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Determining Antibacterial Effect of Yellow Onion (*Allium Cepa*) Peel Extract on Some Pathogen Inoculated in Raw, UHT and Pasteurized Milks

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Abstract

In this research, yellow onion peel extract (YOPE) was tested against some pathogens inoculated into pasteurized, UHT and raw milks. Milk samples were added with YOPE at 10% (v/v) and were left at 4 ºC for 5 days. The pH and colour of milk samples were determined at 1, 3 and 5th day of storage. Moreover, the milk samples were inoculated by 0.0, 5.0 and 7.0 log cfu/mL of *Escherichia coli* ATCC 25922 and by 0.0, 5.0 and 7.0 log cfu/mL of *S. aureus* ATCC 25923. All the above samples were then added with YOPE at a rate of 0.0 or 5.0% (v/v). YOPE had the greatest inhibitory effect on *E. coli* inoculated pasteurized milk at a concentration of 7.00±0.24 log cfu/mL, whereas the lowest inhibitory effect was shown on the raw milk inoculated with 7.00±0.87 log cfu/mL *S. aureus* and 5.00±0.21 log cfu/mL *E. coli*.

Key Words: Antibacterial activity, Milk, *E. coli, S. aureus*

Sarı Soğan (*Allium Cepa*) Kabuğu Ekstraktının Çiğ, UHT ve Pastörize Sütlere İnoküle Edilen Bazı Patojenler Üzerindeki Antimikrobiyal Etkisinin Belirlenmesi

Öz

Bu araştırmada sarı soğan kabuğu ekstraktının (YOPE), pastörize, UHT ve çiğ sütlere inoküle edilen bazı patojenler üzerindeki inhibitör etkisi incelenmiştir. Süt örneklerine %10 (v/v) YOPE ile ilave edilerek

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4 ºC'de 5 gün inkübasyona bırakılmıştır. Süt örneklerinin pH ve rengi depolamanın 1, 3 ve 5. günlerinde belirlenmiştir. Ayrıca, süt numunelerine ayrı ayrı 0,0, 5,0 ve 7,0 log kob/mL konsantrasyonlarında *E. coli* ATCC 25922 ve *S. aureus* ATCC 25923 inoküle edilmiştir. Bahsedilen numunelerin tümüne ayrı ayrı %0,0 ve 5,0 (v/v) konsantrasyonunda YOPE ilave edilmiştir. YOPE en yüksek inhibitör etkiyi 7,00±0,24 log kob/mL konsantrasyonunda *E. coli* ile kontamine edilmiş pastörize sütte gösterirken; en düşük inhibitör etkileri 7,00±0,87 log kob/mL konsantrasyonunda *S. aureus* ile 5,00±0,21 log kob/mL konsantrasyonunda *E.coli* ile kontamine edilmiş çiğ sütte göstermiştir.

Anahtar Kelimeler: Antimikrobial aktivite, Süt, *E. coli, S. aureus*

1. INTRODUCTION

Milk and dairy products are among the most important dietary foodstuffs, providing appreciable amount of protein and peptide structures such as immunoglobulins, enzymes, antibacterial agents, fatty acids, vitamins and minerals [1,2]. The milk has nutritional composition of 11.9-12.7% dry matter, 8.60-9.60% dry matter (ether-extract free); 3.10-3.30% fat; 4.50-5.10% lactose; 3.20-3.40% protein and 0.70% ash [3,4]. The milk is well characterised by its own color, taste and odor [5,6]. The milk is daily subjected to a routine safety monitoring to ensure the legal limits for the levels of bacteria, pathogens and other undesirable substances. However, it is nutritionally an ideal medium for growing microorganisms [7]. The pathogens found in raw milk were primarily *C. jejuni, Salmonella* spp., *Staphylococcus aureus*, *L. monocytogenes*, pathogenic *E. coli* and *Yersinia enterocolitica* [8].

E. coli and *S. aureus* are among the most well-known and studied foodborne pathogens [9]. The detection of these pathogens in food products indicates insufficient heat treatment during production, poor hygiene conditions or contamination after heat treatment [10].

Onion contains 1.4% protein, 0.2% fat, 88.1% water, 8.9% carbohydrate, 0.8 g cellulose and 46 calori [11]. Onion is one of the most grown vegetables in Turkey. The variety of onions with yellow / white skin is juicy and sweet. Yellow onion is mostly used for salads and some types of food [12]. It is one of the vegetables that is a source of vitamin C. Its chemical structure, the amount of phenolic substances and antioxidant activity is high [13]. Water extracts of onion peel has been previously studied as antioxidative and antibacterial agents in many studies [14-18]. However, to our best knowledge there is a lack of research testing the antibacterial effect of water extract of onion peel on the pathogen inoculated milk.

In this research, we aimed to test the effect of enrichment of pasteurized, UHT and raw milks milk on some physical and chemical characteristics (pH and colour) of the milk, and on the inhibition of some pathogens, namely *E. coli* ATCC 25922 and *S. aureus* ATCC 25923, inoculated into the milk.

2. MATERIALS AND METHODS

2.1. Materials

Raw, pasteurized, UHT milks and yellow onion peel were obtained from the local markets in Karaman, Turkey. *E. coli* ATCC 25922 and *S. aureus* ATCC 25923 were kindly provided under aseptic conditions by Selcuk University, Food Engineering Department. UHT milk was used to make a homogeneous comparison with raw and pasteurized milks.

2.2. Methods

2.2.1. Preperation of Yelow Onion Peel Extract (YOPE) and Milks for Analysis

Dry peels separated from yellow onions were cleaned up by washing with tap water. A 5 g of peel was boiled in 100 mL water (5%, v/v) for 10 minutes. The mixture was cooled down to a 25 ºC of temperature and then subjected to a filtration through a Whatman no: 1 paper. A 10

mL of raw, pasteurized and UHT milks was added with 0.0 and 1 mL of YOPE (1 mL). Milk samples were stored at 4 ºC for 5 days. The pH analyzes and color analyzes were performed on 1, 3 and 5th days of storage.

2.2.2. Inoculation of Milks with *E. coli* and *S. aureus*

E. coli and *S. aureus* were inoculated at the level of 0.0, 5.0 and 7.0 log cfu/mL into raw, pasteurized, UHT milks. Milks inoculated pathogens were kept at 25 ºC for 15 minutes. At the end of this period, the milks were added with 5% YOPE (v/v). Milks were again kept at 25 ºC for 15 minutes. Eosin Methylene-blue Lactose Sucrose Agar was used for the enumeration of *E. coli* and Baird Parker Agar used for the enumeration of *S. aureus*. All the milk samples prior to pathogen inoculation were enumerated for total mesophilic aerobic bacteria using a Plate Count Agar. The petri dishes were left to incubate at 35-37 ºC for 24-48 hours. Enumeration was done by counting colonies developed at the end of incubation period, and the results was expressed as log cfu/ml.

2.2.3. Colour and pH Measurements

The exterior surface colour of all samples was measured using a chromameter (Hunterlab Colorimeter Colorflex) according to CIELab system. CIE L^*, a^*, and b^* were determined by the method described by Hunt et al. [19]. The pH values were determined with a pH meter (pH 3110/SET WTW, Germany) [20].

2.2.4. Statistical Analysis

Each treatment was tested in duplicate samples with two replications. All the analyses were performed in two repetitions and two parallels, providing a total of four independent measurements. The results were expressed as means ± standard deviation. A two-way analysis of variance was used to test the effects of treatments at a 5% significance level by using SPSS 22 statistical package for Windows (IBM Corp., Armonk, New York, USA).

3. RESULTS AND DISCUSSION

3.1. Hunter Colour Determination and pH

Table 1 and Table 2 indicates the effects of milk types and storage time on L^*, a^*, b^* and pH values were statistically significant *($p<0.05$)*. The brightest sample (L^*) was obtained from the UHT milk (89.77±0.1) and lowest bright sample was the raw milk with YOPE (70.03±0.78). YOPE has a brightness value of 7.21±0.36. The addition of YOPE resulted in a decrease of L^* value in all milk species. The decrease from 88.90±0.21 to 74.29±0.32 was found to be more profound in pasteurized milk (Table 1).

It is determined that raw milk with YOPE has the highest a^*value (16.10±0.12), as an indication of intense red colour occurrence with addition of YOPE. Sample with at lowest a^* value was obtained from the raw milk (-2.60±0.14) since YOPE has a color of orange. It is inevitable that the milks enriched with YOPE would have a high a^* value. Thus, the addition of YOPE resulted in an increase in the a^* value in all milk samples. The increase from -2.60±0.14 to 16.10±0.12 was found to be more profound in raw milk (Table 1).

Pasteruized milk with YOPE has the highest b^* value as 25.97±0.23 as indication of intensed yellow colour. Sample with at lowest b^* value was the raw milk (4.01±0.58). YOPE per se has a value of 9.79±0.45. The addition of YOPE resulted in an increase in b^* value in all milk samples. The increase from 4.02±0.19 to 25.97±0.23 was more profound in pasteruized milk (Table 1).

UHT milk has the highest pH value of 6.66±0.02. Sample with at lowest pH of 6.49±0.02 was pasteruized milk with YOPE. The pH value of YOPE was determined to be 4.16±0.03. The addition of YOPE resulted in a decrease in the pH value in all milks. The decrease from 6.66±0.02 to 6.55±0.03 was fmore profound in UHT milk (Table 1).

Table 1. Anova comparison test results of the effect on color parameters and pH

Factor	*L**	*a**	*b**	*pH*
P	88.90±0.21^{b}	-2.36±0.12^{ef}	4.02±0.19^{e}	6.55±0.03^{b}
P-O	74.29±0.32^{e}	14.64±0.36^{b}	25.97±0.23^{a}	6.49±0.02^{c}
UHT	89.77±0.41^{a}	-2.15±0.41^{e}	5.04±0.29^{d}	6.66±0.02^{a}
UHT-O	76.41±0.54^{d}	13.58±0.25^{c}	24.63±0.89^{b}	6.55±0.03^{b}
R	84.52±0.58^{c}	-2.60±0.14^{f}	4.01±0.58^{e}	6.63±0.04^{a}
R-O	70.03±0.78^{f}	16.10±0.12^{a}	25.54±0.62^{a}	6.58±0.02^{b}
O	7.21±0.36^{g}	12.57±0.14^{d}	9.79±0.45^{c}	4.16±0.03^{d}

(Comparison of the means of 4 replicates with the Tukey's test shows that there is a significant difference between the a-f means on the averages ($p<0.05$). P: pastorized milk; P-O: pastorized milk with YOPE; UHT: UHT milk; UHT-O: UHT milk withYOPE; R: raw milk; R-O: raw milk with YOPE)

There is waving in *L** and *b** values in all storage days. It was observed that the highest *L** value (70.49±0.06) was reached on 3rd day; the highest values of *a** (7.59±0.06) and *b** (14.79±0.15) was reached on the 5th day; the highest pH value (6.43±0.01) was reached on the 1st day during the storage (Table 2).

Table 2. Anova comparison test results of the effect of storage days on color parameters and pH

Storage (day)	*L**	*a**	*b**	pH
1	69.93±0.03^{b}	6.71±0.05^{c}	13.98±0.03^{b}	6.43±0.01^{a}
3	70.49±0.06^{a}	7.04±0.02^{b}	13.65±0.07^{b}	6.24±0.02^{b}
5	70.07±0.09^{b}	7.59±0.06^{a}	14.79±0.15^{a}	6.02±0.02^{c}

(Comparison of the means of 4 replicates with the Tukey's test shows that there is a significant difference between the a-c means on the averages ($p<0.05$)).

Figure 1 and Figure 2 indicated the effect of interactions of milk species (with/without YOPE) and storage time on *L**, *a**, *b** and pH values, respectively. As can be seen in Figure 1; the highest *L** and *a** values were detected on the 5th day of storage in UHT (90.28±0.11) and raw milk with YOPE (17.57±0.21), respectively. The highest *b** and pH values were detected in raw milk with YOPE (26.92±0.08) and UHT milk (6.86±0.02) on the 5th and 1st day of storage, respectively (Figure 1 and Figure 2). The addition of YOPE reduced the *L** value of all milk samples; increased *a** and *b** values in all storage days.

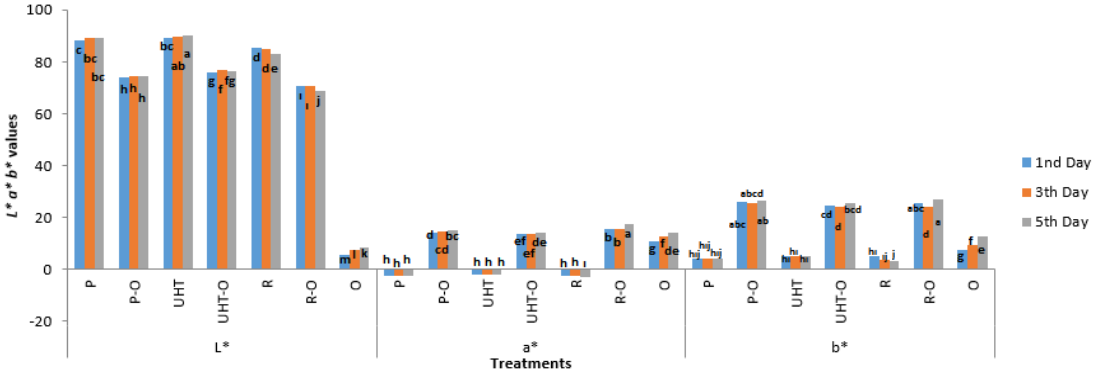

Figure 1. *L**, *a**, *b** values depent on storage time

(P: Pastorized milk; P-O: Pasteurized milk with YOPE; UHT: UHT milk; UHT-O: UHT milk with YOPE; R: raw milk; R-O: raw milk with YOPE; O: YOPE).

Lakade et al., [21] found 77±7.5 as *L**,-5.27±1.8 as *a**, 3.47±3.5 as *b** for control milk; 65±7 as *L**, 37±0.85 as *a**, 5.32±1.7 as *b** for orange milk. Codina-Torrella et al. [22] reported 80.76±0.45 as *L**,-0.48±0.04 as *a**, 14.92±0.17 as *b** for raw milk; 81.85±0.27 as *L**, -0.18±0.018 as *a**, 13.80±0.14 as *b** for homogenized pasteurized tiger nuts' milk product.

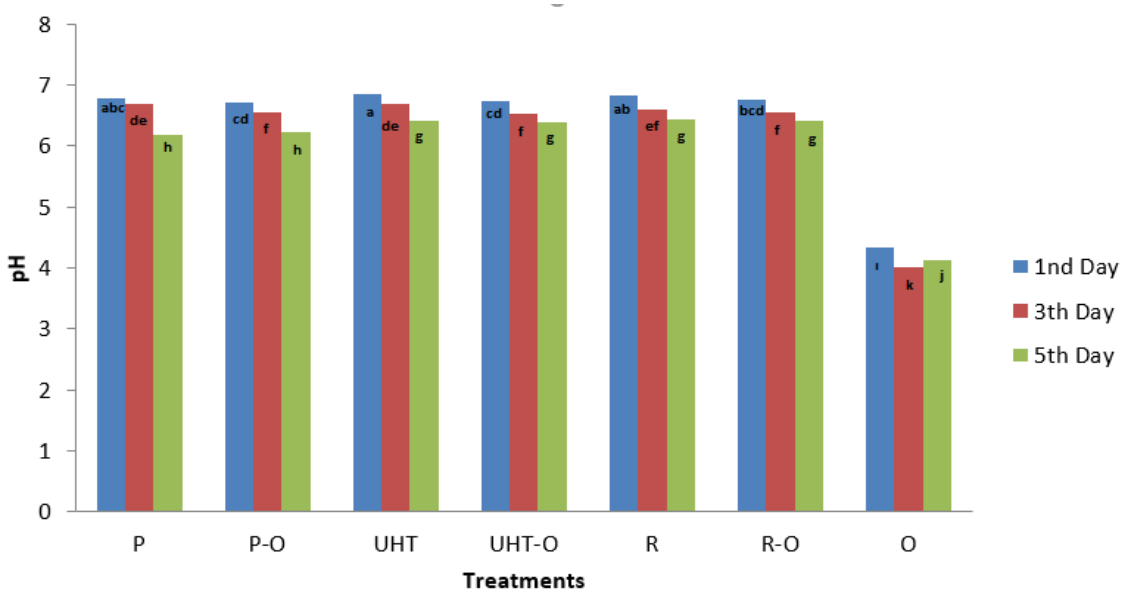

Figure 2. *pH* values depent on storage time
(P: Pastorized milk; P-O: Pasteurized milk with YOPE; UHT: UHT milk; UHT-O: UHT milk with YOPE; R: raw milk; R-O: raw milk with YOPE; O: YOPE).

It was determined that the pH value decreased in all samples during the storage period. This reduction could occur as a result of the activity of microorganisms fermenting milk sugar. A fluctuation in the pH value was observed in only YOPE. But the increase on the 5th day could not reach the ph value on the 1st day. The addition of YOPE reduced the pH value of all milk samples during the all storage days (Figure 2).

Metin and Öztürk [23] comparised pH values and the quality characteristics of the milk in their study. They indicated milk at 6.8-6.6 pH values was accepted as fresh and stated the begining of acidification in milk started at pH 6.3. According to our study, the pH value of pasteurized milk samples fell below 6.3 on the 5 day of storage and samples started to go rancid on the 5 day of storage. Akarca et al., [24] determinated pH value of pasteurized milk start to fell below 6,3 on 7 day of storage. Samples started to go rancid on the 7 day of storage. In the analysis performed with 49 raw milk samples sold in Erzurum, the pH was found as (5.93 ± 0.11) [25]. The pH values of the analyzed milk in two different studies were determined as 5.80-6.05 and 6.41-6.63 [25-26]. Tuncer [27] stated milks pH as 6.45±0.009. Ateş [28] determined the pH value of milk obtained from 24 provinces as 6.66-6.69.

3.2. Microbiological Analysis

The mean values of YOPE on pathogens are shown in Figure 3 (*E. coli*) and Figure 4 (*S. aureus*). As can be seen, YOPE had significant effect (p<0.05) on inhibition of *E. coli* and *S. aureus* (log cfu/ml).

Pasteurized milk samples were inoculated with *E. coli* as 5.00±0.28 log cfu/mL and 7.00±0.24 log

cfu/mL; the results determined as 3.06±0.19 log cfu/mL and 4.69±0.19 log cfu/ml, respectively, with addition of YOPE.

UHT milk samples were inoculated with *E. coli* as 5.00±0.39 log cfu/mL and 7.00±0.35 log cfu/mL; the results determined as 3.30±0.50 log cfu/mL and 4.69±0.19 log cfu/ml, respectively, with addition of YOPE.

Raw milk samples were inoculated with *E. coli* as 5.00±0.21 log cfu/mL and 7.00±0.14 log cfu/mL; the results determined as 4.29±0.06 log cfu/mL and 4.42±0.19 log cfu/mL, respectively, with addition of YOPE.

Before adding pathogens to all milk samples, the total number of mesophilic aerobic microorganisms (TMAB) of milks were examined. As a result, while no microorganisms were found in pasteurized and UHT milk, microorganisms count was made in raw milk at a concentration of 4.33±0.19 log cfu/mL and 7.1±1.5 log cfu/mL. The addition of YOPE reduced the number of microorganisms detected in raw milk to 3,92±0,08 log cfu/mL and 6.34±0.57 log cfu/mL respectively (Figure 3 and Figure 4).

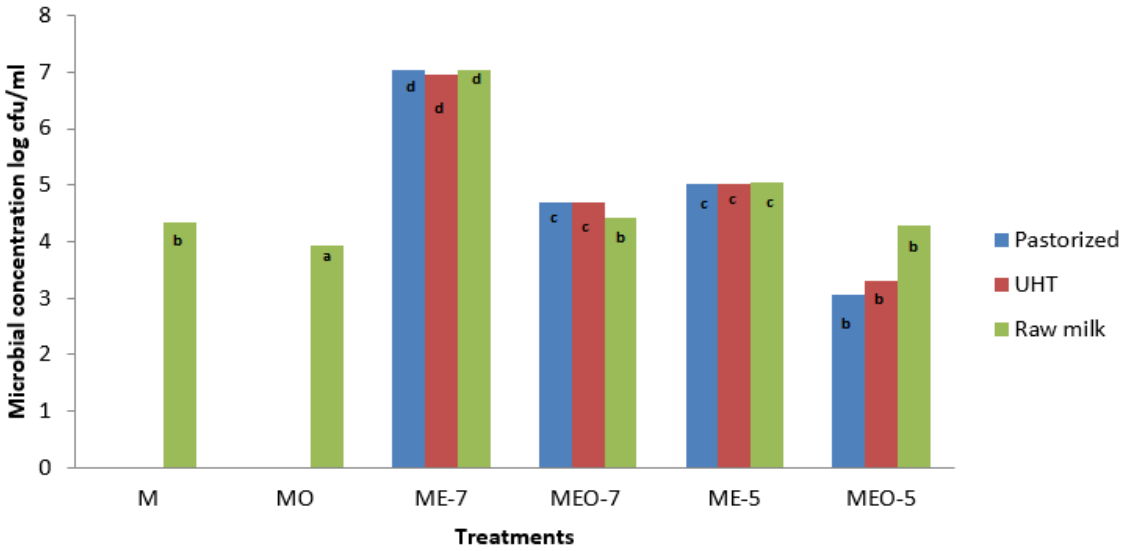

Figure 3. Inhibitory effect on *E. coli* ATCC 25922
(M: milk; MO: milk + YOPE; ME-7: milk+ 7 cfu/mL *E. coli*; MEO-7: milk+ 7 cfu/mL *E. coli* + YOPE; ME-5: milk+ 5 cfu/mL *E. coli*; MEO-5: milk+ 5 cfu/mL *E. coli* + YOPE).

The Communiqué of Turkish Food Codex Raw and Heat Treated Drinking Milk stated that milks with TMAB count above 5 log cfu/mL are not suitable for human consumption [29]. Accordingly, it was determined that one of the raw milk used in our study was not suitable according to food safety criteria. Yapık [30] reported that that only 5 of the 24 milk samples met the values of the Codex. Diler and Baran [25] determined the TMAB count as 5.29 log cfu/mL in the analysis performed with 49 raw milk samples. Çalışkan [31] reported The TMAB count as 5.74-6.29 log cfu/mL in 40 milk samples. Akarca et al. [24] found the TMAB count as 3.86 log cfu/mL in raw milk and 2.98 log cfu/mL in milk added with 0.2% cinnamon on the 5 day of storage.

Pasteurized milk samples were inoculated with *S. aureus* as 5.00±0.68 log cfu/mL and 7.00±0.44 log cfu/mL; the results determined as 4.04±0.09 log cfu/mL and 5.6±0.8 log cfu/mL, respectively, with addition of YOPE.

UHT milk samples were inoculated with *S. aureus* as 5.00±0.08 log cfu/mL and 7.00±0.46 log cfu/mL; the results determined as 3.76±0.38 log cfu/mL and 5.54±0.41 log cfu/mL, respectively, with addition of YOPE.

Raw milk samples were inoculated with *S. aureus* as 5.00±1.36 log cfu/mL and 7.00±0.87 log cfu/mL; the results determined as 4.12±1.19 log cfu/mL and 5.24±0.59 log cfu/mL, respectively, with addition of YOPE (Figure 4).

YOPE has quercetin and derivatives, flavanols such as flavanols in it's structure. Because of these molecules, it has an antibacterial effect. So it is expected YOPE to inhibit pathogens. It was determined that the inhibitory effect of YOPE on pathogens was higher in pasteurized and UHT milk. Since raw milk samples contain a high rate of microorganisms (TMAB) except *E. coli* and *S. aureus* at the beginning of the analysis, YOPE spent its antibacterial effect primarily by reducing the number of TMAB. For this reason, it is thought that YOPE has less inhibitory effect on pathogens in raw milk than pasteurized and UHT.

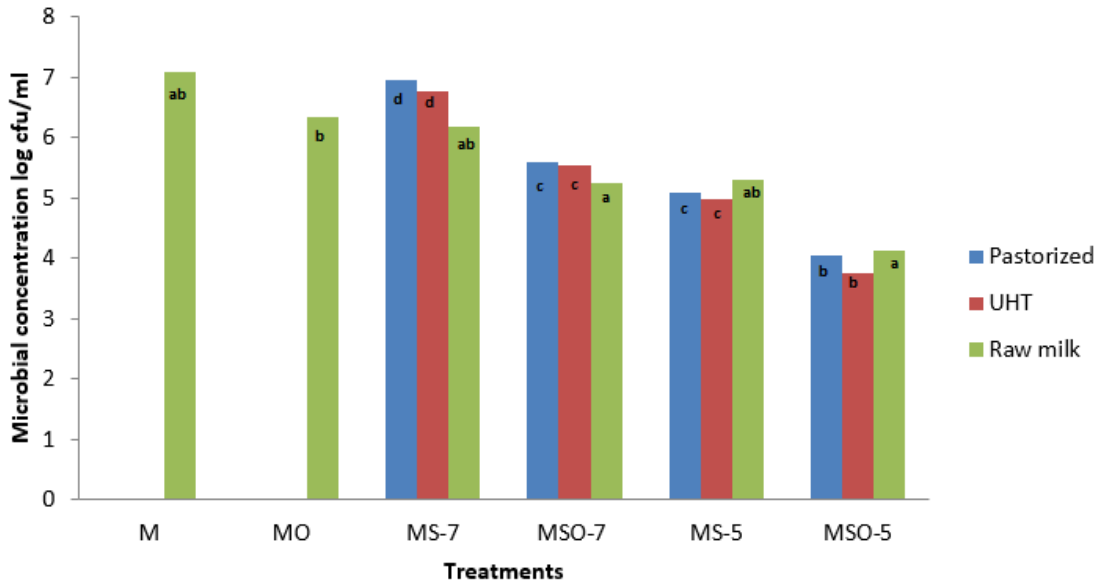

Figure 4. Inhibitory effect on *S. aureus* ATCC 25923
(M: milk; MO: milk + YOPE; MS-7: milk+ 7 cfu/mL *S. aureus*; MSO-7: milk+ 7 cfu/mL *S. aureus* + YOPE; MS-5: milk+ 5 cfu/mL *S. aureus*; MSO-5: milk+ 5 cfu/mL *S. aureus* + YOPE)

Increasing concentration of pomegranate and onion peel extracts increased the antibacterial effect of extracts on *E. coli* [32]. The antibacterial effect was detected as 98.06% when pomegranate and onion peel extracts were used in a combination 50:50%. İlkimen and Gülbandılar [33] found that extracts of lavender and sage has antibacterial activity on *S. aureus* and *E. coli*. Serpi et al. [34] reported extract of lavander has inhibitory effect on some pathogen bacteria. Haşimi et al. [35] stated different plant extracts have antibacterial activity on *S. aureus* and *E. coli*. Harlita et al., [36] reported Dayak onion extracts have antibacterial effect on *S. aureus, B. cereus, Shigella* spp.and *P aeruginosa*. The highest effect was seen on *B. cereus* (139.58 %). Abdel-Rahim et al. [37] stated the yellow onion extract caused a reduction of *A. niger* as 60.46%, 31.09%, 23.04% and 5.95% at different concentration. Al-Delamy [38] determined *S. aureus* growth at 6 log cfu/mL has been completely inhibited by onion extracts. These studies shows different plant extracts including

onion peel extract have antibacterial effect on *E. coli* and *S. aureus*.

4. CONCLUSIONS

The addition of YOPE resulted in a decrease in the *L** value and pH, an increase in the *a** and *b** value in all milk species. Due to the dark color of the YOPE, it was expected that the brightness value of the milk can be lowered by the addition of YOPE. The incerease in *b** value was more profound in pasteruized milk. The highest *L** and *a** values were detected on the 5th day of storage in UHT and raw milk with YOPE. The highest *b** and pH values were detected in raw milk with YOPE and UHT milk on the 5th and 1st days of storage, respectively. Yellow onion contained appreciable amount of quercetin and derivatives, flavanols which may have an antibacterial effect on pathogens of *E. coli* and *S. aureus*. The addition of YOPE in the milk can lower pathogens concentration in milks. The most inhibitory effect on *E. coli* was obtained from the pasteurized milk at a concentration of 7.00±0.24 log cfu / ml. The results show that yellow onion peel extract can be used as a natural antibacterial for *E. coli* ATCC 25922 and *S. aureus* ATCC 25923 in pasteurized, UHT and raw milks.

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