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Determination of antioxidant activity and total phenolic contents in yogurt added with black cumin (*Nigella sativa* L.) honey

Özge Duygu OKUR*

Department of Food Engineering, Faculty of Engineering, Zonguldak Bulent Ecevit University, Zonguldak, Turkey

Abstract. This study aims to reveal the antioxidant activity and phenolic ingredients of yogurts added with black cumin (*Nigella sativa* L.) honey. Therefore, this study aimed to produce healthy food made with black cumin honey with increased total phenolic contents and antioxidant activity. The effect of varying degrees of black cumin honey addition (0, 2.5%, 5%, 10%, 15%) on the antioxidant activity and total phenolic contents of yogurt were examined for 28 days. The antioxidant activity was found between 14.33 and 17.41 mM TE. Total phenolic compounds were between 202.50 and 1415.00 mg GAE/kg. Some important differences were determined between yogurts with black cumin honey and the control with respect to phenolic contents during storage ($p < 0.05$). The results of study reveal that the augmentation of fortification rate increased the total phenolic characteristic and antioxidant activity of yogurt with black cumin honey. Significantly, these outcomes indicate that yogurt with black cumin honey have a high level of polyphenols and could be consumed as bioactive composition.

Keywords: black cumin honey; yogurt; antioxidant activity; total phenolic content.

1. Introduction

As a kind of fermented milk, yogurt is generally used all around the world. Because yogurt has no lactose and includes an important level of Ca^{2+}, it is considered to have a high level of dietary benefit. Additionally, items with prebiotic additives and probiotic bacteria in yogurt make yogurt have a positive bioactive impact [1-3]. Recently, yogurt has been consumed as a natural and healthy food supplement because of an increasing trend in consuming natural and healthy foods in diets [4]. The option of using flavors and smells with essences, honey and fruit concentrates might be a superior alternative than fake flavorings for use in the advancement of new dairy items. This process is generally attractive for use in utilitarian dairy items as it increments nourishing and bioactive qualities of the dairy items and creates an increased acknowledgment by consumers [5]. Because of the natural and healthy reputation of honey, there is a developing interest for its use as an additive sweetener in foods like yogurt [6, 7]. Few scientists have concentrated on the impact of mixing yogurt with honey [8-10]. As a famous ingredient in dairy products [11], honey can decrease the level of dissatisfaction with the solutions which can improve consumer acceptability of acidic items like yogurt [8]. Honey is sweet and syrupy liquid gathered by honeybees from nectar of flowers. Ranging in color from pale yellow to dim golden, the wonderful fragrance and taste of this fluid differs according to location and season. Honey gets its high dietary benefit due to its high level of sugar, modest quantities of amino acids as well as some minerals and vitamins. Honey has great therapeutic and antimicrobial abilities and is preferred in different cuisines [12]. Flavonoids and phenolic acids are phenolic components of honey which can likewise provide some tips for the natural origin of honey [13, 14]. The antioxidant characteristics of these phenolic components benefit to the advantageous properties of honey [15]. Additionally, honey has been proven to have a high level of antioxidant activity including flavonoids, phenolic acids, catalase, ascorbic acid, carotenoid derivatives, glucose oxidase, organic acids, Maillard reaction products, amino acids and proteins [16-18]. In order to reveal the antioxidant activity and phenolic components in honey, some studies have been done [19-26]. In some studies, the antioxidant capability of honey is found to be firmly associated with the concentration of total phenolics [25, 27].

This study aims to reveal the antioxidant activity and phenolic ingredients of yogurts added with black cumin (*Nigella sativa* L.) honey. Therefore, this study aimed to produce healthy food made with black cumin honey with increased total phenolic contents and antioxidant activity. The author thinks that adding black cumin honey to the yogurt may increase its utilization and economic value. Black cumin honey was selected because of its known health effects and its incredibly high economic value. With its exclusive flavor, black cumin honey is only made with the nectar picked up from cumin plants.

2. Experimental

2.1. Materials

The organic black cumin honey obtained from Ankara Destek Company, Turkey. Sterilized full fat cow's milk purchased from a local market in Zonguldak, Turkey was used for the made of yogurt. Non-fat milk powder (NFMP) of Pınar Dairy Product Company Izmir, Turkey

* Corresponding author. *E-mail address*: oduyguokur@beun.edu.tr

was used. Commercial freeze-dried starter culture (YC-180, a blend of *Streptococcus salivarius* subsp. *thermophilus, Lactobacillus delbrucckii* subsp. *bulgaricus* and *Lactobacillus delbrucckii* subsp. *lactis*) was given by Chr. Hansen Co. (A/S, Horsholm, Denmark) and used to produce yogurt. Preceding their usage, the strains were grown and kept up in reconstituted (11%, w/w) skimmed milk is exposed to pasteurization for 20 min at 85 °C and incubated at 42 ± 1 °C. After the incubation, milk that will be used to produce yogurt is immunized with this culture at a ratio of 2.0 % (1:1).

2,2-azinobis (3-ethlybenzthiazolin-6-sulfonic acid) diammonium salt (ABTS), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) and gallic acid were obtained from Acros (Morris Plains, NJ, USA). Folin–Ciocalteu reagent was purchased from Merck (Darmstadt, Germany). Potassium persulphate ($K_2S_2O_8$), sodium carbonate (Na_2CO_3) and ethanol (C_2H_5OH) were purchased from Sigma–Aldrich (St. Louis, MO). All chemicals and reagents were of analytical grade.

2.2. Yogurt production

Yogurt was produced as indicated by the technique revealed by Tamime & Robinson [11]. The milk used to produce yogurt was cow's milk with 3 % fat, 3.60 % protein, 12.1 % solids and 8.0 Soxhlet-Henkel degree (SH°). In order to expand total milk solids to 15 g for every 100 g milk before exposing it to heating 35 min until 85 ºC, non-fat milk powder was used with milk. The black cumin honey was mixed with milk at concentrations of 0, 2.5%, 5%, 10% and 15%. The mix was homogenized (Homogenizer T 65, Ika, Staufen, Germany) and pasteurized at 85 ºC for 30 min and quickly cooled to 45 ºC. DVS YC-180 was used at the rate of 2 % (w/v) as a starter into the mix. Then, milk was incubated at 42±1°C until the pH reached 4.6-4.7 for 3.5 h. Then, milk was kept at 4±1 °C for 12 h. For further analysis, all samples were kept at 4 °C for 28 days. The experiments were performed in duplicate and all analyzes were performed twice on the 1st, 7th, 14th and 28th days.

2.3. Methods

Determination of total phenolic content. Gallic acid technique developed by Folin-Ciocalteu was used as a standard for the determination of phenolic ingredients of yogurts [28]. The absorbance at 760 nm was read in a spectrophotometer (Shimadzu Scientific Instruments, Inc., Tokyo, Japan). An equation taken from the standard gallic acid curve determined the concentration of total phenolic mixes as gallic acid equivalent (GAE). The results were presented as mg GAE (gallic acid equivalent)/kg.

Determination of total antioxidant activity

2,2'-azinobis (3-ethlybenzthiazoline)-6-sulfonic acid (ABTS-TEAC) assay. The reaction of 7 mM ABTS stock solution and 2.45 mM $K_2S_2O_8$ generated 2,2'-azinobis (3-ethlybenzthiazolin-6-sulfonic acid) diammonium salt (ABTS+) radical cation which was diluted to 0.7 (±0.02) absorbance at 734 nm stabilized at 30 °C with PBS, pH: 7.4. Additionally, ABTS+ inhibition against Trolox was measured by spectrophotometer. The absorbance at 734 nm was read in a spectrophotometer (Shimadzu Scientific Instruments, Inc., Tokyo, Japan). Trolox standard curve was used in order to determine the TEAC values of samples. Then, TEAC values of samples were treated as Trolox equivalents (in μmol ml^{-1} of sample) [29].

Statistical analysis. All experiments and analyses were replicated two times. Data were statistically analyzed repeated measure ANOVA using SPSS for Windows 16.0. Differences between data were analyzed by Tukey analysis [30].

3. Results and Discussion

3.1. Evaluation of total antioxidant activity and phenolic contents

Because dietary polyphenols have noteworthy characteristics like antioxidants or antimutagens, they have attracted attention and recognized as possible nutraceuticals [31]. Total antioxidant activity and phenolic contents of samples are given in Figures 1 and 2. Yogurt with 15% black cumin honey (E) had the highest phenolic content and antioxidant values for all samples on the 1^{sh} day of storage.

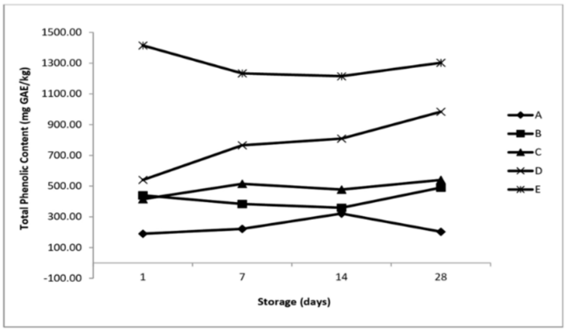

Figure 1. Total phenolic content of the experimental yogurts during storage at 4 °C for 28 days.
A: 0% (Plain control sample); B: Yogurt with 2.5% black cumin honey; C: Yogurt with 5% black cumin honey; D: Yogurt with 10% black cumin hone; E: Yogurt with 15% black cumin honey.

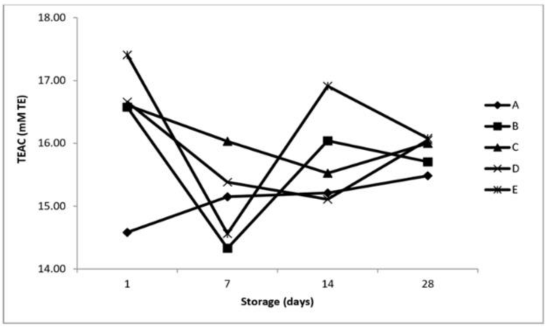

Figure 2. The antioxidant activity of the experimental yogurts during storage at 4 °C for 28 days.
A: 0% (Plain control sample); B: Yogurt with 2.5% black cumin honey; C: Yogurt with 5% black cumin honey; D: Yogurt with 10% black cumin honey; E: Yogurt with 15% black cumin honey.

Some important differences were determined between yogurts with black cumin honey and the control with respect to total phenolic contents during storage ($p < 0.05$). Comparable outcomes were found for yogurt with pine honey [32], for yogurt prepared with heterofloral honey combinations [33]. The total phenolic content of control sample (202.50 mg GAE/kg) is presumably because of the existence of phenols in milk that caused by cattle feed [34]. However, potential intrusive substances such as aromatic amines could react with Folin-Ciocalteu reagent quantitatively [35].

The combination and the characteristic of polyphenolic substances in honey vary depending on the plant origin of honey. The darker colored honey is the phenolic compounds booster than that of light-colored honey [36]. The chemical structure of honey like organic acids, enzymes, amino acids, phenolics and Maillard reaction products determine the antioxidant characteristics of honey [25]. Additionally, the antioxidant and aroma characteristics of honey depend on the flavonoids which are low molecular weight phenolic compounds. In other words, chemical structure of honey such as flavonoids, carotenoids, organic acids and source of origin of honey determines the antioxidant activities [14].

The formation of protein-polyphenol complexes is profoundly affected by the nature of the protein (*i.e*. the number of proline residues), the structure of the polyphenols (*i.e.* the number of aromatic rings), the temperature of the system and the presence of other components (*e.g*. sugar, polysaccharides) that can affect the interaction [37, 38]. These interactions may have a different effect on the *in vivo* solubility of both phenolic compounds and proteins [39]. The potential reduction in the solubility of complexes formed between protein and polyphenol does not necessarily change the bioavailability of such polyphenols [38, 40]. Based on this information, the black cumin (*Nigella sativa* L.) honey may influence water-protein interactions and may result in lower or higher total phenolic content and total antioxidant activity in the milk-honey gel system. During storage, the tendency to decrease and increase in antioxidant activity is possibly because bacterial metabolic activity caused a breakdown of macromolecules that could react with the TEAC reagent [41].

The results of study reveal that the augmentation of fortification rate increased the total phenolic characteristic and antioxidant activity of yogurt with black cumin honey. Significantly, these outcomes indicate that yogurt with black cumin honey have a high level of polyphenols and could be consumed as bioactive composition. At the same time, according to the sensory analysis results made in the first part of this study, yogurt added with black cumin honey was highly appreciated by panelists. Also, the hedonic evaluation results were as follows: the favorite samples on days 1, 7 and 14 were the samples with 10% and 15% *Nigella sativa*-honey content [42].

4. Conclusions

The study describes TEAC and Folin-Ciocalteu method for the antioxidant activity and total phenolic content analysis in yogurts with black cumin honey. Total phenolic content and antioxidant activity of yogurt significantly increased with the amount of black cumin honey added. Consumers seem to gain incredible advantages from the products in question such as high level of protection against pathologies related with free radicals. The yogurt containing black cumin honey might become a popular dairy product for consumers who prefer a unique taste. In conclusion, the results suggest that the black cumin honey can be used for manufacturing functionally fortified yogurt.

Conflict of interest

The author declares no conflict of interest.

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