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Investigation of *E. coli* and propolis interaction in milk: effects on physicochemical properties

Sabire Yerlikaya [1], Yeliz Durgun [2]

[1] Karamanoğlu Mehmetbey University, Engineering Faculty, Food Engineering Department, Karaman, Türkiye
[2] Tokat Gaziosmanpaşa University, Department of Biomedical Equipment Technology Program, Turhal, Tokat, Türkiye

Abstract

Milk is an important food due to its nutritional value and wide consumer base. The microbiological and chemical quality of milk is of critical importance for consumer health. In this study, the effects of *Escherichia coli* ATCC 25922 inoculation and the natural antibacterial agent propolis on the physicochemical properties of milk were investigated. For this purpose, *E. coli* ATCC 25922 pathogen strain was inoculated into pasteurized and UHT milk at a concentration of 4 log cfu/mL and ethanolic propolis extract was added at 1%, 2% and 3% concentrations. Some physicochemical properties (color (*L**, *a**, *b**), pH and brix values) of milk were investigated depending on time (0-30 minutes) at 25ºC.
It was determined that the *L** value of 1% EEP addition (86,95) to pasteurized milk was higher than 2% (81,07) and 3% EEP (82,66) addition. The addition of 1% EEP increased the pH value in both milk samples, the addition of 2% EEP did not change the pH value, and the addition of 3% EEP decreased the pH value. This study suggested the potential of propolis to be used as a natural preservative by increasing the physicochemical stability of milk. It is thought that it can contribute to the development of milk processing and storage techniques.

Keywords: pasteurized, UHT, color, pH, brix

1. Introduction

Milk is an important food due to its nutritional value and wide consumer base. The microbiological and chemical quality of milk is of critical importance for consumer health. In order for milk to be consumed safely by consumers, disease-causing microorganisms must be completely removed from milk, and other microorganisms that will negatively affect its shelf life must be largely eliminated [1]. Consumer, demands for quality food products, have increased in recent years. Therefore, interest in new non-thermal technologies is constantly increasing. In the food industry, traditional processing non-thermal technologies have begun to be used, which are generally carried out at lower temperatures or without heat treatment, thus reducing the negative effect of heat treatment on food quality [2-4].

E. coli generally holds an important place among foodborne pathogens [5]. *Escherichia coli* (*E. coli*), gram negative, facultative anaerobic, mostly a motile, sporeless, rod-shaped bacterium [6-7].

Propolis is a natural resin and is known for its antibacterial properties [8]. Propolis is collected by bees from buds, leaves and similar parts of trees such as pine, oak, poplar, chestnut and some herbaceous plants. It is a sticky, resin-like odor and color ranging from dark yellow to brown. Bees use it for many purposes in the hive by mixing with wax [9]. Propolis usually contains a wide variety of bioactive components, such as polyphenols (flavonoid aglycones, phenolic acid and esters, phenolic aldehydes, alcohols and ketones), terpenoids, steroids, amino acids and inorganic compounds. Phenolic compounds in the structure of propolis increase the capacity of cells to neutralize oxidative stress [10].

* Corresponding author: sabirebattal@kmu.edu.tr

In this study, the metabolic effects of *E. coli* on milk and the potential of propolis to reduce these effects were investigated.

2. Materials and Method

2.1. Materials

Propolis was collected from Yenipazar, Bilecik, Türkiye in September 2023 and brought to the laboratory of Karamanoğlu Mehmetbey University Food Engineering Department under aseptic conditions. *E. coli* ATCC25922 strain was obtained from Selçuk University, Türkiye.

2.2. Methods

2.2.1. Production ethanolic propolis extract

For 1% ethanol propolis extract (EEP), 1 g of propolis in 99 mL 80% ethanol; for 2% EEP, 2 g propolis in 98 mL 80% ethanol; for 3% EEP, 3 g of propolis was dissolved in 97 mL of 80% ethanol. Dissolution was carried out in a closed container, protected from light, for one week. The solution was shaken twice a day and at the end of the period, it was filtered through Whatman No 1 filter paper and placed in sterile bottles. It was stored at +4°C until used for analysis [11].

2.2.2. Pathogen activation and inoculation into milk samples

E. coli ATCC 25922 strain was used in this study. Bacterial strains obtained from stock cultures (−18°C) were activated for 24 h at 37°C in Nutrient Broth (Merck, Darmstadt, Germany). *E. coli* was adjusted to a concentration of 4 log cfu/mL. *E. coli* pathogen was inoculated into 10 mL of pasteurized milk at a concentration of 4 log cfu/mL. The samples were incubated at 25°C for 30 minutes. At the end of the period, ethanolic propolis extract was added to the samples as an inhibitory substance at concentrations of 1%, 2% and 3%, respectively. The samples were incubated again at 25°C for 30 minutes. At the end of the period, each sample was subjected to color (*L**, *a**, *b**), pH and brix analyses. Measurements were made at the 0^{th} and 30^{th} minutes of the treatments. The same analyzes were repeated for UHT milk.

2.2.3. Statistical analysis

The results were analyzed statistically using SPSS 22 (IBM Corp., Armonk, New York, USA) program. Sample means were compared using One Way ANOVA and were evaluated with Tukey test from Post Hoc Test.

3. Results and Discussion

3.1. Color analysis

Figure 1, Figure 2 and Figure 3 demonstrated all extracts significantly affected *L**, *a**, *b** value of milks. Samples are as follows; P: Pasteurized milk; P-70: Pasteurized milk + 70% ethanol; U: UHT milk; U-70: UHT milk+70% ethanol; P-E-4: Pasteurized milk + 4 log cfu/mL *E. coli*; P-E-4-1p: Pasteurized milk+ 4 log cfu/mL *E. coli*+ 1% EEP; P-E-4-2p: Pasteurized milk+ 4 log cfu/mL *E. coli*+ 2% EEP; P-E-4-3p: Pasteurized milk+ 4 log cfu/mL *E. coli*+ 3% EEP; U-E-4: UHT milk+ 4 log cfu/mL *E. coli*; U-E-4-1p: UHT milk+ 4 log cfu/mL *E. coli*+ 1% EEP; U-E-4-2p: UHT milk+ 4 log cfu/mL *E. coli*+ 2% EEP; U-E-4-3p: UHT milk+ 4 log cfu/mL *E. coli*+ 3% EEP.

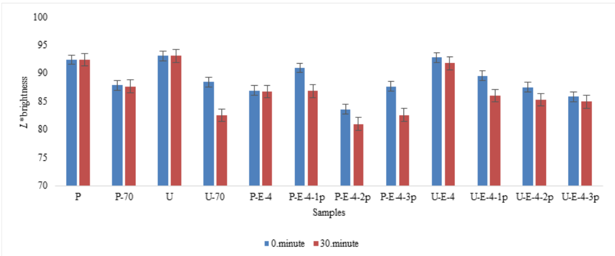

Figure 1. Effect of EEP on *L** value of milks sample

The addition of ethanol caused a decrease in the brightness values of the samples. It is seen that this decrease is higher in UHT milk at the 30^{th} minute of incubation (93,18). With the addition of pathogens, the decrease was higher in the 30^{th} minute of incubation of pasteurized milk (86,82). It was determined that the *L** value of 1% EEP addition (86,95) to pasteurized milk was higher than 2% (81,07) and 3% EEP (82,66) addition. However, the addition of three different EEP decreased the brightness value at the 30th minute of incubation. If the EEP added to UHT milk are compared, the *L** value with the addition of 1% EEP (89,66) was found to be higher than 2% (87,59) and 3% EEP (85,91) at 0 minute of incubation. Since propolis extracts are brown-red-yellowish in color, this decrease in *L** value is expected.

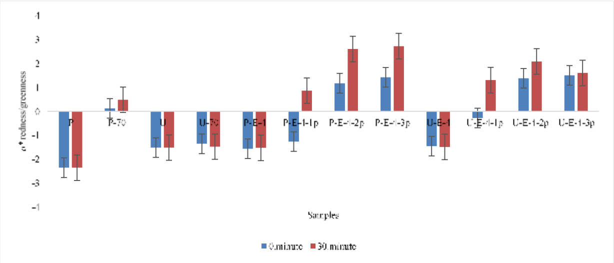

Figure 2. Effect of EEP on *a** value of milks sample

Before the analysis, it was determined that the *a** value of pasteurized milk (-2,36) was lower than UHT milk (-1,52). Ethanol was more effective on the *a** value of pasteurized milk than UHT milk in both incubation minutes. The addition of ethanol to pasteurized milk and the addition of EEP to all samples caused an increase in the *a** value of the samples. The highest increase was obtained at the 30th minute of incubation with the addition of 3% EEP to pasteurized milk (2,72). While negative results were obtained at the 0th minute of incubation in pasteurized (-1,27) and UHT (-0,28) milk with the addition of 1% EEP; positive *a** values determined at the 30th minute. Since propolis extracts are brown-red-yellowish in color, this increase in *a** value is an expected situation.

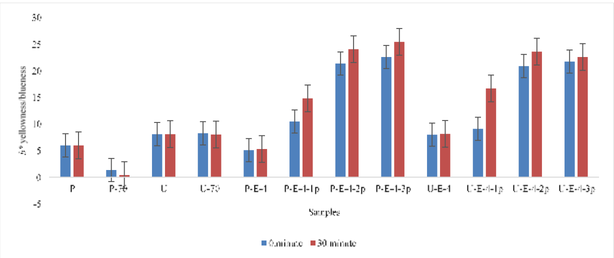

Figure 3. Effect of EEP on *b** value of milks sample

Before the analysis, it was determined that the *b** value of pasteurized milk (5,99) was lower than UHT milk (8,1). The difference in the percentages of nutritional components can be shown as the reason for this situation. The highest *b** value was obtained at the 30th minute of incubation by adding 3% EEP to pasteurized milk (25,43). The lowest yellowness value was determined at the 30th minute of incubation by adding ethanol to pasteurized milk (0,42). The *b** values of the samples to which EEP was added were found to be higher than the other samples. At the 30th minute of incubation, adding 2% EPP to pasteurized milk and 2% EEP to UHT milk (23,58) was more effective in increasing the yellowness value. Since propolis extracts are brown-red-yellowish in color, this increase in *b** value is expected.

3.2. pH and Brix values

Figure 4 and Figure 5 demonstrated all extracts significantly effected pH and brix values of milks.

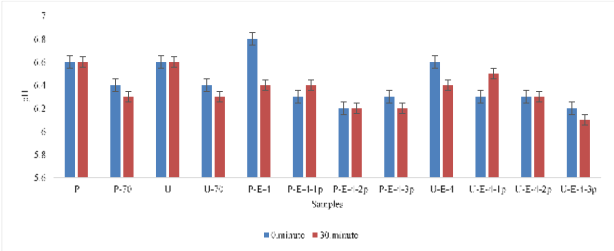

Figure 4. Effect of EEP on pH value of milks sample

When the Figure 4 is looked into, it will be seen that there is fluctuation in the results in both incubation minutes. The highest pH was detected when pathogens were added to pasteurized milk (6,8) in 0.minute of incubation. The lowest pH value was found in samples; P-E-4-2p and U-E-4-3p in 0.minute; P-E-4-2p and P-E-4-3p in 30.minutes of incubation (6,2). The addition of 1% EEP increased the pH value in both milk samples, the addition of 2% EEP did not change the pH value, and the addition of 3% EEP decreased the pH value. These results indicate that propolis may have an effect on pH.

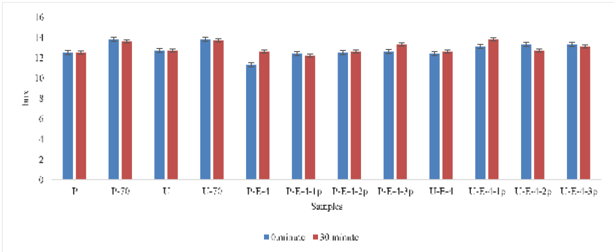

Figure 5. Effect of EEP on brix value of milks sample

The addition of ethanol caused a slight decrease in the brix value in both milk samples. The highest brix value was at the 30th minute of incubation in UHT milk samples with 1% EPP addition (13,8); The lowest value was detected at the 0th minute of incubation with the addition of *E. coli* to pasteurized milk (11,3). While the addition of 1% EEP to pasteurized milk decreased the brix value, the addition of 2% and 3% EEP increased it. The opposite results were observed in UHT milk samples. While 1% EEP addition increased the brix value, 2% and 3% EEP addition decreased it.

It is known that *E. coli* and propolis inoculation has an effect on milk. The effect of *E. coli* ATCC 25922 on the physicochemistry of milk can be characterized by metabolic and physicochemical changes. Chen et al. [5] examined the metabolic profiles of *E. coli* strains and stated that this profile may have an impact on the quality of foodstuffs such as milk. Segueni et al. [8] clearly showed the antibacterial effects of propolis on *E. coli* ATCC 25922. Elbaz et al. [2] investigated the effects of *E. coli* ATCC 25922 in milks. The results obtained concluded that there is a potential effect of *E. coli* on the quality of milk according to the changes in the hematological, immunological and oxidative stress parameters of milk.

4. Conclusion

The results revealed the effects of *E. coli* ATCC 25922 on the physicochemical properties of milks and the potential of propolis in modulating these effects. Propolis can be used as a natural preservative by increasing the microbial stability of milk. These findings provide important information for improving milk processing and storage techniques and suggest natural solutions to improve the microbial and physicochemical quality of dairy products. This research makes significant contributions to the field of food safety and public health and provides a scientific basis for the development of strategies to improve the safety and quality of dairy products. According to the results obtained, *E. coli* inoculation into milk samples affected the physicochemical properties of milk. Ethanolic propolis extracts can be used to reduce these effects. Since propolis is a natural antimicrobial substance, it also has effects on improving the microbiological quality of the food to which it is added. For this purpose, different propolis extracts can be prepared by dissolving in different solvent and experiments can be made with different concentrations to contribute to the literature in this field.

Compliance with Ethics Requirements. Authors declare that they respect the journal's ethics requirements. Authors declare that they have no conflict of interest and all procedures involving human / or animal subjects (if exist) respect the specific regulation and standards.

Disclosure statement. No potential conflict of interest was reported by the authors.

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