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Kinetics of temperature effect on antioxidant activity, phenolic compounds and color of Iranian jujube honey

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Abstract

In the present study, the Iranian jujube honey was evaluated for its total antioxidant activity by DPPH assay, total phenolic content (TPC) by using the Folin–Ciocalteu reagent, and brown pigment formation (BPF). The kinetics of changes in jujube honey samples heated at various temperatures (45, 55 and 65 °C) over 10 days were studied. Increasing treatment temperature and time caused an increase in all three parameters including, antioxidant activity, BPF and TPC. Increases in BPF and TPC followed zero-order kinetics, and the rise in antioxidant activity varied depending on heating temperatures, following second-order, first-order and zero-order kinetics when samples were heated at 45, 55 and 65 °C, respectively. At 45–65 °C, activation energy values of 68 and 64.7 kJ/mol⁻¹ were obtained for BPF and TPC, respectively. Linear relationships were observed between antioxidant activity and BPF, TPC and antioxidant activity, and BPF and TPC, such that the highest phenol content was related to the darkest honey sample. For all three parameters, heating honey to 65 °C was found to be more effective than heating to 45 or 55 °C.

Keywords: Food science, Food analysis

1. Introduction

Honey is a common food product that has been known for its abundant nutritional benefits for centuries. Historical evidence demonstrates that honey has also been used for medicinal applications for thousands of years, such that the Egyptians, Assyrians and Greeks treated diseases with honey in ancient times (Zumla and Lulat, 1989).

Antioxidants are compounds capable of fighting the adverse effects of oxidation in living tissues. However, fake antioxidants are unfortunately sometimes added to food, with abundant evidence confirming their malnutrition effects. Furthermore, laboratory studies on animals have demonstrated that such counterfeit products increase the risk of liver damage and cancer. Hence, the inevitable necessity arises that high efficacy antioxidants with minimal toxicity are needed. In honey, the presence of different compounds with strong antioxidant activity has been confirmed in various studies (Gorjanović et al., 2013; Beretta et al., 2005). These compounds include, ascorbic acid, phenolic acids, glucose oxidase, flavonoids, catalase, amino acids, and proteins. Having said that, evidence suggests that the most significant antioxidants of honey are phenols (Hussein et al., 2011; Aljadi and Kamaruddin, 2004; Blasa et al., 2006).

Due to the fact that the chemical composition of natural honey determines its antioxidant activity (Meda et al., 2005; Socha et al., 2011), phenols or polyphenols can be regarded as the most important categories of compounds present in this food product. Depending on the plant source, the total phenolic content (TPC) of honey varies. Usually, clear honey has a lower content of phenolic compounds than darker honey, suggesting that darker honey samples are associated with greater levels of polyphenols (Jasicka-Misiak et al., 2012).

The Maillard reaction is one of the reactions that food may be subjected to during heating. Foods that have sugars with free amino acids undergo this reaction, leading to the formation of Maillard reaction products (MRPs). Such MRPs are non-enzymatic browning agents with significant antioxidant activity. Hence, the antioxidant activity and brown color of certain foods can be increased by heat treatment (Manzocco et al., 2000).

The antioxidant activities of MRPs are affected by physical and chemical factors during the thermal process. Carbon compounds including ascorbic acid and polyphenols participate in the Maillard reaction (Manzocco et al., 2000). However, in model systems, browning is not directly affected by MRPs produced after extended heating at 100 °C (Morales and Jiménez-Pérez, 2001).

The major components of honey are water, glucose, maltose, fructose and sucrose; its minor components comprise amino acids, proteins, organic acids, flavonoids, vitamins and acetylcholine (da Silva et al., 2016). In honey, thermal treatment modifies its tendency of crystallization, maintains its initial appearance, and eradicates any microbes that may contaminate the product (Turkmen et al., 2006).

Two methods are generally used for honey heating, comprising treatment in hot air (at 45–50 °C over 4–7 days) and in hot water (Turkmen et al., 2006). The non-enzymatic browning that occurs as a result of the Maillard reaction is one of the impacts of heating on honey quality (Ibarz et al., 2000; Wong and Stanton, 1989). Given adequate source bee nutrition, honey usually contains various flavonoids with antioxidant properties (Aljadi and Kamaruddin, 2004). However, a paucity of data demonstrates a relationship between antioxidant activity and BPF when heating at different temperatures and durations. The key goals of the present study were to identify the changes in honey color, TPC and antioxidant activity in the course of thermal treatment and to determine the related kinetic parameters.

2. Materials and methods

2.1. Sample preparation

Jujube honey (80 BX°) was directly obtained from the manufacturer (Delbar Co. Sabzevar, Khorasan-e-razavi province, Iran). Honey samples (5 g) were poured into test tubes and the test tube caps were tightly closed. The samples were then incubated at 45, 55 and 65 °C for 10 days. At 48 h intervals, three tubes of samples from each temperature were cooled down rapidly on ice and stored at –24 °C until the time of analysis. At the time of analysis, 1.0 g samples were dissolved in 5 mL of distilled water using a vortex-mixer, and the resulting solutions were centrifuged for 10 min at 11200 RCF. The centrifuged solutions were then filtered with Whatman No. 1 filter paper and analyzed for antioxidant activity, BPF, and TPC (Turkmen et al., 2006).

2.2. Determination of total antioxidant activity

The method described by Zambiazzi et al. (2016) was slightly modified and then used to determine the DPPH radical scavenging activity. In brief, 0.5 mL of jujube honey dissolved in warm water and 1.5 mL of 0.1 mM DPPH (Sigma) were mixed in methanol. For the control, distilled water was used in place of the extract. After vortex-mixing, the reaction mixture was let to stand at 25 °C in dark conditions for 60 min. Absorbance was determined at 517 nm by utilizing a spectrophotometer (UV-Visible, Model Shimadzu, UV-160A, Japan). The following equation was used to calculate the DPPH scanning effect as a percentage of the DPPH discoloration:

$$AA(\%) = \frac{Abs_{control} - Abs_{sample}}{Abs_{control}} \times 100 \quad (1)$$

To compare the radical scavenging ability of samples, we used from IC_{50} factor that equal the concentration of antioxidant able to inhibit 50 percentages of DPPH free radicals (Zambiazzi et al., 2016).

2.3. Brown pigment formation

For each temperatures and treatment times, samples diluted until 4° in Bx and BPF in samples measured at 420 nm using a spectrophotometer (Turkmen et al., 2006).

2.4. Determination of total phenolic content

The reformed Folin–Ciocalteu method (Beretta et al., 2005) was employed to measure TPC. Each honey sample (5 g) was diluted in 50 mL of distilled water. Next, 100 μ L of the ensuing solution was obtained (containing 10 mg of fresh honey) and added to 1 mL of 10% Folin–Ciocalteu reagent. The blend was then vortexed for 2 min, and after 20 minutes, the sample absorbance was identified at 750 nm against the sugar analog. In order to obtain the calibration curve, gallic acid (0–200 μ g/ml) was used as the standard. TPC was expressed as mg of gallic acid (GA) per kg of honey (Beretta et al., 2005).

2.5. Kinetics calculations

Regression analyzes (Sigma chart 41) were used to determine the rate constants for antioxidant activity, TPC, and BPF. By employing the Arrhenius equation, the activation energy (E_a) at different temperatures was calculated from the rate coefficients (Labuza, 1984).

In order to obtain the antioxidant, phenol and browning activities of the jujube honey samples, the general kinetic models of zero, first and second order reactions were used, presented in Eqs. (2), (3), and (4), respectively (Ritzoulis, 2013).

$$[A]_0 - [A] = kt. \quad (2)$$

$$[A] = [A]_0 \exp(-kt). \quad (3)$$

$$1/[A] - 1/[A]_0 = kt. \quad (4)$$

In these equations, k (day^{-1}) is the reaction constant; t is the reaction time (day); $[A]_0$ and $[A]$ are the initial and final amounts of reactants respectively, at the different the times t and temperatures ($^\circ\text{C}$).

The effect of temperature on k was determined using Eq. (5) (Arrhenius equation). The activation energy (E_a) for the formation of each parameter was determined by

linear regression of $\ln k$ curve versus $1/T$.

$$(\ln k = \ln k_0 - Ea/RT) \quad (5)$$

Here, E_a (kJ/mol^{-1}) denotes the activation energy for the reaction; R (J/mol.K) denotes the universal gas constant; T (K) denotes the temperature and k denotes the reaction constant.

Regression models for each of the parameters at various temperatures were developed with Excel software in accordance to the graph of $[A]$ diagram versus time. Using Excel software, the initial models were obtained by plotting the $[A]$, $\ln [A]$ and $1/[A]$ diagrams versus the time and temperature of analysis for each of the various parameters, corresponding to zero, first and second order kinetics, respectively.

The final regression models were obtained by matching and analyzing the initial models obtained by Excel software with general equations of zero, first and second order reactions presented in Eqs. (2), (3), and (4), respectively.

3. Results and discussion

3.1. Antioxidant activity, BPF and TPC

Fig. 1A, B and C portray changes in the antioxidant activity, BPF and TPC of honey samples exposed to thermal treatments at 45–65 °C for different times, respectively. The results showed that TPC and BPF continuously increased over 10 days at 45, 55 and 65 °C, and the antioxidant activity increased in different ratios over 10 days at 45 and 55 °C and over 8 days at 65 °C. With increasing temperature, all three

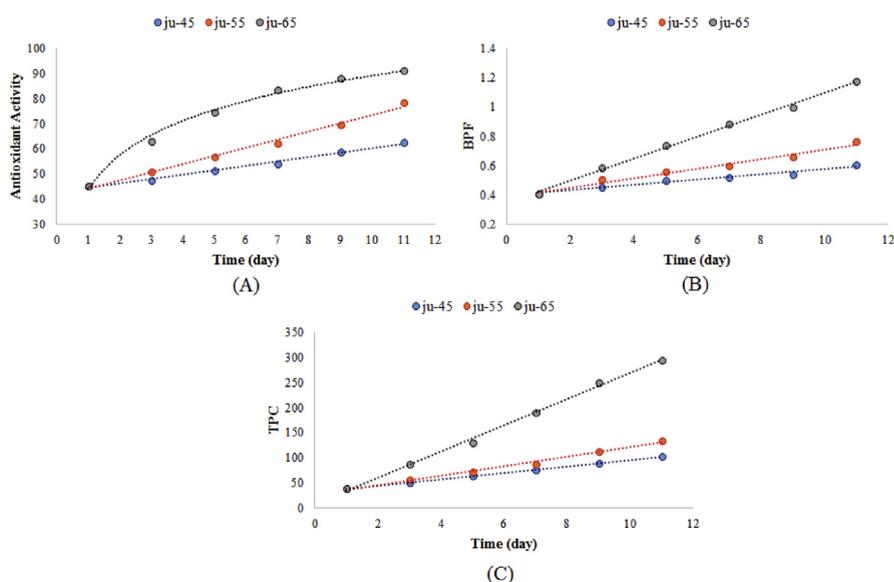


Fig. 1. Antioxidant activity (A), BPF (B) and TPC (C) of honey as a dependent of heating time at 45, 55, 65 °C.

parameters increased. This increase was more pronounced in samples heated to 65 °C rather than those heated to 45 or 55 °C, which indicates a high dependence of the antioxidant activity, BPF and TPC on the time and temperature of heating. With increase in temperature of process, the BPF was increased, and its consequence the amount of TPC increased. This could be due to the production of different compounds in maillard reaction. These results observed by other researchers (Giovannelli and Lavelli, 2002; Mastrocola et al., 2000; Turkmen et al., 2006).

3.2. The relationship between antioxidant activity and BPF

As shown in Table 1, a strong correlation was identified between antioxidant activity and BPF at various temperatures. In Fig. 2, increasing antioxidant activity along with increasing sample browning can be observed, which can be attributed to the formation of MRPs that have antioxidant properties, as previously reported for food and model systems (Manzocco et al., 2000; Wagner et al., 2002).

Over time, both antioxidant activity and BPF linearly increased during heating at 45 and 55 °C. However, a antioxidant activity has a logarithmic rise 65 °C (Fig. 1A and B). The increase in both antioxidant activity and browning can be due to products from non-enzymatic browning reactions (Maillard) as they include various compounds depending on the processing conditions (Manzocco et al., 2000; Van Boekel, 2001). Wagner et al. (2002) identified that melanoidin fractions with different molecular weights had clear differences in radical suppressing capability (Wagner et al., 2002). Also, different antioxidant activities have been reported for melanoidins isolated from various food (Morales and Jiménez-Pérez, 2004). Morales and Jiménez-Pérez (2001) concluded that in a sugar-amino acid mixture heated at 100 °C, free radical scavenging activity increased dramatically over the

Table 1. Regression equations and correlation coefficients (R²) of antioxidant activity as a dependent of BPF and TPC quantity, and TPC as a dependent of BPF quantity for heated instances at different temperatures.

Function	Temperature (°C)	Regression equations	R ²
Antioxidant activity as a dependent of BPF	45	$y = 94.228x + 5.6954^a$	0.955
	55	$y = 97.261x + 3.822^a$	0.982
	65	$y = 44.895\ln(x) + 86.922^a$	0.989
Antioxidant activity as a dependent of TPC	45	$y = 0.274x + 34.555^b$	0.980
	55	$y = 0.3419x + 31.968^b$	0.988
	65	$y = 23.046\ln(x) - 38.844^b$	0.996
TPC as a dependent of BPF	45	$y = 343.03x - 104.89^c$	0.970
	55	$y = 283.44x - 81.724^c$	0.976
	65	$y = 346.93x - 112.29^c$	0.988

^a y: antioxidant activity; x: BPF.

^b y: antioxidant activity; x: TPC.

^c y: TPC; x: BPF.

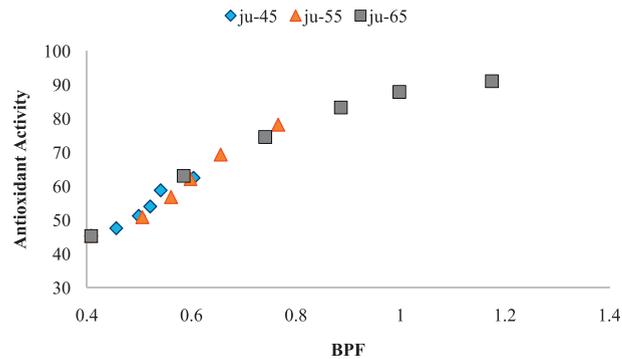


Fig. 2. Antioxidant activity-BPF relation in heated honey samples at 45, 55, 65 °C.

first 12 h, which was followed by a steady-state condition with regular increases in browning. At elevated temperatures, the antioxidant properties of milk have also been reported to increase due to the formation of brown pigments (Morales and Jiménez-Pérez, 2001).

3.3. The relationship between antioxidant activity and TPC

The high correlation coefficient observed between the data for antioxidant activity against TPC at different temperatures (Table 1) indicates that there was a strong correlation between these parameters. In Fig. 3, an increase in antioxidant activity along with an increase in the TPC of the samples can be observed. With an increase in heating time, both antioxidant activity and TPC linearly increased at 45 and 55 °C, while a logarithmic increase in antioxidant activity was observed at 65 °C (Fig. 1A and C). The TPC of jujube honey at 65 °C increased from 20.7 µg/g jujube honey at the initial time ($t = 0$ days) to 287 µg/g jujube honey at the terminal time ($t = 10$ days), corresponding to an increase of approximately 14 times. The results were consistent with previous studies for TPC. Ferreres et al. (1992), Gil et al. (1995) and Martos et al. (1997) found that the TPC of honey was between 500–2000, 700–2000 and 20–2400 µg/100 g honey, respectively.

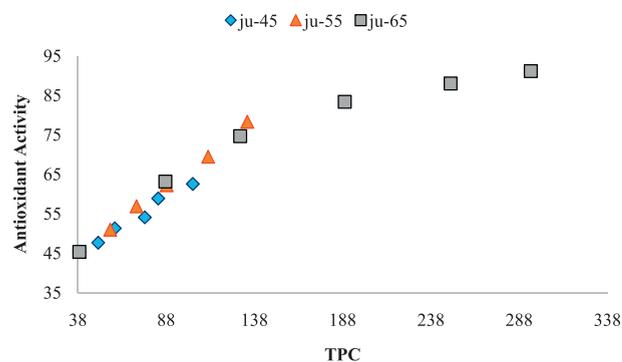


Fig. 3. Antioxidant activity- TPC relation in heated honey samples at 45, 55, 65 °C.

The results of the present study indicate that phenolic compounds may be the responsible for the antioxidant activity of jujube honey. In the study of [Koca and Karadeniz \(2009\)](#), which has findings consistent with those of the present study, a direct relationship was established between the antioxidant properties and the amount of phenolic and anthocyanin compounds in blueberry (a type of fruit that is found in the Black Sea area). Phenolic compounds are important compounds due to their antioxidant properties, which facilitate the removal of free radicals and prevent the conversion of hydroperoxides to free radicals ([Jimoh et al., 2008](#)).

Some reports indicate a direct relationship between TPC (analyzed by Folin–Ciocalteu method) and anti-oxidant activity (analyzed by the diphenyl picryl hydrazyl assay, trolox equivalent antioxidant capacity assay and the antioxidant potential of iron regeneration assay) ([Stratil et al., 2006](#)). Many researchers have investigated the phenolic compounds of honey and their relationship with antioxidant activity.

[Jahan et al. \(2015\)](#) stated that with an increase in the TPC of Bangladesh honey, its antioxidant properties increased. [Khalil et al. \(2012\)](#) reported that there was a strongly positive correlation between the TPC and antioxidant activity of Algerian honey samples. [Aljadi and Kamaruddin \(2004\)](#) showed that there were direct relationships between TPC and antioxidant activity in both Malam and Malaysian coconut honey.

3.4. The relationship between BPF and TPC

A strong correlation was determined between BPF and TPC at various temperatures, as shown by the large correlation coefficient values in [Table 1](#). In [Fig. 4](#), it can be observed that the increase in BPF was associated with an increase in the TPC of the honey samples.

Both BPF and TPC in jujube honey linearly increased with an increase in heating time at 45, 55 and 65 °C ([Fig. 1B and C](#)). The results of BPF show that, with increasing temperature and heating time, the color of honey samples darkened,

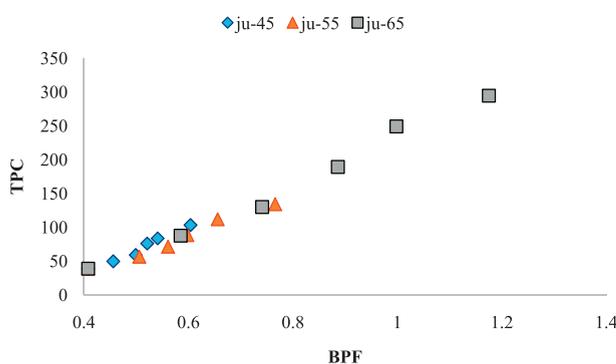


Fig. 4. TPC – BPF relation in heated honey samples at 45, 55, 65 °C.

and this darkening reached its maximum at 65 °C. Because the phenolic compound content is usually lower in clear honey compared to darker honey, higher levels of polyphenols in the honey samples may be associated with darker color (Jasicka-Misiak et al., 2012).

The relationship between different honey colors and TPC has been explored in various studies. Sant'Ana et al. (2014) reported that there was a direct correlation between the increase in darkening of Brazilian honey samples and their phenolic content. Khalil et al. (2012) stated that there was a direct correlation between an increase of TPC and flavonoids and the darkening of Algerian honey samples.

3.5. Reaction kinetics for antioxidant activity, BPF and TPC

In order to explain the phenomena of changing color, antioxidant activity and TPC, the data were fitted by employing kinetic models. The values obtained from the fitted parameters are given in Table 2. Consistent with previous studies, the zero-order kinetic model explained both BPF and TPC at all temperatures. The phenomenon of non-enzymatic browning has been evaluated by other researchers using the zero- and first-order kinetic models (Bozkurt et al., 1999; Yu et al., 2017).

First- and second-order models were also evaluated but had lower correlation coefficients. However, first-order kinetic models were suitable for both BPF and TPC. On the other hand, changes in antioxidant activity with treatment time were fitted to second-order, first-order and zero-order reaction kinetic models at 45, 55 and 65 °C, respectively. The different kinetic models used for antioxidant activity at different heating temperatures highlighted the effect of the activity of different reactants in the Maillard reaction, which may be due to differences in the temperature sensitivity of the various steps of the Maillard reaction (Van Boekel, 2001). No study has reported the dynamics of antioxidant activity during the heat treatment process of honey. On the other hand, Suh et al. (2004) reported that the antioxidant ability of

Table 2. Kinetic parameters for BPF, antioxidant activity and TPC in honey at different temperatures.

	Temperature (°C)	Zero-order		First-order		Second-order		Ea (kJ/mol ⁻¹)
		k (day ⁻¹)	R ²	k (day ⁻¹)	R ²	k (day ⁻¹)	R ²	
Brown color formation	45	0.017	0.973	0.036	0.969	-0.073	0.956	68
	55	0.032	0.973	0.057	0.966	-0.103	0.934	
	65	0.074	0.996	0.101	0.960	-0.147	0.875	
Total antioxidant activity	45	1.746	0.990	0.032	0.993	-0.0006	0.996	
	55	3.220	0.990	0.053	0.998	-0.0009	0.982	
	65	4.459	0.918	0.065	0.863	-0.001	0.802	
Total phenolic content	45	6.411	0.999	0.096	0.981	-0.0033	0.932	64.7
	55	9.422	0.992	0.120	0.985	-0.0017	0.921	
	65	26.04	0.997	0.195	0.935	-0.0019	0.741	

mulberry fruit extract undergoing treatment at 80–100 °C fitted both first- and zero-order models. However, the aforementioned authors recommended the latter model, which is consistent with the results acquired at 65 °C in the present study. [Table 2](#) shows an obvious increase in kinetic constants with temperature, suggesting that the increase in treatment temperature contributed to rises in all three of antioxidant activity, BPF and TPC.

The relationship between BPF and TPC indicates that both of these parameters followed zero-order kinetic models, which suggests that browning in jujube honey is probably related to phenolic compounds. This is backed by the studies of [Mantell et al. \(2002\)](#), [Patil et al. \(2009\)](#) and [Jaiswal et al. \(2010\)](#), in which anthocyanins have been determined to be responsible for orange, red, purple and blue color in a large group of plants. [Elliott \(1999\)](#) stated that azelaic acid, a phenolic compound and the most important chemical compound present in pomegranate skin, has antioxidant properties.

Heat treatment has a positive effect on the phenolic content such that the phenolic content in honey increases with increasing heat ([Fig. 1C](#)). This study is similar to the case reported for [Turkmen et al. \(2006\)](#) that antioxidant activity linearly increases with increasing temperature (50, 60 and 70 °C) over a period of 12 days ([Turkmen et al., 2006](#)). Also our findings were similar to those reported for Manuka honey from New Zealand, where the phenolic content was reported to be 20.1 and 16.3% under heat treatments at 50 and 70 °C, respectively ([Akhmazillah et al., 2013](#)). In the aforementioned study, the increase in phenolic amounts may be due to protein denaturation and the opening of active protein positions, degradation of the intrinsic antioxidants agents, and the production of some non-nutritive antioxidants agents such as those of the Maillard reaction products, as reported by [Kusznierewicz et al. \(2008\)](#) and [Serpen et al. \(2012\)](#).

Using the Arrhenius equation, the calculated activation energy for brown pigment and phenol formation at 45–65 °C was found to be 68 and 64.7 kJ/mol, respectively. These values were lower than the amount of 132 kJ/mol for boiled grape juice ([Bozkurt et al., 1999](#)) and 77.1 kJ/mol for apple puree ([Ibarz et al., 2000](#)), showing that the formation reaction of browning colors and phenolic compounds in jujube honey is more sensitive to heat compared with boiled grape juice and apple puree. Differences in the rate of browning in honey compared with grape juice and apple juice may be related to differences in their amino acid and reducing sugar contents. Other factors that may affect the Maillard kinetics are the types and thermal stabilities of amino acids and reducing sugars participating in the reaction.

4. Conclusion

In the present study, Iranian jujube honey was heated at 45, 55 and 65 °C for 10 days, and antioxidant activity, total phenol content and browning of the heated honey

samples were evaluated. In general, the increase in the values of all three parameters was more pronounced at 65 °C, indicating a strong dependence of all three parameters on temperature. Regular and prolonged heat can increase the antioxidant activity of honey by gradually increasing the amount of compounds with antioxidant properties, such as phenolic compounds, as well as forming Maillard reaction products that can have a positive effect on human health, but consumers tend to reject honey that has dark brown color. Hence, positive and negative effects should be balanced to achieve high antioxidant activity and low BPF simultaneously.

Declarations

Author contribution statement

Mohammad Molaveisi: Performed the experiments; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Adel Beigbabaei, Mostafa Shahidi Noghabi: Conceived and designed the experiments; Analyzed and interpreted the data.

Ehsan Akbari: Performed the experiments; Wrote the paper.

Morteza Mohamadi: Analyzed and interpreted the data; Wrote the paper.

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Competing interest statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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