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Scientific Research

The Effect of Hot Chocolate Powder Fortification with Jujube Fruit on Its Quality Characteristics

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ABSTRACT

Hot chocolate (a cocoa-based beverage) has gained popularity in recent decades due to its many health benefits. However, during the processing of the base powder, some of the nutrients in the product are lost. Therefore, fortification is an effective method to increase the overall nutrient content of hot chocolate. Jujube fruit powder contains ascorbic acid, triterpenic acids, phenolic acids, amino acids, saponins, cerebrosides, flavonoids, polysaccharides, and minerals. In this study, jujube fruit powder was added to hot chocolate powder formulations at 0 (control), 10, 20, and 30%, and the physical, chemical, sensory, and microbial properties; phenolic and flavonoid content, and antioxidant capacity of the product were evaluated. The results showed that the addition of jujube powder significantly increased the total phenolic compounds, flavonoids, and antioxidant activity of the hot chocolate powder. Jujube powder reduced the sugar, fat and pH of the product and increased the total ash and acid-soluble ash. Adding jujube powder up to 20% improved the sensory properties of the product, but adding 30% jujube powder reduced the overall acceptance score of the product from the sensory evaluators' perspective. Therefore, adding 20% jujube powder to the hot chocolate powder formulation can be acceptable.

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1-Introduction

Hot chocolate is a cocoa-based beverage that has been consumed for a long time. Traditionally, hot chocolate beverages were consumed in South and Central America and in Europe by people of all ages, without a specific time of consumption [1]; however, today the market for this product is expanding rapidly worldwide. They are usually prepared either from a cocoa powder mixture, from hocolate pieces or chocolate blocks by spreading them in milk or water and are marketed at very different prices. The color, appearance, aroma, taste and texture contribute to the acceptance of the beverage and are decisive factors for consumer preference. The cocoa in the beverage formulation plays a prominent role in creating the overall sensation. The type of cocoa strongly determines the sensory characteristics such as color, taste, mouthfeel, consistency and bitterness[2].

The production of hot chocolate base powder is a multi-step process that includes fermentation, drying, roasting, grinding and refining of cocoa beans, conching and tempering of cocoa beans and its formulation. During this long processing, there is a significant loss of nutrients. The polyphenol content decreases by approximately 10 times. Some flavonoids are completely reduced during the formation of Maillard reaction products. For this reason, the antioxidant composition varies from raw materials to finished products [3]. Approximately 50% of epicatechin is lost during drying. Faster drying speeds lead to the production of acetic acid, which gives off undesirable flavors, and drying too slowly, leads to excessive mold growth and lack of desirable color in the chocolate [4]. Anthocyanin degradation occurs during fermentation due to the hydrolysis and polymerization of dense tannins [3]. During fermentation, the content of epicatechin, polyphenols and procyanidins decreases drastically. Even anthocyanidins become undetectable after fermentation [5]. To compensate for this high loss of phytonutrients, fortification is an effective way, and the fortification of cocoa-based products has attracted much attention in recent years.

Jujube fruit is a good source of bioactive components including polyphenols, triterpenic acids, polysaccharides, nucleosides and nucleobases; therefore, jujube is known as a rich source of functional food. For example, in a study, it was found that in jujube fruit pulp, total phenolic compounds ranged from 1.1 to 2.4 g and flavonoid content ranged from 0.7 to 1.8 g per 100 g of dry matter. In addition, jujube fruits contained several flavonoid compounds such as procyanidin B2, epicatechin, quercetin-3-O-rutinoside, quercetin-3-O-galactoside and kaempferol-glucosyl-rhamnoside. Wojdyło et al. (2016) reported that 25 polyphenolic compounds were present in four Spanish jujube cultivars, and the total polyphenol content ranged from 1442 to 3432 mg/100 g dry matter. According to their study, a total of 10 flavan-3-ols, 13 flavonols, 1 flavanone, and 1 dihydrochalcone were identified in Spanish jujube. Flavan-3-ols, the major group of polyphenols in jujube, accounted for about 92% of the total polyphenol content, while flavonols represented only 8% [6]. Jujube also contains small amounts of triterpenic acids, nucleosides, and nucleobases [7].

Studies have shown that jujube fruit powder has not been used to enrich hot chocolate powder. The aim of this study was to add jujube fruit powder in amounts of 10, 20, and 30 percent to the hot

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chocolate powder formulation and to investigate the physical, chemical, microbial, and sensory properties of the products.

2 –Materials and Methods

2-1 –Materials

Magush brand hot chocolate powder (containing cocoa powder, chocolate base powder, sugar, non-dairy creamer powder, powdered milk and E410 stabilizer) was obtained from Aynaz Maku Company, jujube powder was purchased from local apothecaries, all chemicals and reagents from Merck, Germany, and solvents of the highest purity were obtained.

2-2 –Sample preparation

First, the hot chocolate product was formulated according to the Aynaz Maku Company formulation in the Aynaz Maku Company Research and Development Unit (Magush Coffee Factory), and jujube fruit powder was added to the hot chocolate powder formulation at 0 (control), 10, 20 and 30% w/w. Then, they were mixed in a mixer and packaged in metallized bags to prevent moisture penetration.

2-3- Physical, chemical, sensory and microbial analyses

The physical, chemical, sensory and microbial properties of hot chocolate were analysed according to the Iranian National Standard No. 16884 (2010) [8].

2-4- Determination of total phenolic compounds (TPC)

The amount of total phenolic compounds was determined by the Singleton-Rossi method. In this way, 0.2 ml of a sample was mixed with 1 ml of Folin-Ciocalteau reagent previously diluted in distilled water (1:10) and 0.8 ml of 7.5% (w/v) sodium carbonate [13]. The absorbance value was measured at 765 nm with a spectrophotometer after 30 minutes. The results were expressed as milligrams of gallic acid (GAE) per hundred grams of sample [9].

2-5- DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity

In this study, antioxidant capacity was measured based on the anti-radical activity of the samples using the DPPH method and according to the method of Saviz et al. (2015). Thus, 100 µL of the samples were added to 10 mL of DPPH solution with a concentration of (100 µM) in methanol and shaken vigorously. After 30 minutes of incubation at 27 °C, The absorbanc value was measured at 517 nm against the control sample. In this experiment, TBHQ with a concentration of 100 ppm was used as a control sample. The percentage of DPPH free radical scavenging was calculated using the following equation [10]:

I%= (A Control –A sample /A Control) × 100

2-6- Determination of flavonoid content

The total flavonoid contents of the samples were determined by Udayaprakash et al. method (2015). 5 mL of 0.1 M aluminum chloride was mixed with 200 µL of the extract. The absorbance was measured at 415 nm after incubation for 40 min at room temperature by using a spectrophotometer. The total flavonoid content was expressed as milligrams of

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quercetin equivalent per gram of dry weight (mg QE/g DW) of the sample using a quercetin standard curve [11].

2-7- Microbiological Properties Test Method

2-7-1- Sample Preparation

The samples were prepared according to the Iranian National Standard No. 4-8923, in which the powdered product was thoroughly mixed in the primary container using a sterile spatula or spoon using aseptic methods and then weighed. To reduce osmotic shock to the microflora of the product, it was carefully added to a volume of diluent (buffered peptone water) that had been previously distributed in sterile containers [12].

2-7-2- Total count of microorganisms, mold and yeast

For total count of microorganisms, Iranian National Standard No. 5272-1 was used, and for count of mold and yeast, Iranian National Standard No. 10899-3 was used [13].

3-1- The effect of jujube fruit powder on the physical and chemical properties of hot chocolate powder

Hot chocolate powder is an instant beverage that is prepared based on cocoa powder with the addition of varying amounts of non-dairy creamer powder, sugar, milk powder, emulsifiers, and stabilizers. The presence of a significant amount of sugar and creamer can negatively affect its nutritional value. Therefore, the addition of jujube fruit powder can enhance the nutritional properties of the product and provide a functional product. Jujube fruit is a rich source of nutritional compounds and vitamins, and for this reason it is known as the "king of vitamins." In addition, it is a dietary supplement with a high content of bioactive compounds, and due to its potential antioxidant activity, it can be used as a natural antioxidant element in foods and medicines. Studies have shown that jujube has anti-cancer, anti-inflammatory, anti-diabetic and heart-protecting properties. It also has protective, antioxidant, and anti-insomnia of liver and stimulates the nervous and immune systems. Jujube contains significant amounts of carbohydrates, minerals, vitamins, fiber, amino acids, fatty acids, and phenolic compounds such as gallic, chlorogenic, and caffeic acids, which are considered essential components for human health. Jujube fruit is classified as a “functional food” due to its health benefits that can reduce the risk of certain chronic diseases and also affect target functions beyond its primary nutritional functions [14]. Some properties of jujube fruit powder added hot chocolate samples are given in Table 1.

Table 1 Effect of jujube on some physicochemical properties of hot chocolate powder

Treatment	Appearance	pH	Moisture	Acid-soluble ash	Ash	Fat	Total sugar
control	Acceptable	7.55±0.01^{d}	1.6 ± 0.3 ^{a}	0.1 ± 0.01^{a}	2.2±0.3^{a}	7.1 ± 0.2^{b}	58.1 ± 0.1^{c}
10%	Acceptable	7.46±0.02^{c}	1.8 ± 0.3^{a}	0.15± 0.03^{b}	2.8±0.2^{b}	6.5 ± 0.3	55.7 ± 0.95 ^{c}
20%	Acceptable	7.34±0.01^{b}	2.46 ± 0.3^{b}	0.018± 0.04^{c}	3.3±0.1^{c}	6.2 ± 0.2 ^{b}	52.1 ± 1.2 ^{b}
30%	acceptable	7.32±0.03^{a}	3.06 ± 0.3^{c}	0.22 ± 0.01^{c}	3.6±0.2^{d}	5.8±0.1^{a}	50.5 ± 0.98 ^{a}

Different letter between columns indicates significant statistical difference at level 5%

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The results showed that the addition of jujube fruit powder had no significant effect on the appearance of hot chocolate powder ($P > 0.05$) and all treatments were in accordance with the standard in terms of appearance properties and no foreign materials were observed in the samples. The results also showed that with increasing the level of jujube fruit powder in the product formulation, the moisture content increased significantly ($P < 0.05$). The lowest moisture content was observed in control sample and the highest moisture content was observed in 30% jujube powder added sample. Considering the high initial moisture content of dried jujube fruit compared to other ingredients, increasing the moisture content of the product seems reasonable. Similar results have been reported by Kumar Jatava and Kumar Bahat (2019) [15], Bahrasmani Kohestani et al. (2019) [16] and Shahbazi et al. (2022) [17] who added jujube powder to toffee and dark chocolate formulations, respectively.

The results also showed that with increasing the level of jujube fruit powder in formulation, total sugar decreased significantly ($P<0.05$). Given that increasing the level of jujube powder in the final product reduces the amount of sugar in the formulation, it is logical to reduce the total sugar content of the product. However, the sugar percentage of all treatments was within the standard range. Similar results have been reported by Shahbazi et al. (2022) [17].

The results of this study showed that with increasing the level of jujube fruit powder, the fat content decreased and the total ash and acid-soluble ash increased. Considering that the treatments containing jujube fruit powder have less fat than the control treatment (due to the reduction in the nondairy creamer level), these results seem quite logical. On the other hand, considering that jujube fruit has more minerals than other hot chocolate components, the total ash and acid-soluble ash of the product will also be higher. The results of this study are consistent with the studies of Ario Emamifar et al. (2019) who added jujube fruit powder to cake formulation [17] and also with the findings of Go et al. (2016) who added jujube fruit to sauce formulation [18].

The results showed that the pH value decreased with increasing jujube fruit powder. The highest value was observed for the control sample and the lowest pH value was observed for the 30% jujube powder treatment (Table 1).

3-2 -Effect of jujube on the amount of TPC, flavonoids and antioxidant capacity of hot chocolate powder

Jujube fruit is considered as a good source of bioactive components including polyphenols, triterpenic acids, polysaccharides, nucleosides and nucleobases; therefore, jujube is known as one of the rich sources of functional food. As secondary metabolites and phenolics are important groups of plant compounds that are formed in response to environmental stresses. Due to their hydroxyl groups, these compounds can counteract free radicals and act as electron or hydrogen donors. These compounds also show antioxidant activity by inhibiting the decomposition of hydroperoxides into free radicals [14].

The results of the effect of jujube fruit powder on the amount of TPC and flavonoids are given in Figures 1 and 2. The results showed that with increasing the level of jujube fruit powder, the amount of phenolic and flavonoid compounds in the samples increased significantly ($P<0.05$).

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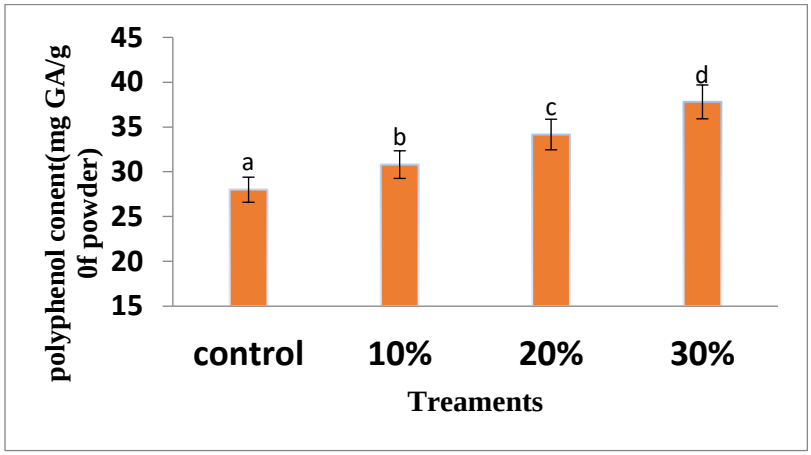

Fig 1 The effect of jujube fruit powder on the phenolic content of hot chocolate
Values with different letters in the same column represents the difference significant. (P<0.05)

The highest amount of total phenolic compounds was found in the 30% jujube powder added sample (37.8 ± 1.5) and the lowest amount was found in the control sample (28 ± 1.6).

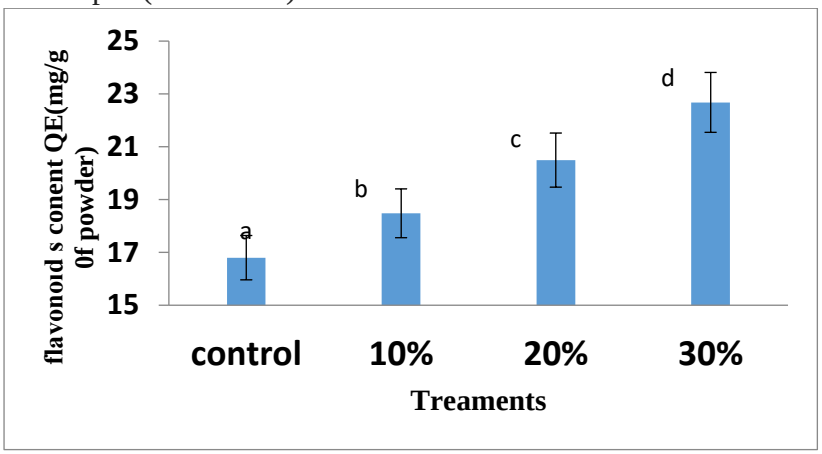

Fig 2 The effect of jujube fruit powder on the Flavonoid compounds of hot chocolate
Values with different letters represents the difference significant. (P<0.05)

The results show that the amount of flavonoid compounds increases with increasing percentage of jujube fruit powder. The results of this study are consistent with the findings of Najja et al. (2020), who added jujube powder to functional cake formulation [19] and also Shahbazi et al. (2020) [14], who enriched dark chocolate with jujube fruit powder. The findings of this study are also consistent with the report of Rashwan et al. (2022) [20].

The DPPH antioxidant assay is widely used to assess the ability of antioxidants to scavenge or neutralize the DPPH radical. The DPPH radical is more stable than the hydroxyl and superoxide anion radicals, which is one of its advantages [21]. Aromatic essences and extracts of medicinal plants such as jujube also have a high potential to scavenge DPPH radicals due to their high levels of phenolic compounds and total antioxidant capacity [22].

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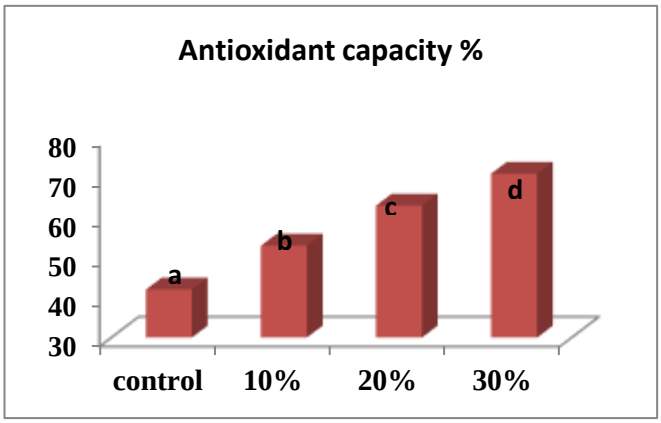

Fig 3 Inhibition of DPPH radicals by samples at different concentrations.
Values with different letters represents the difference significant. (P<0.05)

The results showed that hot chocolate powder as a control sample had significant antioxidant activity (42.32 ± 0.2) due to the significant amount of cocoa powder in its formulation, which was significantly (P < 0.05) increased by adding different percentages of jujube. The highest inhibitory activity of hot chocolate was observed for the treatment containing 30% jujube powder (71.58 ± 1.00%) (Figure 3).

3-3 -Effect of jujube fruit powder on total microbial count

The effect of jujube fruit powder on the microbial load of hot chocolate powder is shown in Table 2. The microbial load values of different treatments are shown in terms of (standard deviation ± mean) in the table.

Table 2 Effect of jujube powder on the total microbial count of the product

Treatment	Microbial load (logcfu/g)
Control	2.47 ±0.01 [a]
10%	2.54 ± 0.05 [b]
20%	2.81 ± 0.03 [c]
30%	2.85 ± 0.05 [c]

According to the above table, the microbial load between the different experimental treatments is significant at the 5% error level. The highest microbial load was observed in %30 treatment and the lowest in the control treatment. Comparison of the experimental results showed that with increasing the percentage of jujube powder the microbial load increased. The higher microbial load with increasing the percentage of jujube powder is probably a result of the high microbial load of jujube added to the product.

3-4 -Effect of jujube fruit powder on the amount of mold and yeast in hot chocolate powder

The effect of jujube fruit powder on the amount of mold and yeast in hot chocolate powder was also evaluated. The results showed that mold and yeast were not observed in control group and jujube fruit powder incorporated samples.

3-5 -Effect of jujube fruit powder on sensory properties of the product

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Cocoa-based products are very popular among different groups of people due to their distinctive taste, aroma and color. Today, chocolate food and beverage consumers are not only specific groups, but also all levels of society. This indicates an encouraging development in the outlook of the chocolate food and beverage market. However, consumers are also focused on the positive impact of food and beverage on health. One of the most popular food products is chocolate drinks, which has been transformed into simple products such as "instant hot chocolate" and "ready-to-drink chocolate". Small amounts of several active compounds are present in chocolate drinks, including catechins, procyanidin B1 and procyanidin B2, at concentrations of 0.01–0.12% (w/w). Cocoa beans contain polyphenolic compounds, which is why cocoa products have antioxidant properties that make them potential health beverage products [23]. In this study, 10 trained sensory evaluators were used to evaluate the sensory properties of the product, and the sensory evaluators assigned a score of 0–5 to the treatments. The results of this evaluation are summarized in Table 4.

Table 4 Effect of jujube powder on the sensory properties of hot chocolate

Treatment	Appearance color	Taste	Aroma	Consistency	Overall acceptance
Control	4.56 ±0.6 [b]	4.5±0.3 [a]	4.75 ± 0.3 [a]	4.6 ± 0.7 [b]	4.40±0.3 [b]
10%	4.77 ± 0.5 [b]	4.65 ± 0.4 [a]	4.75 ± 0.4 [a]	4.57 ± 0.5 [b]	4.65 ± 0.2 [b]
20%	4.87 ± 0.3 [b]	4.79 ± 0.6 [a]	4.79 ± 0.6 [a]	4.67 ± 0.2 [b]	4.83 ± 0.7 [b]
30%	3.85 ± 0.5 [a]	4.85 ± 0.2 [b]	4.85 ± 0.2 [a]	3.32 ± 0.3 [a]	3.28 ± 0.3 a

Different letter between columns indicates significant statistical difference at level 5%

The findings of this study showed that the color acceptability of the different experimental treatments was significant (P < 0.05). The highest color score was given to the 0, 10%, and 20% treatments, and the lowest was given to the 30% jujube powder treatments. Comparison of the experimental results showed that increasing the percentage of jujube powder to 20% had no effect on the color acceptability of the different treatments, but adding 30% jujube powder reduced the color score, which was due to the lower color and solubility of jujube powder. The findings of this study also showed that the taste and flavor acceptability of the different experimental treatments was significant at the 5% (P < 0.05). The highest taste and flavor score was given to the 30% treatment, and the lowest was given to the control treatment. Comparison of the test results showed that the taste score increased with increasing the percentage of jujube powder. The higher taste score with increasing the percentage of jujube powder is probably due to the taste of jujube powder added to the product due to its high fructose content. Analysis showed that the aroma and flavor scores of the different test treatments were not significant (p.0.05). In other words, increasing the jujube powder had no effect on the taste and flavor of the product. The results of the analysis of variance showed that the texture or consistency of the different test treatments was significant (p<0.05) from the sensory evaluators' perspective. The highest texture evaluation score was given to the 0, 10, and 20% treatments, and the lowest was given to the 30% treatment. Comparison of the test results showed that with increasing the percentage of jujube powder, the acceptability of the texture increased, but adding 30 percent of jujube powder had a negative effect on the consistency of the product due to the high concentration of the

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product. The findings of this study showed that the overall acceptability of the different experimental treatments is significant. The highest overall acceptability score was given to the 20% treatment and the lowest to the 30% treatment.

4 - Conclusion

Hot chocolate powder is an instant beverage based on cocoa powder, which is being produced and marketed in various formulations. During the processing of the hot chocolate base, some of its nutritional components are lost. On the other hand, the desire to consume functional products containing bioactive compounds is also in demand by consumers. In this study, the effect of adding functional jujube fruit powder in amounts of zero (control sample), 10, 20 and 30% was added to the hot chocolate powder formulation and its appearance, physical and chemical properties, sensory and microbial properties were evaluated. In addition, the amounts of total phenolic compounds, flavonoid compounds and antioxidant capacity were evaluated in all treatments. The results showed that jujube fruit powder had no significant effect on the appearance of hot chocolate powder and all treatments were in accordance with the standard in terms of appearance properties. With increasing jujube fruit powder level in the product formulation, moisture, total ash and acid-soluble ash and pH increased, but total sugar and fat contents decreased. However, all physical and chemical properties of the experimental samples were within the standard and acceptable range. With increasing jujube fruit powder level, the amount of phenolic and flavonoid compounds in the product increased significantly. The treatment containing 30% jujube powder had 35 and 38% more phenolic and flavonoid compounds, respectively, than the control sample. The antioxidant activity of the samples enriched with jujube powder was higher than the control sample and the treatment containing 30% jujube powder had 29% more DPPH free radical scavenging properties than the control sample. Adding jujube powder up to 20% did not have a negative effect on the sensory properties of the product, but samples containing 30% jujube powder scored lower than the control sample. The best treatment for enriching hot chocolate powder with jujube was the 20% treatment.

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مقاله علمی_پژوهشی

تأثیر غنی‌سازی پودر شکلات داغ با میوه‌ی عناب بر ویژگی‌های کیفی آن

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کلمات کلیدی:

عناب، شکلات داغ،
ترکیبات فنولی،
ظرفیت آنتی‌اکسیدانی،
پودر کاکائو،
فراسودمند

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* مسئول مکاتبات:

چکیده

شکلات داغ (یک نوشیدنی بر پایه کاکائو) به دلیل فواید سلامتی فراوانی که دارد در دهه‌های اخیر محبوبیت پیداکرده است؛ اما در طی فرآوری پودر پایه‌ی این محصول، مقداری از مواد مغذی این محصول از بین می‌رود؛ بنابراین، غنی‌سازی یک روش مؤثر برای افزایش محتوای کلی مواد مغذی شکلات داغ است. پودر میوه‌ی عناب، حاوی اسید اسکوربیک، اسیدهای تری‌ترپنیک، اسیدهای فنولیک، اسیدهای آمینه، ساپونین‌ها، سربروزیدها، فلاونوئیدها، پلی ساکاریدها و مواد معدنی است. در این تحقیق پودر میوه عناب به میزان ۰، ۱۰، ۲۰ و ۳۰ درصد وزنی–وزنی به فرمولاسیون شکلات داغ افزوده شد و خصوصیات فیزیکی، شیمیایی، حسی و میکروبی؛ میزان ترکیبات فنلی، فلاونوئیدی و ظرفیت آنتی‌اکسیدانی محصول مورد ارزیابی قرار گرفت. نتایج حاصل نشان داد که افزودن پودر عناب توانست سبب افزایش معنی‌دار ترکیبات فنلی کل، ترکیبات فلاونوئیدی و فعالیت آنتی‌اکسیدانی پودر شکلات داغ شود. پودر عناب، سبب کاهش میزان قند کل، چربی و pH محصول و سبب افزایش خاکستر کل و خاکستر محلول در اسید گردید. افزودن پودر عناب تا سطح ۲۰ درصد وزنی خواص حسی محصول را بهبود داد ولی افزودن ۳۰ درصد پودر عناب امتیاز پذیرش کلی محصول را از دیدگاه ارزیابان حسی کاهش داد؛ بنابراین افزودن پودر عناب به میزان ۲۰ درصد به فرمولاسیون پودر شکلات داغ می‌تواند موردپذیرش قرار گیرد.

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