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Research Article

Effect of heat treatment on antioxidant potential of honey-milk beverage

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Abstract

This study aimed to investigate the effects of honey addition and different heat treatments, including pasteurization and sterilization, on the antioxidant activity of milk of cow. The antioxidant capacities of milk, honey, and honey–milk mixtures were evaluated using three widely accepted analytical methods, i.e., ABTS, DPPH, and reducing power assay. The results demonstrated that honey incorporation (5%) significantly enhanced the antioxidant potential of milk. Among the tested samples, sterilized honey–milk exhibited the highest antioxidant activity, indicating a strong positive effect of high-temperature treatment on antioxidant activity. In addition, pasteurized honey–milk showed higher antioxidant activity compared to raw honey–milk samples. Heat treatment, particularly sterilization, significantly increased the antioxidant activity of milk, honey, and their combinations, which may be attributed to the formation of antioxidant compounds during thermal processing. Overall, the findings suggest that both honey addition and heat treatment play important roles in improving milk antioxidant properties. Therefore, honey-enriched heat-treated milk can be considered a functional beverage with excellent antioxidant activity and potential health benefits for consumers.

Keywords: Antioxidant activity, Heat treatment, Honey, Honey-milk, Milk.

Introduction

In recent years, new functional beverages have attracted the attention of consumers. The addition of honey to milk can result in the production of a functional beverage due to the nutritional value of milk and the health advantages of honey. Milk is considered a complete food because it contains valuable nutrients (fats, proteins, amino acids, vitamins, and minerals) (Lindmark-Månsson & Åkesson, 2000). Owing to its complex composition, milk exhibits various biological activities. For example, compounds such as lactoferrin, lactoperoxidase, and lysozyme have antibacterial effects (Clare et al., 2003; Floris et al., 2003), while other compounds such as vitamin E, vitamin C, beta-carotene, and enzymatic systems (superoxide dismutase and catalase), have antioxidant activity (Lindmark-Månsson & Åkesson, 2000). The antioxidant potential of different types of casein (alpha, beta, and kappa) has been demonstrated (Cervato, 1999). The antioxidant effects of casein, peptides resulting from casein, and peptides resulting from beta-lactoglobulin have been reported previously (Díaz & Decker, 2004; Hernández-Ledesma et al., 2005). Antioxidants, such as vitamin A, carotenoids, and ubiquinol in the lipid phase, and vitamin C in the aqueous phase, act as free radical scavengers. Other antioxidants, such as flavonoids, are active in both lipid and aqueous phases (Lindmark-Månsson & Åkesson, 2000).

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Antioxidants play an important role in preventing lipid peroxidation and preserving the quality of milk. Antioxidants may have health-promoting effects on consumers.

The health benefits of honey are mainly related to its phenolic compounds and flavonoid content. These compounds exhibit antimicrobial and antioxidant activities. Honey is considered as a rich source of natural antioxidants (Wilczyńska & Żak 2024; Tlak Gajger et al., 2025). Antioxidants play an important role in protecting food and the human body against the detrimental effects of oxidation and free radicals. Thus, honey antioxidants can prevent cardiovascular diseases, cancers, Alzheimer's disease, and various inflammatory processes (Beretta et al., 2005; Liu et al., 2013). The antioxidant efficiency of phenolic compounds in honey milk beverages may be affected by milk proteins. Various studies on tea, coffee, cocoa, and fruit beverages have indicated that the addition of milk or milk proteins can reduce the antioxidant capacity of phenolic compounds due to interactions between polyphenols and proteins (Arts et al., 2001; Dubeau et al., 2010; Niseteo et al., 2012). However, other researchers have found no significant reduction in the antioxidant activity of phenolic compounds in tea, coffee, and cocoa after the addition of milk (Dupas et al., 2006; Keogh et al., 2007). These contradictory findings can be attributed to the different antioxidant methods used in these studies (Dubeau et al., 2010; Zulueta et al., 2009).

Thermal treatments (e.g., pasteurization and sterilization) are among the major processes used in the dairy industry. These thermal treatments can reduce the nutritional value of milk, decrease the number of phenolic compounds, carotenoids, and ascorbic acid, and consequently decrease its antioxidant capacity. However, a study showed that thermal treatment, particularly sterilization, increased the antioxidant activity of milk (Khaledian et al., 2025). This increase was attributed to Maillard reaction products (melanoidins) with antioxidant potential (Lan et al., 2026). A large number of studies have evaluated the impact of milk or milk proteins on the antioxidant activity of beverages containing phenolic compounds (e.g., cocoa, coffee, and particularly tea). However, no study has focused on the effects of adding honey to milk and various thermal treatments on the antioxidant capacity of milk. Therefore, the objective of the present study was to investigate the effects of honey addition and various heat treatments on the antioxidant potential of milk.

Materials and Methods

Chemicals

Ferric chloride, potassium ferricyanide, 2,2 -diphenyl-1-picrylhydrazyl (DPPH), Butylated hydroxyl toluene(BHT), 2,2-azinobis-3-ethylbenzothiazoline-
6-sulphonic acid (ABTS), and potassium persulfate were obtained from Sigma-Aldrich Chemie (Steinheim, Germany). Disodium hydrogen phosphate (Na_2HPO_4), sodium dihydrogen phosphate (NaH_2PO_4), sodium carbonate, NaOH, trichloroacetic acid, and Folin-Ciocalteu's phenol reagent were purchased from Merck (Darmstadt, Germany).

Thermal treatments

Cow raw milk was obtained from the Department of Animal Husbandry, Faculty of Agriculture, Urmia University (Iran). Honey was purchased from a local market. Three raw samples, including milk, honey (5%), and honey-milk (5%), were prepared. The samples were subjected to thermal processing (pasteurization and sterilization). For pasteurization, an aliquot of each raw sample (milk, honey, and honey-milk) was poured separately into test tube, placed in a water bath and heated to 63 °C for 30 min (He et al., 2015). The tubes were immediately cooled under tap water. The pasteurized milk was mixed with the pasteurized honey to prepare honey-milk (5%). For sterilization, a volume of each sample (milk, honey and honey milk) was separately transferred to Erlenmeyer flasks. Then, the samples were placed in an autoclave for 10 min at 121°C (He et al., 2015). The Erlenmeyer flasks were removed from the autoclave and cooled immediately under tap water. The sterilized milk was mixed with sterilized honey. The antioxidant activities of the treatments were examined using three antioxidant methods. The samples were kept frozen until used for antioxidant tests.

Antioxidant tests

ABTS (2,2′-azinobis-(3-ethylbenzothiazoline-6-sulphonic acid))

The ABTS solution (7 mL) was mixed with potassium persulfate (2.45 mL) solution and placed for 16 h at room temperature. The solution was then diluted with ethanol to obtain an absorbance of 0.7 ± 0.02 at 734 nm. Then, 2 mL of the solution was mixed with 20 µL of the sample. After 6 min, the absorbance of the samples was measured using a spectrophotometer (Novaspec II; Pharmacia LKB, Uppsala, Sweden) at 734 nm (Chen et al., 2003). ABTS radical scavenging activity of the samples was determined using the following equation:

ABTS radical scavenging activity (%) = $[(A_b - A_s)/A_b] \times 100$

Where A_b and A_s absorbances of the blank and sample, respectively.

DPPH (2, 2′-Diphenyl-1-picrylhydrazyl)

Two milliliters of methanol solution of DPPH (24 µg/mL) was mixed with 100 µL of the sample. The mixture was placed in the dark for 60 min and then centrifuged at 4000 rpm for 5 min. Finally, the absorbance of the samples was measured using a spectrophotometer at 517 nm (Bučević-Popović et al., 2014). The percentage of free radical removal was calculated using the following formula:

DPPH radical scavenging activity (%) = $[(A_b - A_s)/A_b] \times 100$

Where A_b is the absorbance of the blank and A_s is the absorbance of each sample.

Reducing power

The reducing power of the samples was measured according to the method described by Oyaizu (1986). A total of 500 µL of the sample was mixed with 2.5 mL sodium phosphate buffer (pH 6.6) and 2.5 mL potassium ferricyanide (1%). After incubation at 50 °C for 20 min, 2.5 mL trichloroacetic acid (10%) was added to the mixture and centrifuged at 4000 rpm for 10 min. Then, 2.5 mL of the upper layer of the mixture was taken and mixed with 2.5 mL distilled water and 0.5 mL ferric chloride (0.1%). The solution was then centrifuged at 4000 rpm for 5 min. Finally, the absorbance of the samples was measured using a spectrophotometer at 700 nm. Higher absorbance indicates a higher reducing power.

Statistical analysis

Statistical analyses were conducted using SPSS version 18 (SPSS Inc., Chicago, IL, USA). One-way analysis of variance (ANOVA) and Tukey's test were applied to determine the statistical differences among treatments.

Results and Discussion

The results of the ABTS radical scavenging activity of the samples are presented in **Table 1**. In the raw treatment, a significant difference was observed between milk, honey, and honey milk ($p < 0.05$). Raw honey-milk sample exhibited the highest antioxidant activity (65.04%). In the case of thermal treatments, significant differences were found among pasteurized samples, and pasteurized honey-milk showed the strongest antioxidant activity (68.86%). The antioxidant potential of pasteurized honey-milk was higher than that of the mixture of pasteurized honey and pasteurized milk. Similar to pasteurized samples, a significant difference was observed among sterilized samples, and the results showed that the antioxidant strength of the sterilized honey milk was significantly higher than that of other sterilized samples. Regarding the effect of different treatments on samples, no significant difference was observed between the raw and pasteurized milk samples ($p > 0.05$), while the sterilized milk showed a higher antioxidative strength than the other two samples. Chen et al. (2003) performed the ABTS test and showed that the pasteurization heat does not change the anti-oxidative capacity of milk. The results indicated that the thermal treatment had no significant effect on the antioxidant activity of honey. In the case of effect of thermal treatments on honey-milk, a significant difference was observed between the raw and sterilized samples ($p < 0.05$); the sterilized honey-milk showed a higher antioxidant strength compared to the raw honey-milk. However, no significant difference was observed between the pasteurized honey-milk and raw or sterilized honey-milk samples ($p < 0.05$).

Results of the DPPH test are shown in **Table 2.** In the raw treatments, no significant differences were observed between the raw milk and raw honey-milk samples ($p < 0.05$). In this test, raw honey showed the lowest DPPH radical scavenging activity. Among the pasteurized treatments, a significant difference was observed between the pasteurized honey-milk and the mixture of pasteurized milk and pasteurized honey ($p < 0.05$), with the pasteurized honey milk showing a higher antioxidant strength than the other pasteurized treatments. Among the sterilized treatments, significant differences were observed among all samples ($p < 0.05$), and the strongest DPPH radical scavenging activity was exhibited by sterilized honey milk. No significant difference was observed between the antioxidant activities of raw and pasteurized milk samples ($p < 0.05$), whereas sterilization significantly increased the DPPH radical scavenging activity of milk. Thermal treatment had no significant effect on antioxidant activity of honey. However, thermal processing intensified DPPH radical scavenging activity of honey-milk. The antioxidant activity of sterilized honey-milk was higher than that mixture of sterilized honey and milk.

Table 1. ABTS radical scavenging activity (%) of milk, honey and honey-milk under various heat treatments

Sample	Treatment		
	Raw	**Pasteurization**	**Sterilization**
Milk	51.18 ± 1.59^{Ba}	53.21 ± 2.68^{Ba}	58.39 ± 2.82^{Bb}
Honey (5%)	7.71 ± 0.70^{Aa}	6.58 ± 0.86^{Aa}	7.45 ± 0.55^{Aa}
Honey (5%)- milk	65.04 ± 2.50^{Ca}	$68.86 \pm 2.22^{Da,b}$	71.71 ± 3.65^{Db}
Pasteurized honey (5%) + Pasteurized milk	NA	63.62 ± 1.39^{C}	NA
Sterilized honey (5%) + Sterilized milk	NA	NA	65.05 ± 1.26^{C}

A-C, different capital letters in the same column show statistical differences among samples. a-c, different small letters in the same row show statistical differences among treatments. NA, not applicable.

Table 2. DPPH radical scavenging activity (%) of milk, honey and honey-milk under various heat treatments

Sample	Treatment		
	Raw	**Pasteurization**	**Sterilization**
Milk	21.01 ± 1.79^{Ba}	17.88 ± 2.17^{Ba}	45.11 ± 2.75^{Bb}
Honey (5%)	6.46 ± 1.16^{Ab}	2.16 ± 1.14^{Aa}	$4.69 \pm 0.73^{Aa,b}$
Honey (5%)- milk	22.28 ± 1.97^{Ba}	29.00 ± 1.39^{Cb}	89.21 ± 1.58^{Dc}
Pasteurized honey (5%) + Pasteurized milk	NA	31.96 ± 0.31^{C}	NA
Sterilized honey (5%) + Sterilized milk	NA	NA	59.02 ± 2.17^{C}

A-C, different capital letters in the same column show statistical differences among samples. a-c, different small letters in the same row show statistical differences among treatments. NA, not applicable.

The results of the reducing power assay are presented in **Table 3.** Significant differences were observed among raw samples ($p < 0.05$), and the reducing power of honey-milk was higher than that of other raw samples. Similar to DPPH results, there was no significant difference between reducing powers of pasteurized honey-milk and mixture of pasteurized honey and pasteurized milk. However, reducing power of sterilized honey-milk was significantly higher that of sterilized honey and sterilized milk ($p < 0.05$). Sterilized honey milk showed the highest reducing power. Despite pasteurization, sterilization significantly increased the reducing power of both milk and honey-milk ($p < 0.05$). However, both thermal treatments increased reducing power of honey. No significant difference was observed between raw honey-milk and pasteurized honey-milk ($p < 0.05$). However, a significant difference was observed between the sterilized honey-milk sample and raw or pasteurized honey milk.

Table 3. Reducing power of milk, honey and honey-milk under various heat treatments

Sample	Treatment		
	Raw	**Pasteurization**	**Sterilization**
Milk	0.191 ± 0.021^{Ba}	0.170 ± 0.018^{Aa}	0.402 ± 0.029^{Bb}
Honey (5%)	0.064 ± 0.013^{Aa}	0.175 ± 0.020^{Ab}	0.191 ± 0.012^{Ab}
Honey (5%)- milk	0.350 ± 0.028^{Ca}	0.448 ± 0.019^{Ba}	1.078 ± 0.112^{Db}
Pasteurized honey (5%) + Pasteurized milk	NA	0.451 ± 0.040^{B}	NA
Sterilized honey (5%) + Sterilized milk	NA	NA	0.878 ± 0.040^{C}

A-C, different capital letters in the same column show statistical differences among samples. a-c, different small letters in the same row show statistical differences among treatments. NA, not applicable.

In a study, the effects of different thermal treatments on milk antioxidant activity were investigated. The findings of this study showed that short-term thermal treatments reduced the antioxidant activity of milk. This reduction in the antioxidant activity of milk was attributed to the thermal degradation of natural antioxidants in milk and the production of new oxidative compounds during the early stages of the Maillard reaction. In contrast, long-term heat treatment results in increased milk antioxidant properties due to formation of brown melanoidins (Calligaris et al., 2004; Khaledian et al., 2025; Lan et al., 2026). These findings are consistent with those of the current study. In another study, the antioxidant capacities of raw, pasteurized, and sterilized milk were investigated (Şanlidere Aloğlu, 2013). The findings of this study showed that neither pasteurization nor sterilization had a significant effect on the antioxidant capacity of milk, while after digestion, the antioxidant capacity of the three milk types increased significantly. The effect of thermal treatment on the antioxidant properties of human milk was investigated, and it was shown that long-term pasteurization (63 °C, 30 min) resulted in a significant reduction in the antioxidative capacity of milk. However, the antioxidant potentials of raw and pasteurized milk samples under short-term pasteurization (75 °C, 15 s) were similar (Silvestre et al., 2008). In the case of honey, the results indicated that heat treatment had no influence on DPPH and ABTS radical scavenging activity, which is in agreement with previous findings (Šarić et al., 2013). However, sterilized honey exhibited a higher reducing power.

In recent years, functional beverages, particularly those containing milk and fruit juice, have attracted consumer attention. In the current study, the antioxidant potential of honey milk (as a functional beverage) was investigated. The proteins and minerals in milk have high nutritional value. On the other hand, honey may have health-promoting effects on consumers due to its phenolic compounds and antioxidant properties. Therefore, honey-milk beverages can prevent the incidence of cancers, cardiovascular diseases, inflammation, Alzheimer's disease, diabetes, and other illnesses induced by oxidative stress (He et al., 2015; Rodríguez-Roque et al., 2013).

Conclusion

The current study showed that sterilized milk had the highest antioxidant capacity among the milk samples (raw, pasteurized and sterilized). In addition, sterilized honey-milk showed the highest antioxidant activity compared to raw and pasteurized honey-milk. In general, sterilization enhanced the antioxidant activity of milk and honey-milk. This can be attributed to production of antioxidant compounds during Maillard reaction at sterilization temperature. Further studies are needed to investigate the interactions between milk proteins and honey polyphenols.

Conflicts of interest

The authors declare no conflicts of interest.

Disclaimer

Author declare that she was not used any AI in article preparation.

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