect on the L*, a*, and b* value and it is statistically significant ($p \leq 0.05$).

In the studies, L* and a* values decreased with cooking processes, while b* values increased [40]. In another study, it is explained by the inactivation of the chlorophylase enzyme with temperature in cooking processes [31].

Table 1. Color determination results by conventional extraction. Differences in letters show that temperature, time, and interaction of temperature and time are statistically effective on colour values (p≤0.05).

	L*	a*	b*
20 °C / 15 dk	25.23±0.11^{Aa}	7.24±0.07^{Aa}	16.72±0.09^{Aa}
20 °C / 30 dk	24.59±0.45^{Aa}	7.73±0.23^{Aa}	16.58±0.53^{Aa}
20 °C / 45 dk	17.63±0.09^{Aa}	6.12±0.06^{Aa}	5.37±0.73^{Aa}
40 °C / 15 dk	21.09±0.35^{Aa}	5.59±0.25^{Ba}	10.72±0.58^{Aa}
40 °C / 30 dk	19.90±0.29^{Aa}	7.03±0.49^{Ba}	8.25±0.49^{Aa}
40 °C / 45 dk	20.84±0.36^{Aa}	5.57±0.29^{Ba}	10.45±0.62^{Aa}
60 °C / 15 dk	16.66±0.44^{Ba}	4.27±0.19^{Ca}	2.67±0.07^{Ba}
60 °C / 30 dk	17.56±0.18^{Ba}	3.61±0.07^{Ca}	4.21±0.07^{Ba}
60 °C / 45 dk	17.76±0.12^{Ba}	4.69±0.02^{Ca}	5.04±0.03^{Ba}

4 Conclusions

Phenolic substances are mostly defined as heat sensitive components. The antioxidant activity and phenolic components depend on the composition of the food, the amount and ratio of the substances in the composition of the food, the interaction of these components with each other, the technological processes applied during the process, the duration and temperature of the heat treatment, the ratio of solvents that extract phenolic components such as water in the environment, the type of solvent used during the analysis and greatly affected by the rate. According to these results, it was stated that the total amount of phenolic substance in the extracts obtained in the classical solvent extraction ranged between 290.94-690.94 mg GAE/kg, the EC50 value, which is the antioxidant activity value, ranged between 5.08-10.57, and the amount of ascorbic acid varied between 37.13-66.74 mg/100g. According to the data obtained, the best combination of temperature and time for the extraction process can be expressed as 20°C and 15 minutes. The data in this study, in which the effects of different temperature and time parameters on extraction from broccoli are evaluated, are expected to shed light on future studies on extraction parameters.

Conflict of interest

The authors declare that there is no conflict of interest.

Similarity rate (iThenticate): 16%

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