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The antioxidant activity of lycopene and chlorophyll oleoresin and phenol stability of Berberis vulgaris extracts in cupcake formulation

Running title: The natural colored cakes

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Abstract

The application of natural ingredients in food formulations plays a key role in public health. The application of natural colorants is helpful in human health. Most of the natural colorants have additional roles such as antioxidant or antimicrobial activities. Natural colorants in foods especially in cake formulation, make an attractive view for Childs. In this study, the effects of three natural colorants of lycopene, chlorophyll, and Berberis Vulgaris extracts were investigated in cupcakes. The amounts of 0, 0.5, 1.5, and 2% of extracts were used. The moisture, volume increment, porosity, color parameters, total phenols, antioxidative effects of extracts, and sensory properties were evaluated. Results showed that all-natural colorants increase the final volume, porosity, oxidative stability, phenol content. Also, they reduced the moisture and lipid oxidation of samples. The red, green, and blue parameters of cakes decreased, especially in samples with Berberis Vulgaris extract. The lightness of all samples was significantly reduced after cooking but the lightness of samples with 2% lycopene oleoresin was not high. Sensory evaluation showed that the color, flavor, and odor of cakes prepared with lycopene, and chlorophyll oleoresin had the highest scores. The cakes prepared with Berberis Vulgaris extract had the lowest scores in color, flavor, and odor, but the texture and porosity were the same as other samples. Finally, it can be concluded that lycopene oleoresin showed significant acceptance, antioxidant effect, and acceptable physical properties. Results can be helpful for researchers and food industries because the lycopene oleoresin showed a significant antioxidant activity after 3 weeks and the total phenols of Berberis Voulgaris extract showed significant stability after 3 weeks. Especially, the panelists have a significant reflection of the colored food. They propose them as an attractive materials for consumption.

Keywords: Natural colorant, Cupcake, Functional food, Natural antioxidant

Introduction

Bakery products are used by a wide range of consumers. Different types of cakes are a good choice for children and teenagers who are in an important period of their growth. Thus, by changing in food’s formulation and making them functional or healthier foods, we can try to increase the health benefits of life.

Color is an important parameter in bakery products and has a great role on consumer purchasing decision. Color is the first factor that shows a wide range of quality factors like flavor, freshness, odor, healthy processing and handling, and make an attractive image for the consumer to choose. Since the color is the first factor for the attraction of consumers to choose a food product, and nowadays consumers are aware of the importance of application the natural ingredients in food formulations, application of natural colors in food formulation is a great step to make healthier foods. Also, some natural colors have other characteristics like antioxidant (Delgado-Vargas et al., 2000, Bao et al., 2005), antimicrobial (Ranjbar and Ranjbar, 2016), and also health benefit properties.

Lycopene, as an important natural colorant, is investigated for nutritional, healthy, and nutraceutical properties. Lycopene has a reddish color, and also antioxidant and

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antimicrobial properties (Vitaglione et al., 2005, Ozkan, Akyuz et al., 2012, Palermo, Panozzo et al., 2014, Peker Akalin et al., 2016, Ranjbar and Ranjbar, 2016, Urbanoviciene and Viskelis, 2017). Studies on the health role of lycopene, emphasis on its antioxidant property as a key factor to preventing diseases in the digestive system, glands, different types of cancers, bones, and so on (Sies and Stahl, 1998; Shi and Maguer, 2000; Stacewicz-Sapuntzakis and Bowen, 2005; Sheriff and Devaki, 2013; Sroynak et al., 2013, Sahin et al., 2014, Sachdeva and Chopra, 2015; Sahin et al., 2015).

Phenols are chemical constituents in plants that can play a different role in plants and also in human health. They can be found in many fruits and vegetables like grapes, berries, and tomatoes. They can promote health benefits through their antioxidant role. They can reduce the risk of metabolic syndromes and contribute to preventing some diseases (Laamech et al., 2017). In recent years, medicinal plants are highly used in functional foods, and plants that contain antioxidants and other health beneficial components are sources of novel food formulations. The *Berberis vulgaris* extract has some effects like reducing blood pressure, cholesterol, and triglycerides, makes the good effects on diabetics, preservation effects on neurons, and antibacterial and antioxidant effects like most antibiotics (Rahimi-Madiseh et al., 2017).

Oxidation is an important problem in processed foods that can affect the final physicochemical, shelf-life of product and consumer health. The addition of antioxidants can reduce the unwanted effects of oxidation. But today because of some doubts about health effects of synthetic antioxidants, the natural antioxidants can be a good candidate for processed foods. The addition of some antioxidants to cake batter is a good approach to make functional foods. Ranawana et al. (2018) studied the effect of beetroot on oxidative stability and functional characteristics of cakes. They found beetroot can significantly improve the antioxidant and phenolic profile, oxidative stability, and shelf-life of sponge cakes (Ranawana et al., 2018). Pasukamonset et al. (2018) also found the addition of *Clitoria ternatea* extract to cupcakes can increase the antioxidant activity and reduce the lipid peroxidation of sponge cakes (Pasukamonset et al., 2018). Lu et al. (2010) studied the quality and antioxidant property of green tea powder in sponge cake as a flour replacer and found green tea cake is a good product with effective antioxidant properties (Lu et al., 2010). Seo et al. (2010) optimized the amount suitable to add turmeric powder in sponge cake. They reported that by increasing the amount of turmeric powder in sponge cake batter, the hardness, gumminess, and chewiness, also increased but the sensory values decreased. They found the optimal ingredients were 1.6% turmeric powder, and 14.9% oil. Thus the turmeric powder can be easily used as a functional ingredient in sponge cakes (Seo et al., 2010).

Celik et al. (2007) studied the effect of soapwort extract on the physical and sensory properties of sponge cake. They found egg white proteins can be replaced by soapwort extract and its application in cake formulation up to 75% did not change the physical properties of sponge cake (Çelik, Yılmaz et al., 2007). Toyosaki and Koketsu, (2007) investigated the antioxidant effects of silky fowl eggs and white leghorn eggs on the water-soluble part of baked sponge cake. They found that the silky eggs increased but the leghorn egg decreased the antioxidant effect and then the use of silky fowl eggs can improve the quality and oxidative properties of cakes.

In this study, cupcakes were prepared with natural colorants of lycopene and chlorophyll oleoresins, and *Berberis vulgaris* extract. The goal of this work was to investigate the stability of natural colors during baking and study the effect of natural colorant application in cake properties and consumer acceptance.

**Materials and methods**

Lycopene oleoresin (20% purity from fresh tomato (*Solanum lycopersicum*)) chlorophyll oleoresin (30% purity from spinach (*Chenopodiaceae* species)) and *Berberis*
vulgaris extract were purchased from a local company (San’at Pooya Torang CO. Iran). All other ingredients including flour, sugar, fresh eggs, vanilla, baking powder, and sunflower oil were purchased from a local market. Pure water was used.

**Making the cupcakes**
All samples had 100 g flour, 25 g fresh egg, 36 g sugar, 35 g water, 15 g oil, 1 g vanilla, and 1 g baking powder., the lycopene and chlorophyll oleoresins, and Berberis vulgaris extract was added to batters in 0, 0.5, 1.5, and 2% levels. These levels obtained from the pretreatment studies. At first, the oil and sugar were mixed to make a cream. Then eggs and vanilla were added and mixed for 5 min. after all, the flour (mixed with baking powder) and water were added gradually and mixed gently (Katomo, Japan). The equal weights of batter were poured into the same form of cups. Baking was done at 170°C for 25 min (Beikzadeh, Peighardouz et al. 2016). After reaching room temperature, the cakes were packaged into plastic zipper bags. Nine different formulations were prepared in this study.

**Determination of total phenolic content and antioxidant property**
Diluted samples of Berberis vulgaris extract cakes (0.50 ml) were added to 2.5 ml of diluted (1:10) Folin-ciocalteu reagent and kept for 4 min. Then 2 ml saturated sodium carbonate solution was added and after 2 h incubation at room temperature, the absorbance was measured by a spectrophotometer (LKB Novaspec II; Pharmacia, Sweden) at 760 nm against Gallic acid as a standard reference (mg GAE/g) (Li et al., 2014). The total phenolic content was calculated as gallic acid equivalent (GAE) by the following equation:

\[ TCVM = \frac{T \times C}{V \times M} \]

(T is the total phenolic content in mg g⁻¹ of the extracts as GAE, C is the concentration of gallic acid established from the calibration curve in mg ml⁻¹, V is the volume of the extract solution in ml and M is the weight of the extract in g (Abdelhady et al., 2011).

The antioxidant property of cakes with lycopene and chlorophyll was determined according to the method of Lu et al. (2010). In order to measuring the peroxide value, about 1.0 g sample was treated with 25 ml of organic solvent mixture (chloroform/ acetic acid, 2/3: v/v). The mixture was shaken vigorously. Then 1 ml of saturated potassium iodide (Merck) solution was added and the mixture was kept in the dark for 5 min. After that, 75 ml of distilled water and 0.5 ml of starch solution (1%, w/v) was added as an indicator, and PV was determined by titrating the iodine liberated from potassium iodide with standardized 0.01 N sodium thiosulfate solution. The PV was expressed as milli equivalents (meq) of peroxide per kg of lipid.

**Physicochemical evaluations**
Moisture content was determined by AACC (2000) method, about 2 h after the baking time. The volume index was determined as the sum of three height of the right side, left side, and the center of the midsection of the cakes. The porosity was measured by Image J processing software. About 2 h after the baking time, a central section cut was made and the color parameters (lightness (L*), Red, Green, and Blue) were determined. To determining the porosity, a 2×2×2 cm cut was made and scanned with a scanner (HP Scanjet G3010). With image J processing software and calculating the light and dark points, the porosity of cakes was measured (Khalilian Movahhed et al., 2016).

**Sensory evaluation**
Twenty-five semi-trained panelists evaluated the sensory parameters of colored cake samples. A 5- Hedonic test (1= extremely dislike and 5= extremely like) was done. Panelists were from the food science students and the lab employees of the food science department of Semnan University. The samples were randomly numbered with three-digit numerical codes. The color, flavor, texture, porosity, and total acceptance of samples were studied (Khalilian Movahhed et al., 2016).

**Statistical analysis**
All measurements were done in triplicate except for sensory evaluation. The significance
of difference among treatment means was determined by one-way analysis of variance (ANOVA) and Tukey test using SigmaPlot software (version 14). A $P$ value of less than 0.05 was considered statistically significant. The sensory data were analyzed by chi-square (Khalilian Movahhed et al., 2016).

![Graphs](a, b, c)

Fig. 1. The moisture content (a), Volume index (b), and porosity (c) of colored cakes. The (●), (■), and (▲) represents lycopene oleoresin, chlorophyll oleoresin, and Berberis Voulgaris extract, respectively.
**Results and discussion**

**Physico-chemical properties of cupcakes**

Figure 1 shows the changes in moisture content, volume index, and porosity of colored cake samples. According to the figure, the moisture content of all samples was reduced after adding natural colorant. Adding the chlorophyll oleoresin and 2% lycopene oleoresin to the cake batter, led to a significant reduction in moisture content. The *Berberis vulgaris* extract and lycopene oleoresin at 0.5, and 1.5% had a moderate effect on moisture reduction. A reduction in moisture might due to the oily nature of oleoresins. Because oil can lead to a decrease in water activity. The lycopene and chlorophyll oleoresins are a mixture of colorants, lipids, phospholipids, protein-phospholipid components, fatty acids (Shi and Maguer, 2000; Liu *et al.*, 2010, Saini and Keum, 2018) that some of them can play as macromolecules and bind with free water molecules (Khalilian Movahhed *et al.*, 2016). Also, all colorants showed a significant effect on the volume index of colored cakes. The oily nature of lycopene and chlorophyll oleoresins can have a positive effect on the volume increasing of batter during baking the cake. Brooker (1996) suggested the melting of lipid crystals develops the porosity of bread. He believed that melted crystals move to the interface of developing the air-crystals interface and helps the porosity and volume increasing of bread during the baking process.

As can be seen in figure 1-c, the porosity of samples increased after adding the natural colorants to the cake batter. This increase in samples with lycopene oleoresin is significant and can be explained by Brooker (1996) theory. Brooker (1996) also explained the crystal size has a significant reverse effect on the porosity of bread or cake. Oils contain small crystals can have a better effect on porosity than the oils with bigger crystals. The difference in the effect of lycopene oleoresin and chlorophyll oleoresin can be derived from this fact that perhaps the crystal sizes of the two oleoresins are different.

This can be further studied in future. Also, because of the emulsifying effect of phospholipids, the observed increase in volume and porosity was possibly due to the oleoresins composition (Khalilian Movahhed *et al.*, 2016).

It can be concluded that adding the lycopene and chlorophyll oleoresins to the cake batter, have a good effect on final product physical properties due to moisture reduction and increasing in volume. Also, lycopene oleoresin showed a good effect on the cake porosity that can be important if we consider the final interesting yellow-red color of the product.

**Total phenols and antioxidant properties**

Figure 2 shows the total phenol content of colored cake samples after baking. The total phenols of samples were monitored for 3 weeks and there were no significant changes during the 3-week storage of cake samples (Table 1). The antioxidants are sensitive colorants that their stability is important during the food formulations. Many factors such as pH, temperature, light, chelating agents, etc have a significant effect on the anthocyanin’s stability (Taghvaei and Jafari, 2015; Zhao, Yu *et al.*, 2017; Chatham *et al.*, 2020). Thus different studies have done for increasing their stability and then their antioxidant activity (Martins *et al.*, 2016, Ziabakhsh Deylami *et al.*, 2016; Bastos *et al.*, 2017; Rodriguez-Sanchez *et al.*, 2017). For example, Pasukamonset *et al.* (2018) used *Clitoria ternatea* extract in sponge cake and found a significant increase in the phenol content of sponge cakes.

In this study, the reduction of phenol content in all samples was not so significant in 3 weeks (Table 2). Also, it was shown that the application of *Berberis Vulgaris* extract in the studied cake batter was effective on the stability of extract phenols (Table 1). This can be helpful to decide about selecting the food formulations that can be considered as a low-cost way for stabilizing the natural colorants without further processes.
Fig. 2. Total phenol content of cake samples contains *Berberis Vulgaris* extract (the signs show the comparison between weeks)

Table 1 - Total phenol content ANOVA during 3 Weeks

<table>
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<tr>
<th>Week 1 and 2</th>
<th>Source of Variation</th>
<th>DF</th>
<th>SS</th>
<th>MS</th>
<th>F</th>
<th>P</th>
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<td>Between Groups</td>
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<td>682.667</td>
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<td>23901.333</td>
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<tr>
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<td>96288.000</td>
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<th>Source of Variation</th>
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<th>SS</th>
<th>MS</th>
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<tr>
<td>Total</td>
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<td>5</td>
<td>106845.333</td>
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Table 2 - Comparison of Antioxidant activity of lycopene oleoresin during 3 Weeks

<table>
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<tr>
<th>Week 1</th>
<th>Comparison</th>
<th>Diff of Means</th>
<th>t</th>
<th>P</th>
<th>P&lt;0.050</th>
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<th>Comparison</th>
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<th>t</th>
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<th>P&lt;0.050</th>
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<th>Comparison</th>
<th>Diff of Means</th>
<th>t</th>
<th>P</th>
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<td>3.041</td>
<td>0.038</td>
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As can be seen in Figure 3, the addition of lycopene oleoresin significantly increases the oxidative stability of cakes (Table 2). The peroxide value in all lycopene content samples is lower than a control for 3 weeks. In the first week, the lycopene oleoresin at 1.5% showed the lowest peroxide value. The peroxide value in week 2 is near week 1 and at week 3 increases. But the increase in samples with lycopene oleoresin at week 3 was almost lower than the peroxide value of control at week 1. It can be concluded that the addition of lycopene as a strong antioxidant can significantly help to maintain the lipid stability of cakes without the need to add synthetic antioxidants. Lu et al. (2010) showed the addition of green tea powder to the cake is effective in the antioxidant property of cakes (Lu et al., 2010).
Fig. 3. Total peroxide value of colored cake samples with lycopene oleoresin during 3 weeks (The signs show the comparison between each week)

Fig. 4. The color of batters colored with lycopene oleoresin (a), chlorophyll oleoresin (b), and *Berberis Vulgaris* extract (c), respectively.

**Color of samples**
Color is the most important factors in consumer acceptance of processed food. Color is effective in attracting consumers in purchasing a food product. But the most important challenge of food colors is the maintenance of color during processing. The changes in pH, acidity, the interaction between food components, and the temperature of the food processing can affect the final color of processed foods. Natural colorants have a sensitive structure out of their source (Tønnesen et al., 2002; Shahid et al., 2013; Taghvaei and Jafari, 2015; Torres et al., 2016). Therefore, their application and formulation must be carefully chosen. This needs a wide study about the natural colorants in different food formulations and processing conditions. Calvo and Salvador. (2000) have examined the stability of four natural colorants of annatto (orange), chlorophyllins (green), cochineal (red), and curcumin (yellow) during gel making. They made their samples with gelatin, a mixture of xanthan and locust bean gum,
sugar, and natural colorant. Then they measure the color (with a Hunter Labscan II colorimeter) and sensory parameters (a team of 10 judges). They found that the cochineal and curcumin natural colorants can replace the synthetic ones in jellies (Calvo and Salvador 2000). Pasukamonset et al. (2018) found the application of Clitoria ternatea extract in sponge cake can decrease the lightness, redness, and yellowness (Pasukamonset, Pumalee et al. 2018). Lu et al. (2010) showed that the addition of green tea powder to cake formulation decrease crust \( L \), \( a \), \( b \), and crumb \( L \), \( b \) values of samples.

Figures 4 and 5 show the color of batter (before baking) and cakes (after baking) containing the lycopene and chlorophyll oleoresin, and Berberis vulgaris extract. Seo et al. (2010) reported that turmeric powder decreased the brightness, and increased redness and yellowness in sponge cakes. In this study, the color parameters were determined for batters and final cakes, and the data are shown in Table 3.

![Fig. 5. The color of cakes colored with lycopene oleoresin (a), chlorophyll oleoresin (b), and Berberis Vulgaris extract (c), respectively.](image)

Table 3 shows the lightness, redness, greenness, and blueness parameters of the cake colors. The lightness of all samples was reduced after baking but the lightness of samples with 7% lycopene oleoresin was not high. After baking, the redness, greenness, and blueness parameters of cakes also decreased. It is demonstrated that the addition of lycopene, chlorophyll, and Berberis vulgaris extracts to the batter, changes the color to the red, green, and mixture of them in each sample, respectively. Lycopene makes a red-yellow color in cakes and chlorophyll changes the final color of cakes to green-blue. The samples with Berberis vulgaris extract have a mixture of colors of red, green, and blue. But after baking, the decrease in color in samples with Berberis vulgaris extract was more than other oleoresins. This may be due to the oily nature of lycopene and chlorophylls and also due to their color intensity in pH about 6-7 (Dabas and Kean, 2015). The Berberis vulgaris extract is pH sensitive because of the chemical structure of colorants (Li et al., 2014) and perhaps this significant reduction in color parameters in cakes with Berberis vulgaris extract was due to its sensitivity to pH of baking powder which was added to the cake batter.

**Sensory Evaluation**

Panelists scored overall acceptance of colored cakes as good. They believed that colored cakes are interesting for consumption in comparison with a non-colored cake. As can be
seen in Figure 6 and Table 4, the panelists recorded the high scores for cakes containing lycopene oleoresin. The cake with 0.5% of lycopene oleoresin had the highest overall acceptance. The cakes with 0.5 and 1.5% chlorophyll oleoresin had the second scores in overall acceptance. The color, flavor, and odor of cakes with lycopene and chlorophyll had the highest scores. The texture was approximately the same in all samples. Pasukamonset et al. (2018) reported that the Clitoria ternatea extract did not affect overall acceptability between the control and the cake containing Clitoria ternatea extract.

The panelists did not give high scores for cakes with Berberis vulgaris extract. The cakes with Berberis vulgaris extract had the lowest scores in color, flavor, and odor, but the texture and porosity were the same as other samples. Lu et al. (2010) reported that there was no difference between the control and cakes with different levels of green tea powder except the 30% level of green tea powder samples that obtained the lower rate in sensory evaluation.

**Conclusion**

Natural colorants are suitable ingredients for coloring the processed foods. Some of the natural colors have also shown other characteristics like antioxidant, emulsifying, antibacterial property, and also health benefits. It is worthy to study the different natural colorants in different foods according to their physicochemical conditions like the method of extraction, stabilization, and application of colors and the food parameters like pH, acidity, composition, and type of processing on the final effects of colorant in foods. This study may be helpful for industries that apply natural colorants. In this study, it was shown that lycopene, chlorophyll, and Berberis vulgaris extracts could make an interesting color to attract consumers. Also, data have shown that the application of these natural colors, helps to improve some physical properties of cakes, and increases the phenol contents and oxidative property. It is a good step for human health, especially when we know that the major consumers of cakes are children.
Fig. 6. The sensory evaluation of colored cake: (a) lycopene oleoresin, (b) chlorophyll oleoresin, (c) *Berberis Vulgaris* extract, respectively.
By increasing the natural additives in food formulations, and the tendency towards the natural sources used in the food industry, it has been a good area of study about the effect of different food formulations and processing on natural additives which can be linked by their health effects in human. Finally, it can be concluded that lycopene oleoresin is a good natural colorant to make colored cakes. It showed significant acceptance, antioxidant effect, and cake physical properties. The lycopene oleoresin at 0.5% level is proposed according to the overall acceptance of panelists and physico-chemical analysis of the final cake.

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فعالیت آنتی اکسیدانی اولئورزین لیکوپن و کلروفیل و پایداری فنولی عصاره زرشک در فرمولاسیون کیک فنجانی

آزاده رنجبر ندامانی*

تاریخ دریافت: 1399/5/0
تاریخ پذیرش: 1399/10/0

چکیده
کاربرد عناصر رنگی در فرمولاسیون مواد غذایی نقش کلیدی در سلامت عمومی بازی می‌کند. کاربرد رنگدانه‌های طبیعی در سلامت بشر مفید است. بیش‌تر رنگدانه‌های طبیعی حجم کیک، تخلخل و پایداری اکسیداتیو را افزایش داده و همچنین باعث کاهش رطوبت و اکسیداسیون عصاره زرشک شدند. پارامترهای قرمز، سبز و آبی کیک‌ها در نمونه حاوی عصاره زرشک بعد از پخت کاهش یافتند. ارزیابی حسی نشان داد که کیک‌های حاوی لیکوپن و کلروفیل بیشتر امتیاز را به داشتند. نتایج نشان دادند که هر یک از عناصر رنگی در فرمولاسیون کیک فنجانی نقش مهمی داشته و افراد مصرف‌کننده آن می‌توانند از نظر سلامتی بهتر باشند.

واژه‌های کلیدی: رنگدانه طبیعی، کیک فنجانی، عصاره زرشک، آنتی اکسیدانی طبیعی.

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