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The effect of barberry powder fortification on the quality characteristics of hot chocolate powder

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ABSTRACT

Since some of the nutrients, including polyphenols, flavonoids, ascorbic acid, and thiamine, are lost during the processing of hot chocolate powder, fortification is an effective method to increase the overall nutrient content of hot chocolate powder. In this study, the effects of barberry powder incorporation at 0, 2.5, 5, and 7.5% (w/w) on phenolic compounds, flavonoids, and anthocyanins, antioxidant capacity, ascorbic acid content, and physical, chemical, microbial, and sensory properties of the hot chocolate powder were evaluated. The results showed that barberry powder had no significant effect on the appearance of hot chocolate powder, but with increasing barberry powder level in the hot chocolate powder formulation, moisture content increased but total sugar and the fat content of the product decreased ($p<0.05$). With increasing barberry powder ratio, total ash content increased and pH decreased. Barberry powder increased the content of anthocyanins and ascorbic acid, phenolic compounds, flavonoids, and antioxidant activity. Based on thethe sensory evaluators' perspective, the highest overall acceptability score was given to the 5% added barberry powder treatment. Using barberry powder at a level of 5% in the formulation of hot chocolate powder can improve its nutritional properties and meet consumers' needs for bioactive compounds.

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1- Introduction

Hot chocolate drinks were traditionally 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 hot chocolate is expanding rapidly worldwide due to its popularity among people, especially children and adolescents. This product is usually prepared either from a mixture of cocoa powder, from chocolate pieces or from chocolate blocks by spreading them in milk or water and marketing at very different prices. Although a wide range of flavors and formulations are available on the market, its main ingredients are cocoa powder or cocoa liquor, sugar and milk components. Due to the insolubility of cocoa particles and depending on the fat content, chocolate drinks tend to separate by settling and becoming creamy. In most cases, consumption is recommended immediately after preparation.

During the multi-step processing of hot chocolate base powder production, which includes fermentation, drying, roasting, grinding and refining of cocoa beans, conching and tempering of cocoa beans, and its formulation, there is a significant loss of cocoa nutrients. For this reason, the fortification of hot chocolate has received attention in recent years, and various ingredients, including blackberries, lemon balm extract, cinnamon powder, prebiotics and probiotics, have been used for fortification [2,3,4,5,6].

Barberry (*Berberis vulgaris*) is a red fruit that grows in Asia, North Africa, and Europe. This fruit is rich in phytochemicals and bioactive compounds. It is used to prevent various diseases and also has various health-promoting properties. It has antibacterial, anticancer, antidiabetic, anti-inflammatory, antihypertensive, and lipid-lowering properties [5,6]. The fruit can be used fresh, dried, or in the form of syrup, jam, and jelly. Its powder also has many applications as a spice or as an ingredient in food product formulations [7]. Adding barberry powder to hot chocolate powder formulations can meet the needs of consumers, especially children and adolescents, for bioactive compounds. Studies have shown that barberry fruit powder has not been used to hot chocolate powder fortification so far. In this study, barberry fruit powder was added to hot chocolate powder formulation in amounts of 0 (control), 2.5, 5, and 7.5 percent, and the physical, chemical, microbial, sensory properties, and bioactive compounds of the product were investigated.

2 -Materials and Methods

2-1- Materials

Hot chocolate powder, With Magush coffee trade name, (Ingridients: cocoa powder, non-dairy creamer, sugar, chocolate base powder, powdered milk, and E410 stabilizer), barberry powder was obtained from Aynaz Maku Company (Maku, Iran) and Zare Company (Qaen, Iran) respectively. All chemicals and solvents were of analytical grade or higher available purity.

2-2- Sample preparation

First, the hot chocolate product was formulated according to the Aynaz Maku Company formulation in the Research and Development Unit (Magush Coffee Factory) and barberry fruit powder was added to the hot chocolate powder formulation in amounts of 0 (control sample), 2.5, 5 and 7.5 % (w/w). Then, it was mixed in a mixer for 15 minutes and inorder to prevent moisture penetration, packaged in metalized bags until analysis.

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

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

2-4 -Total phenolic, flavonoid and antioxidant capacity

The total phenolic content (TPC) was determined according to Singleton-Rossi method. Briefly, 0.2 ml of a sample was

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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. After 30 minutes stand in the dark at room temperature the absorbance was recorded at 765 nm using UV-Visible spectrophotometer (model 6305, Jenway, UK). The results were expressed as milligrams of gallic acid (GAE) per hundred grams of sample [9].

The total flavonoid content of the extract was determined by the method of Udayaprakash et al. (2015). 5 mL of 0.1 M aluminum chloride was mixed with 200 µL of the extract. The absorbance was recorded at 415 nm after incubation for 40 min at room temperature by spectrophotometer. The total flavonoid content was expressed as milligrams of quercetin equivalents per gram of dry weight (mg QE/g DW) of the sample using a quercetin standard curve [10].

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. Thus, 100 microliters of the samples were added to 10 ml of DPPH solution with a concentration of 100 micromolar in methanol and shaken vigorously. After 30 minutes of incubation at 27 °C, the optical absorption of the samples was read at a wavelength of 517 nm against the control extract. In this experiment, TBHQ with a concentration of 100 ppm was used as a control sample. The inhibition (%) of radical scavenging activity was calculated by the following equation: [11]:

$I\% = (A_{Control} - A_{sample} / A_{Control}) \times 100$

2-5- Vitamin C (ascorbic acid) and anthocyanins

Vitamin C was measured by titration using 2,6-dichlorophenol-indophenol [12]. The total anthocyanin content of the extract was measured spectrophotometrically using the pH difference method [13]. Two buffer systems were used, potassium chloride buffer (0.25 M) with pH = 1 and sodium acetate buffer (0.4 M) with pH = 4.5. Briefly, in this method, 400 µL of the extract solution was mixed with 3.6 mL of each buffer separately and each was read at two wavelengths of 510 and 700 nm. The results were expressed in mg/L equivalent of cyanidin-3-glycoside in the extract according to the following formula:

$$Monomeric\ anthocyanin\ pigment\ (mg/L) = \frac{A \times MW \times DF \times 1000}{\varepsilon \times 1}$$

In the above formula, DF, MW, ε and A represent the dilution factor of the samples, the molecular weight of cyanidin, the molar coefficient of cyanidin and the absorbance value, respectively. The absorbance value is calculated as follows:

$A = (A_{510} - A_{700})_{pH1} - (A_{510} - A_{700})_{pH4.5}$

A_{510} and A_{700} represent absorption at wavelengths of 510 nm and 700 nm, respectively.

2-7- Microbiology Characteristics Test Method

Sample Preparation

The samples were prepared according to the Iranian National Standard No. 4-8923, which was followed by aseptic methods, mixing the powdered product thoroughly in the initial container using a sterile spatula or spoon and then weighing. 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.[۱۴]

Total count of microorganisms, molds, and yeasts

The Iranian National Standard No. 1-5272 was used for the total count of microorganisms, and the Iranian National Standard No. 3-10899 was used for the count of molds and yeasts [15].

3. Results

3-1- The effect of barberry Powder on physical and chemical properties of hot chocolate powder

The effect of adding barberry powder on the properties of hot chocolate powder is given in Table 1.

Table 1 Effect of Barberry powder on the physicochemical properties of hot chocolate powder

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Treatment	Appearance	pH	Moisture	Ash	Fat	Total sugar
control	Acceptable	7.55±0.01^{d}	1.6 ± 0.3 ^{a}	2.2±0.03^{a}	7.1 ± 0.2^{b}	58.1 ± 0.1^{c}
2.5%	Acceptable	6.95±0.02^{c}	1.75 ± 0.2^{ab}	2.32±0.06^{b}	6.9 ± 0.3^{b}	55.7 ± 0.8 ^{c}
5%	Acceptable	6.66±0.01^{b}	1.83 ± 0.3^{b}	2.56±0.02^{c}	6.8 ± 0.2 ^{b}	56.1 ± 0.7 ^{b}
7.5%	Not Acceptable	6.27±0.03^{a}	1.96 ± 0.3^{b}	2.9±0.1^{d}	6.5±0.1^{a}	54.5 ± 1.2 ^{a}

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

The findings showed that barberry fruit powder up to 5% had no significant effect on the appearance of hot chocolate powder ($p>0.05$) and all treatments in terms of appearance properties except for the 7.5% sample were in accordance with the standard and no foreign substances were observed in the samples. Also, with increasing the level of barberry fruit powder in the hot chocolate formulation, the moisture content increased significantly. The lowest moisture content (1.6 ± 0.1%) was observed for the control treatment and the highest moisture content (1.96 ± 0.3%) was observed for the 7.5% barberry treatment. The findings also showed that with increasing the level of barberry fruit powder in the hot chocolate formulation, total sugar and fat decreased significantly ($p<0.05$). Considering that increasing the level of barberry powder in the final product reduces the amount of sugar and creamer in the formulation, it is logical to reduce the total sugar and fat content of the product. However, the percentages of sugar and fat in all treatments was within the standard and acceptable range. Similar results were reported by Bakmohammadpour et al. (2021) and Jaberi et al. (2020) who added barberry powder to puffed corn extrude and hamburger formulations, respectively [7,16].

Also, according to the data given in Table 1, it was observed that with increasing the level of barberry fruit powder, the amount of total ash and acid-soluble ash increased. Given that barberry fruit is rich in minerals such as phosphorus, manganese, sodium, potassium, iron and zinc, the total ash and acid-soluble ash of the product will also be higher.

The results showed that with increasing the level of barberry powder, the pH value decreased. The highest value was observed for the control sample and the lowest pH value was observed for the 7.5% barberry powder treatment. The pH of barberry fruit varied from 2.49 to 3.91 depending on its variety, and the low pH is due to compounds such as chlorogenic acids, ascorbic acid and citric acid; the low pH of the formulated product seems logical [17]. The findings of this study are largely consistent with the findings of Матюшев et al. (2016), who added barberry powder in 1, 3, 5, and 7 % to the toffee formulation and in 3, 7, 11, and 15 % to the cake formulation [18].

3-2- The effect of barberry on the content of anthocyanins, ascorbic acid, phenolic, flavonoids compounds and antioxidant capacity of hot chocolate powder

The results of the effect of barberry fruit powder on the levels of anthocyanin, ascorbic acid, total phenolic compounds, flavonoids, and antioxidant capacity are presented in Table 2.

Table 2 Effect of barberry powder on bioactive compounds of hot chocolate

Treatment	**control**	**2.5%**	**5%**	**7.5%**
Anthocyanin (mg/kg of product)	9.5 ± 0.7 ^{a}	30.75 ± 0.4 ^{b}	52 ± 0.8 ^{c}	69.5 ±1.1^{d}
Ascorbic acid (mg/kg of product)	0.2 ± 0.03 ^{a}	0.7 ± 0.01 ^{b}	1.5 ± 0.05 ^{c}	2.7± 0.06 ^{d}
TPC (mg of GA acid / kg)	28100 ±12 ^{a}	28250 ±11 ^{b}	28425 ±13 ^{c}	28570±18 ^{d}
Flavonoid compounds (mg quercetin/kg)	16830 ± 14 a	17970 ± 15 ^{b}	18480±12 ^{c}	19710 ±25 ^{d}
Antioxidant capacity (%DPPH)	42^{a}	54^{b}	69^{c}	78^{d}

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Different letter within rows indicates significant statistical difference at level 5%

Anthocyanins have several health-promoting properties. They can reduce total cholesterol and low-density lipoproteins, which are important risk factors for cardiovascular diseases. These compounds are also effective in preventing and reducing the risk of diabetes. Anthocyanins have free radical scavenging activity and prevent oxidation. With these positive properties, it is recommended to fortification food products with these compounds. Hot chocolates have a small amount of bioactive components such as anthocyanins. However, they are a favorite food product that is often consumed by many people, especially children. Hot chocolate powder fortified with barberry powder can serve as a good source of bioactive components, since barberry is a rich source of bioactive components such as anthocyanins [19].

The findings of this study showed that the addition of barberry powder had a significant effect on the anthocyanin and ascorbic acid (vitamin C) content of hot chocolate powder. The lowest anthocyanin content was observed in the control treatment (9.5 ± 0.7) and the highest content was found in the 7.5% barberry powder sample, which was evaluated as 69.5 ± 1.1 mg per kilogram of hot chocolate powder. The results also showed that barberry powder had a significant effect on the vitamin C content of hot chocolate. Addition of 7.5% barberry powder, increased the vitamin C content of hot chocolate powder from 0.2 mg to 2.7 mg per kilogram of product. These results are consistent with the findings of Bakmohammadpour et al. (2021), who added barberry powder in 2.5, 5, 7.5, and 10% to the puffed corn extrude formulation (7). Similar results were reported by Jabri et al. (2019) and Sarraf et al. (2019) [16, 17].

The results of the analysis of variance showed that barberry powder had a significant effect on the amount of TPC in the fortified hot chocolate powder. The highest amount of TPC was found in the 7.5% barberry powder treatment (28570 ± 18) and the lowest amount was found in the control (28100 ± 12) mg/kg of powder. The results also showed that the amount of flavonoid compounds increased with increasing ratio of barberry powder. The results of this study are consistent with the findings reported by Polniski et al. (2022) who fortified dark chocolate with elderberry [20].

The findings also showed that hot chocolate powder as a control sample had significant antioxidant activity (42.32 ± 0.2) due to the ratio of cocoa powder in its formulation, which was significantly increased by adding different percentages of barberry ($P<0.05$). The highest inhibitory activity of hot chocolate was observed for the treatment containing 7.5% barberry powder (78 ± 1.00%).

3-3- Total microbial and mold and yeast count

The effect of barberry powder on the total microbial count of hot chocolate powder is shown in Table 3. The microbial count values of different treatments are shown in terms of standard deviation ± mean in the table.

Table 3 Effect of barberry powder on the total microbial count of the product

Treatment	Microbial load (logcfu/g)
Control	2.47 ±0.01 [a]
2.5%	2.48 ± 0.05 [a]
5%	2.45 ± 0.03 [a]
7.5%	2.51± 0.07 [a]

According to the table 3, the microbial load between the different experimental treatments was not significant ($P>0.05$). The microbial count of all samples was within the standard and acceptable range, and barberry powder did not affect the total

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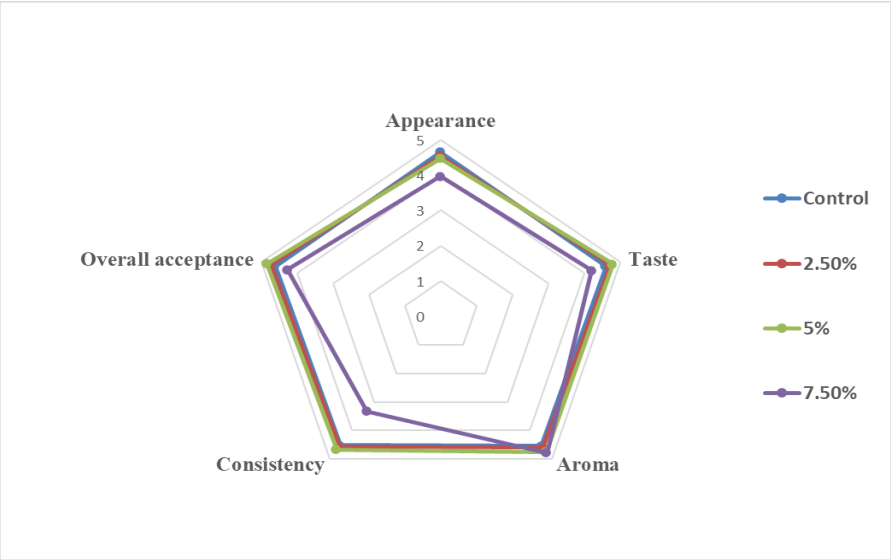
microbial count of the product. When Bak Mohammadpour et al. added barberry powder to the puffed corn extrude formulation, the addition of barberry powder did not affect the total microbial count of the product, which is consistent with the findings of the present study [7].
Comparison of the test results showed that with increasing the percentage of barberry powder, mold and yeast were not observed in any of the treatments. Considering that hot chocolate powder and different treatments have low moisture content, the absence of mold and yeast in the samples is not far from expectation.

3-4- The effect of barberry fruit powder on the sensory properties of the product

One of the characteristics of food products is their sensory properties, which has a significant impact on the selection of food products and their consumption, and special attention should be paid to it in the production of new products. Considering the acidity and also the bitter aftertaste in barberry fruit powder, adding it up to 5% had a positive effect on sensory properties and their scores were comparable to the scores given to the control sample, but at the level of 7.5%, the scores given and the acceptability of the product decreased (Figure 1). The reasons for the decrease in acceptability were the bitter aftertaste and the appearance of the products in the barberry powder treatments.
The findings of this study showed that the color acceptability of different treatments was significant (P<0.05). The highest color score was given to 0, 2.5%, and 5% treatments, and the lowest was given to 7.5% barberry powder treatments. Comparison of the test results showed that increasing the percentage of barberry powder to 5% had no effect on the color score of different treatments, but adding 7.5% barberry powder reduced the color score, which was due to the darker color of barberry powder.

Also, analysis of variance showed that the difference in taste scores of different experimental treatments was significant (p<0.05). The highest taste score was assigned to the 5% barberry powder treatment and the lowest was assigned to the 7.5% treatment.



.Fig. 2 The effect of barberry powder on the sensory properties of hot chocolate powder

The results also showed that the aroma score of different experimental treatments was significant (P<0.05). Barberry powder affected the sensory panel score due to its

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specific aroma. The results also showed that the texture or consistency of the different experimental treatments were significant from the perspective of sensory evaluators. The highest texture evaluation score were given to the treatments with 0, 2.5 and 5 % barberry powder, and the lowest was given to treatment with the 7.5 %. Comparison of the experimental results showed that with increasing the percentage of barberry powder, the acceptability of the texture increased to the level of 5 %, but adding 7.5 % barberry powder had a negative effect on the consistency of the product due to the high concentration of the product. The findings of this study showed that the overall acceptability score of the different experimental treatments were significant at the 5 % error level. The highest overall acceptability score was given to the 5 % treatment and the lowest was given to the 7.5 % treatment.

4- Conclusion

Barberry fruit contains high amounts of phytochemicals and bioactive compounds and has antibacterial, anticancer, antidiabetic, anti-inflammatory, antihypertensive and lipid-lowering properties. This fruit can be used fresh, dried or in the form of syrup, jam and jelly. Its powder also has many uses as a spice or as an ingredient in food product formulations [7]. Adding barberry fruit powder can enhance the nutritional properties of the product and provide a functional product. Barberry fruit is also a good source of carotenoids, flavonoids, phenolic compounds, anthocyanins and some alkaloids. The amount of anthocyanins, phenolic compounds, flavonoid compounds and ascorbic acid in barberry fruit, depending on its varieties and cultivation location, varies from 0.17 to 0.93, 2.18 to 7.8, 0.58 to 3.95 and 107 to 136 mg per gram of fruit respectively; 1. Also, the presence of tannin compounds in the fruit causes an astringent and bitter taste in the fruits[17].

For this reason, the functional barberry fruit powder was added to the hot chocolate powder formulation in amounts of 0 (control sample), 2.5, 5 and 7.5 % and the appearance, physical and chemical properties, sensory properties, microbial properties and bioactive compounds of experimental samples were evaluated. The results showed that the incorporation of barberry fruit powder up to 5 % had no significant effect on the appearance of the hot chocolate powder and all treatments in terms of appearance properties except the 7.5 % sample were in accordance with the standard and no foreign substances were observed in the samples. With increasing the level of barberry fruit powder in the hot chocolate powder formulation, the moisture content increased significantly but the total sugar and fat of the product decreased. Also, the amount of total ash and acid-soluble ash increased and the pH decreased. The addition of barberry powder increased the amount of anthocyanin and ascorbic acid (vitamin C), phenolic compounds, flavonoids and antioxidant activity of hot chocolate powder. On the other hand, the microbial load of all samples were within the standard and acceptable level, and barberry powder did not affect the total microbial count of the product. The highest overall acceptability score from the sensory evaluators' perspective was given to the 5% barberry powder treatment and the lowest was given to the 7.5% treatment. Considering that hot chocolate is a popular product and its consumption is increasing day by day, and during its processing and formulation, a large part of the bioactive compounds of cocoa are lost, and on the other hand, the presence of functional bioactive compounds in barberry powder, the use of this additive in hot chocolate formulation can meet some of the consumers' needs for these compounds. The results of this study showed that the use of this valuable material up to the level of 5% can be used in hot chocolate powder formulation.

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* مسئول مکاتبات:

چکیده

به دلیل اینکه در طی فرآوری پودر شکلات داغ، مقداری از مواد مغذی آن ازجمله پلی فنل‌ها، فلاونوئیدها، اسید اسکوربیک و تیامین از بین می‌رود، غنی‌سازی یک روش مؤثر برای افزایش محتوای کلی مواد مغذی شکلات داغ خواهد بود. در این پژوهش پودر زرشک به میزان ۰، ۲/۵، ۵ و ۷/۵ درصد (وزنی-وزنی) به فرمولاسیون شکلات داغ اضافه‌شده و میزان ترکیبات فنلی، فلاونوئیدی، آنتوسیانین‌ها، ظرفیت آنتی‌اکسیدانی، میزان اسید اسکوربیک، خصوصیات فیزیکی، شیمیایی، میکروبی و حسی محصول مورد ارزیابی قرار گرفت. نتایج نشان داد که پودر زرشک تأثیر معنی‌داری بر روی ظاهر پودر شکلات داغ نداشت ولی با افزایش سطح پودر زرشک در فرمولاسیون پودر شکلات داغ، رطوبت به‌طور معناداری افزایش ولی قند کل و چربی محصول کاهش یافت. با افزایش پودر زرشک، میزان خاکستر کل افزایش و pH کاهش یافت. پودر زرشک مقدار آنتوسیانین و اسید اسکوربیک، ترکیبات فنلی، فلاونوئیدی و فعالیت آنتی‌اکسیدانی افزایش داد. از دیدگاه ارزیابان حسی بالاترین امتیاز مقبولیت کلی به تیمار ۵ درصد پودر زرشک داده شد. استفاده از پودر زرشک در سطح ۵ درصد در فرمولاسیون پودر شکلات داغ می‌تواند سبب بهبود ویژگی‌های تغذیه‌ای آن شده و نیازمندی‌های مصرف‌کنندگان را به ترکیبات زیست فعال تأمین نماید.

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