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RESEARCH ARTICLE

Effects of Ice Cream Produced with Lemon, Mandarin, and Orange Peel Essential Oils on Some Physicochemical, Microbiological and Sensorial Properties

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ABSTRACT

In this study, some physicochemical, microbiological and sensorial properties of ice cream produced with essential oils obtained from lemon (L), mandarin (M), and orange (P) peels at different ratios (0.1%, 0.3%, and 0.5%) were investigated. The highest amount of dry matter was determined in sample P3 which contains 0.5% lemon peel essential oil as 39.36%. The pH and % acidity values of the samples were found to vary between 6.36-6.45 and 0.22-0.27, respectively. At the end of the study, the lowest total aerobic mesophilic bacteria (3.80 log CFU/g), psychrophilic bacteria (4.01 log CFU/g), yeast and mold (3.71 log CFU/g) and *Pseudomonas* spp. (2.04 log CFU/g) counts were determined in ice cream sample P3. The total coliform group bacteria, *Salmonella* spp., *Staphylococcus aureus, Escherichia coli, and Listeria monocytogenes* were not detected in any of the ice cream samples. As a result of the sensory evaluation, it was determined that the most admired sample was P3 and the least preferred sample was the control sample.

Keywords: Essential Oil, Ice Cream, Lemon, Mandarin, Orange

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Limon, Mandalina ve Portakal Kabuk Esansiyel Yağlarıyla Üretilen Dondurmaların Bazı Fizikokimyasal, Mikrobiyolojik ve Duyusal Özellikleri Üzerine Etkileri

ÖZ

Bu çalışmada farklı oranlarda (%0.1, %0.3, %0.5) limon (L), mandalina (M) ve portakal (P) kabuklarından elde edilen esansiyel yağlarla üretilen dondurmaların bazı fizikokimyasal, mikrobiyolojik ve duyusal özellikleri araştırılmıştır. En yüksek kuru madde miktarı 39.36% ile %0,5 oranında limon kabuk esansiyel yağı içeren P3 örneğinde saptanmıştır. Örneklerin pH ve % asitlik değerlerinin ise sırasıyla; 6.36-6.45 ve 0.22-0.27 arasında değiştiği belirlenmiştir. Araştırma sonucunda en düşük toplam aerobik mezofilik bakteri (3.80 log kob/g), psikrofilik bakteri (4.01 log kob/g) maya küf (3.71 log kob/g) ve *Pseudomonas* spp. (2.04 log kob/g) sayılarıyla P3 dondurma örneğinde tespit edilmiştir. Dondurma örneklerinin hiçbirinde toplam koliform grup bakteri, *Salmonella* spp., *Staphylococcus aureus, Escherichia coli ve Listeria monocytogenes* türü bakteri gelişimi tespit edilmemiştir. Duyusal değerlendirme sonucunda en çok beğenilen örnek P3 olmuşken, kontrol örneği ise en az tercih edilen örnek olduğu tespit edilmiştir.

Anahtar Kelimeler: Dondurma, Esansiyel Yağ, Limon, Mandalina, Portakal

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INTRODUCTION

Ice cream which is claimed to be made by the Chinese 3000 years ago for the first time (Tekinşen 2000) is one of the oldest dairy products preferred all over the world (Manoharan and Ramasamy 2013). Nowadays, its sales at the global level increase nearly by 5% every year and have exceeded 73.8 billion dollars (Goff and Hartel 2013). Although its definition varies among the countries, ice cream is expressed as a milk product which is produced by cooling and mixing and transforming into pasty (Clark 2012). In addition to milk obtained from different dairy animals (Marshall et al. 2012) ice cream may contain many more components as well as sugar, oil, stabilizer and emulsifier in the mixture (Corvitto 2011).

Ice cream is a food product with an extremely rich nutritional value. The nutritional value of ice cream directly depends on the nutritional values of the components that make up ice cream. Ice cream also has all nutritional elements owned by milk. Furthermore, ice cream contains 3-4 times more oil and more proteins by 12% - 16% compared to milk. In addition to these, it is a richer product than milk due to the addition of additives such as fruits, nuts, and eggs (Arbuckle 2011).

Citrus fruits are the plants that belong to the *Rutaceae* family and are grown in the Mediterranean countries such as Italy, Spain, Turkey and Egypt (Di Vaio 2010). Citrus fruits are composed of many species, mainly sweet orange (*Citrus aurantium* L.subsp. *dulcis*), sour orange (*Citrus aurantium* L. subsp. *amara*), lemon (*Citrus limon* L.), mandarin (*Citrus reticulata* L.) and grapefruit (*Citrus paradisi* L.) (Bampidis and Robinson 2006). Citrus fruits take an important place in human nutrition since they have high vitamin and mineral content. Furthermore, citrus fruits and their process wastes are also used in various industrial areas (Baratta et al. 1998).

Citrus peels are frequently used in various fields in the industry due to their high essential oil content (Bampidis and Robinson 2006). In general, citrus essential oils are rich in monoterpenic hydrocarbons (at ratios reaching 97.3g /100 g) and basically contain d-limonene (IARC 1993). As a result of these studies, it has been revealed that these oils have antioxidant, antibacterial, antimicrobial and antiparasitic activity (Singh et al. 2010).

Lemon (*Citrus limon*) has many important natural chemical components including citric acid, ascorbic acid, minerals, flavonoids, and essential oils. Lemon leaves are used in folk medicine for the treatment of obesity, diabetes, blood lipid lowering, cardiovascular diseases, brain disorders and certain types of cancer (Lidianne et al. 2011). The essential oils extracted from lemon peel are commonly used in perfume, cosmetics and food industries (Aloisi et al. 2002).
Mandarin (*Citrus reticulata* L.) peel essential oils are predominantly composed of hydrocarbons. Limonene is the main component (52.2 - 96.2%), and ϒ-terpinene (tr-36.7%), α-Pinene (0.1-2.1%), linalool (0.1-2.5%), myrcene (1.3- 1.8%) and sabinene (0.1-1.3%) are other components (Lota et al. 2000). Mandarin essential oils are frequently used among people due to their relaxing, antiseptic and antifungal properties (Azadi et al. 2012).

Orange (*Citrus aurantium* L.subsp. *dulcis*) essential oils mainly contain β-pinene, limonene, trans-β-ocimene, linalool and α-terpineol (Sarrou et al. 2013). Orange blossom and blossom juice are used as tranquilizers and diuretic agents in folk medicine (Azadi et al. 2012).

In this study, it was aimed to investigate the effects of essential oils obtained from lemon, orange and mandarin peels on physical, chemical, microbiological and sensorial quality properties of the products obtained by the use of them in ice cream production.

MATERIALS and METHODS

Hydrodistillation

After fresh lemon (*Citrus limon* L.) and mandarin (*Citrus reticulata* L.) and orange (*Citrus aurantium* L.subsp. *dulcis*) peels were crushed in a blender (Waring 8010S/G, USA), 200 g was taken and subjected to hydrodistillation for 3 hours up to the point when the oil in matrix was depleted using a Clevenger type apparatus in accordance with the European Pharmacopoeia Specification (Anonymous 1996). The essential oils collected were dried on anhydrous sodium sulfate and stored at 4 °C until use.

Production of ice cream

The production of ice cream was performed by modifying the formulation indicated by Sağdıç et al. (2012). 7 L of cow's milk (4% fat) was used in ice cream production. At first, the fat content of ice cream was standardized to 9% using milk cream (60% fat) (400 mL). Then, the mixture was subjected to heat treatment at a moderate temperature. At the same time, milk powder (650 g), salep (30 g), emulsifier (20 g) (commercial mixture of mono- and diglycerides), stabilizer (gelatine) (8 g) and sugar (1500 g) were combined and mixed continuously and then slowly added to the mixture at 45-55 °C. After the mixture was pasteurized at 85 °C for 1 min, it was cooled to 43 °C. The mix was rested for 4-5 h until the temperature decreased to 4 °C. The mix ready for processing was divided into 10 equal parts under aseptic conditions. The samples were formed by adding lemon, mandarin, and orange peel essential oils at different ratios to the mixes divided (Table 1).

Then, the mixes were frozen by mixing at -5 °C in the ice cream freezer (CRM,-GEL 25C, Italy). 250 g of each mixture was taken into sterile glass containers and hardened at -24 °C. Then, the samples were stored at -18 °C until their analyses were completed.

Physicochemical Analyses
The dry matter, oil and % acidity values of the ice cream produced by adding different citrus essential oils were calculated according to AOAC (2016). The pH values of the samples were measured using the Ohaus (ST 5000) device.

Microbiological Analyses
10 g of ice cream samples were taken into a stomacher bag under sterile conditions, 90 ml of sterile ringer solution (1:9 w/v) was added, and it was homogenized in the stomacher (Lab-Blender 400, London, UK) for 3 minutes. 1 ml of this sterilized mixture was taken and added to the tube containing 9 ml of sterile ringer solution and mixed with vortex (IKA MS3, Germany). Thus, 10^{-2} dilution was prepared. The process was maintained in the same way, and serial dilutions were prepared until series 10^{-6} (Anonymous 2001).

Ice cream samples were subjected to total aerobic mesophilic bacteria (ISO 2013), psychrophilic bacteria (ISO 2013), yeast and mold (ISO 2008), total coliform (ISO 1991a) , *Pseudomonas* spp. (ISO 2010), *Salmonella* spp. (ISO 2017a), *Staphylococcus aureus* (ISO 1991b), *Escherichia coli* (ISO 2001, ISO 2015), and *Listeria monocytogenes* (ISO 2017b, ISO 2017c) count analyses.

Sensory Analyses
In the sensory evaluation of the ice cream samples, the scorecards created by modifying the sensory test parameters indicated by Akarca et al. (2016) were used, and the differences were determined by the scoring system on a scale. Sensory panel was carried out by twenty trained panelist that were from the Food Engineering Department of Afyon Kocatepe University. Sample were evaluated for, color and appearance, texture and consistence, taste and odor and general appreciation with a hedonic scale between 1-9 as follows: 1-3 (not acceptable), 4-5 (fairly acceptable), 6-7 (good aceptable), 8-9 (very good) (Önoğur and Elmacı 2012, Anonymous 2012).

Statistical Analysis
The statistical analysis of the results was performed using the SPSS statistical package program (SPSS Inc., USA). Data obtained from the study were analyzed by one-way analysis of variance in order to test for significant differences between treatments. Significance of differences was defined as $P < 0.05$. When we observed any differences among the groups, Duncan test was also applied in order to determine the significance levels.

RESULTS and DISCUSSION

Physicochemical Analyses
The physicochemical analysis results of ice cream samples are presented in Table 2. In our ice cream samples, the highest dry matter value was determined by 39.36% in the sample with 0.5% lemon peel addition in its production, and the lowest dry matter value was determined in the control sample by 38.17% ($P > 0.05$).

Similarly to the results of our study, Macit et al. (2017) produced ice cream samples using some spice essential oils and reported that they determined the highest dry matter value in the samples with 0.4% lemon peel addition in their production by 39.81% and the lowest dry matter value in the control sample by 39.21%.

In the study, the pH values of the samples were found to vary between 6.36 and 6.45, and the lowest pH value was determined in the sample with 0.5% lemon peel essential oil addition in its production, while the highest pH value was determined in the control sample. It was determined that the difference between the samples was not statistically significant ($P > 0.05$). Patır et al. (2004) indicated that the pH values of 50 samples taken from ice cream offered for sale in Elazığ varied between 6.05 and 7.41.

The % acidity values of the samples were determined to vary between 0.22 and 0.27. It was determined that the sample with 0.5% lemon essential oil addition in its production had the lowest degree of acidity ($P > 0.05$). Öksüztepe et al. (2005) reported that they determined that the % acidity values of 50 samples taken from fruit and flavored ice cream in Elazığ varied between 0.12 and 0.48.

The pH and % acidity values obtained in other relevant studies are different from the results of this study. It is thought that the difference is caused by the substances used in the formulation of the mixture applied in ice cream production and the usage rates.
It was determined that the amounts of oil of the ice cream samples produced by adding different citrus peel essential oils were very close to each other (14.24% - 14.26%). Similarly to the results of our study, Macit et al. (2017) reported that oil ratios varied between 14.10% and 14.50% in ice cream samples produced by adding lemon essential oil at two different ratios, and they stated that the essential oils added had no effect on the oil ratio of ice cream.

Microbiological Analyses
In our study, it was determined that the highest total aerobic bacteria (Figure 1) and psychrophilic bacteria counts (Figure 2) were 5.12 and 4.94 log CFU/g, respectively, in the control sample ($P < 0.05$) and that the lowest total aerobic bacteria and psychrophilic

bacteria counts were 3.80 and 4.01 log CFU/g, respectively, in the samples with 5% lemon peel essential oil addition in their production ($P < 0.05$).

In their study, Akarca and Kuyucuoğlu (2009) reported that the total aerobic bacteria counts varied between 2.99 and 6.17 log CFU/g in unpacked ice cream samples sold across Afyonkarahisar province. Similarly, Bostan and Akın (2002) investigated the microbiological qualities of industrial ice creams in their study, and they determined that the total aerobic bacteria counts of the samples varied between 2.00 and 4.26 log CFU/g.

Çubukçı (2016) reported that the psychrophilic counts of ice cream sold in Erzurum market varied between 1.5 and 7.26 log CFU/g, while Mukan and Evliya (2002) determined that the psychrophilic bacteria counts of plain-vanilla ice-cream sold in Adana market varied between 0 and 4.46 log CFU/g. The results determined by the researchers are similar to our results.

It was determined that the minimum and maximum yeast and mold counts of ice cream samples produced by adding two different citrus peel oils were 3.71 and 4.59 log CFU/g, respectively ($P < 0.05$) (Figure 3).

Korel et al. (2005) stated that the yeast and mold counts in unpacked ice cream samples sold in Manisa province were between <1 and 4.48 log CFU/g. Çınar (2010) reported that the yeast and mold counts in ice cream samples offered for sale in Tekirdağ province were between <1 and 4.62 log CFU/g.

In our ice cream samples, it was determined that the lowest *Pseudomoas* spp. count was 2.04 log CFU/g in the sample with 0.5% lemon peel essential oil in its production and that the highest count was 3.68 log CFU/g in the control sample ($P < 0.05$) (Figure 4).

Erol et al. (1998) determined that *Pseudomonas* spp. counts in ice cream samples sold in various pastry shops in Ankara varied between < 2.30 and 4.90 log CFU/g. The values obtained by the researchers are higher compared to the results of our study. The difference between them is thought to be due to the failure to adequately comply with the hygiene and sanitation rules in the production, storage, and sale of ice cream.

In our study, the total coliform group bacteria, *Salmonella* spp., *Staphylococcus aureus*, *Escherichia coli*, and *Listeria monocytogenes* type bacterial growth was not detected in any of the ice cream samples. As a result of the microbiological analyses, it was determined that all our ice cream samples were in conformity with all criteria specified in the Turkish Food Codex, Communique on microbiological criteria, Food safety criteria (Anonymous 2011).

In their study, Keskin et al. (2007) determined that the total coliform count in ice cream samples was between 2.48 and 5.68 log CFU/g. Coşkun (2005) stated that the presence of coliform group bacteria was determined in all plain ice cream samples sold in the provincial borders of Tekirdağ and that their count varied between 1.47 and 4.38 log CFU/g.

Erol et al. (1998) reported that they determined the development of *Escherichia coli* and *Salmonella* spp. in 2% of 100 ice cream samples offered for sale in pastry shops operating in the provincial borders of Ankara. Çubukçı (2016) reported that the presence of *Escherichia coli* and *Listeria monocytogenes* type bacteria was not detected in any of a total of 75 samples taken from ice cream offered for sale in Erzurum market.

In their study, Yücel et al. (2000) reported that *Staphylococcus aureus* counts in 30 ice cream samples offered for sale in Ankara varied between 2 and 3.48 log CFU/g. Uraz et al. (2001) carried out a study on 39 ice cream samples offered for sale in pastry shops located in different districts of Ankara province, and they reported that *Staphylococcus aureus* counts varied between 2.48 and 4.30 log CFU/g.

The total coliform group bacteria, *Salmonella* spp., *Staphylococcus aureus,* and *Escherichia coli* type bacteria counts obtained from similar studies by the researchers are higher than the values obtained in our study (Yücel et al. 2000, Uraz et al. 2001). It is thought that the differences were caused by the microbial quality of raw materials, auxiliaries, and additives used in the production of ice cream, and by the contaminations due to the failure to pay adequate attention to hygiene and sanitation rules in production, packaging and storage conditions.

According to the results of the microbiological analysis of ice cream samples produced by the addition of two different citrus peel essential oils, it was determined that the sample produced by adding 5% lemon essential oil had the lowest total aerobic mesophilic bacteria, psychrophilic bacteria, and yeast and mold counts. According to the analysis results, it was determined that the microorganism counts of the samples decreased as the amount of essential oil added increased.

It was indicated that the composition of lemon essential oils contained Limonene (61.68%), Neral (21.66%), β-Pinene (10.23%), ϒ-Terpinene (6.42%) (Ammada et al. 2018). The antibacterial and antifungal effect of lemon essential oils in samples is thought to be caused especially by limonene, β-Pinene, and ϒ-Terpinene.

The sensory analysis results of ten different ice cream samples are presented in Table 3. Panelists gave the highest score in terms of color and appearance to the control sample by 8.66. This sample was followed by the sample with 1% lemon peel essential oil addition in production by 8.21 points. The lowest score, 7.23, was given to the sample with 5% mandarin peel oil addition in production. The increase in the amount of essential oil used in production had a negative effect on color and appearance.

In the sensory analyses conducted, in the evaluation of the structure and consistency, the panelists gave all the samples the values that were very close to each other. It was determined that the essential oils added at different ratios and types in production had no negative effect on the structure and consistency of the ice cream.

In the evaluation made in terms of taste and smell, the product with 5% lemon peel essential oil addition in its production had the highest score by 8.93. This product was followed by the sample with 2% lemon peel essential oil addition in production with a score of 8.76 and the sample with 1% lemon peel essential oil addition in production with a score of 8.27. The samples with citrus essential oils addition were scored higher than the control sample by the panelists participating in the analysis. Similarly, the samples with lemon peel essential oil addition had higher taste and smell scores than the samples with orange and mandarin peel essential oil addition.

In terms of general admiration, the sample with 5% lemon peel essential oil addition had the highest score of 8.85 among ten different ice cream samples. It was determined that the control sample was the least admired sample by the panelists with a general admiration score of 7.56.

Table 1. Essential oils and ratios

Samples	Added essential oils and ratios
K	Control Sample
L1	% 0.1 Lemon peel essential oil
L2	% 0.3 Lemon peel essential oil
L3	% 0.5 Lemon peel essential oil
M1	% 0.1 Tangerine peel essential oil
M2	% 0.3 Tangerine peel essential oil
M3	% 0.5 Tangerine peel essential oil
P1	% 0.1 Orange peel essential oil
P2	% 0.2 Orange peel essential oil
P3	% 0.5 Orange peel essential oil

Table 2. Physicochemical Analysis Results of Ice Cream Samples

Samples	Dry Matter (%)	pH	Acidity (%)	Fat (%)
K	38.17	6.45	0.27	14.26
L1	38.41	6.41	0.25	14.25
L2	38.86	6.39	0.23	14.25
L3	39.24	6.36	0.22	14.24
M1	38.39	6.43	0.26	14.26
M2	38.88	6.39	0.25	14.25
M3	39.33	6.38	0.24	14.25
P1	38.40	6.43	0.26	14.26
P2	38.84	6.38	0.25	14.25
P3	39.36	6.37	0.23	14.24

Table 3. Sensory evaluation results of ice cream samples

Samples	Color and Appearance	Structure and Consistency	Taste and Smell	General Appreciation
K	8,66a	8,85a	7,63d	7,56e
L1	8,08b	8,93a	8,27b	8,42b
L2	7,65c	8,88a	8,76a	8,56ab
L3	7,49d	8,95a	8,93a	8,85a
M1	8.16b	8.78a	7.80c	7.58e
M2	7.46d	8.80a	7.95c	7.85d
M3	7.23e	8.89a	8.12b	8.04c
P1	8,21b	8,77a	8,06b	8,27b
P2	7,76c	8,82a	8,24b	8,35b
P3	7,32de	8,91a	8,82a	8,42b

Means within a column with different letters are significantly different ($P < 0.05$).

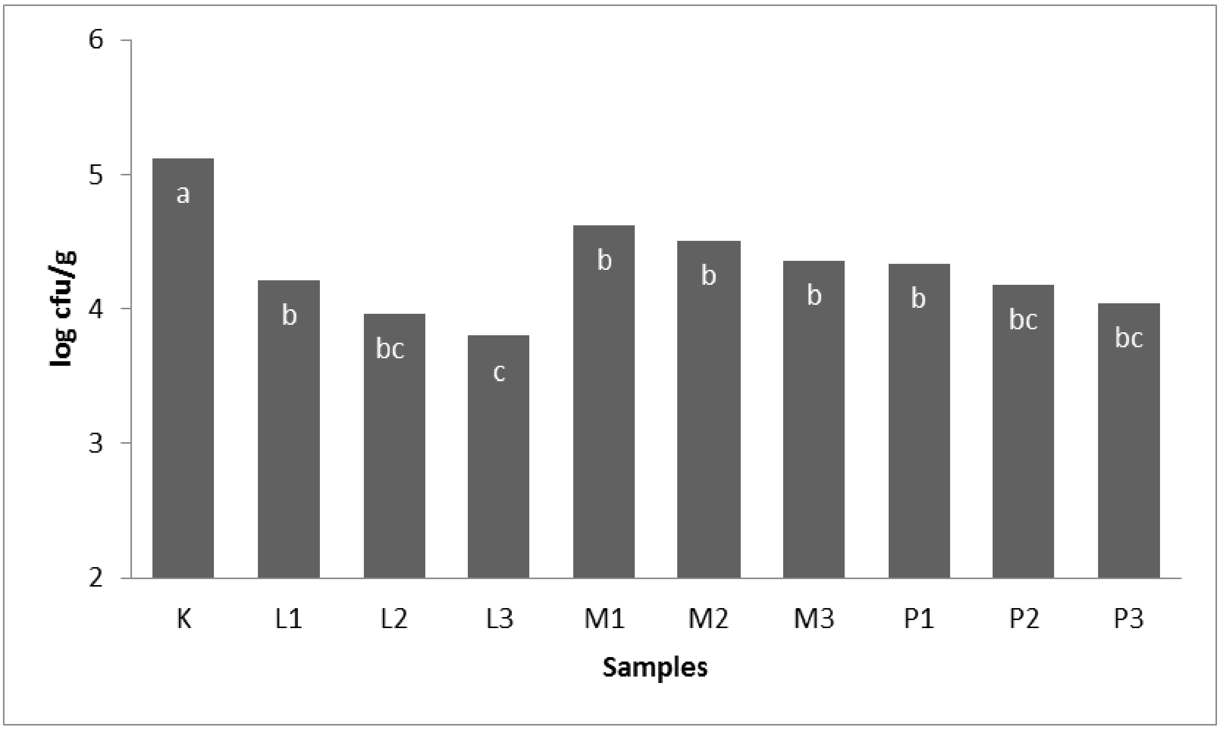

Figure 1. Total aerobic mesophilic bacteria counts of ice cream samples (Log CFU /g).
Means within a factor with different letters are significantly different (P < 0.05).

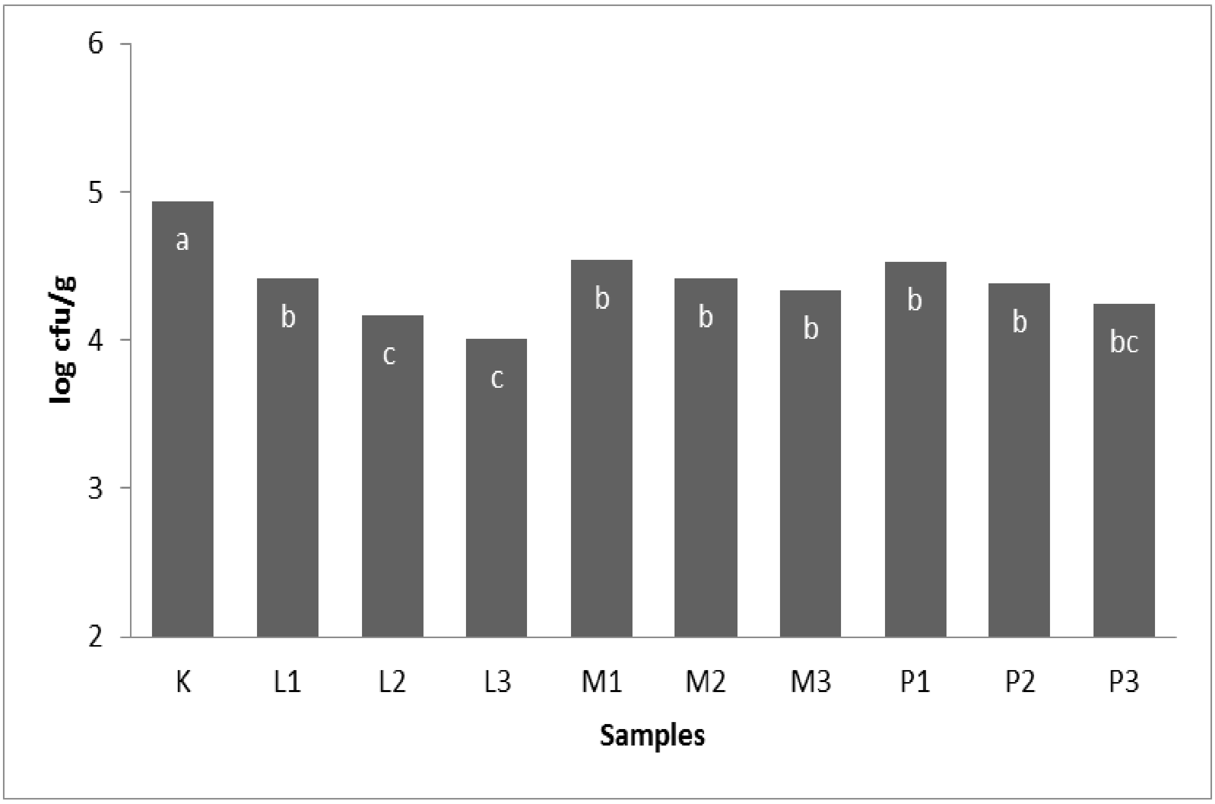

Figure2. Psychophilic bacteria counts of ice cream samples (Log CFU/g).
Means within a factor with different letters are significantly different (P < 0.05).

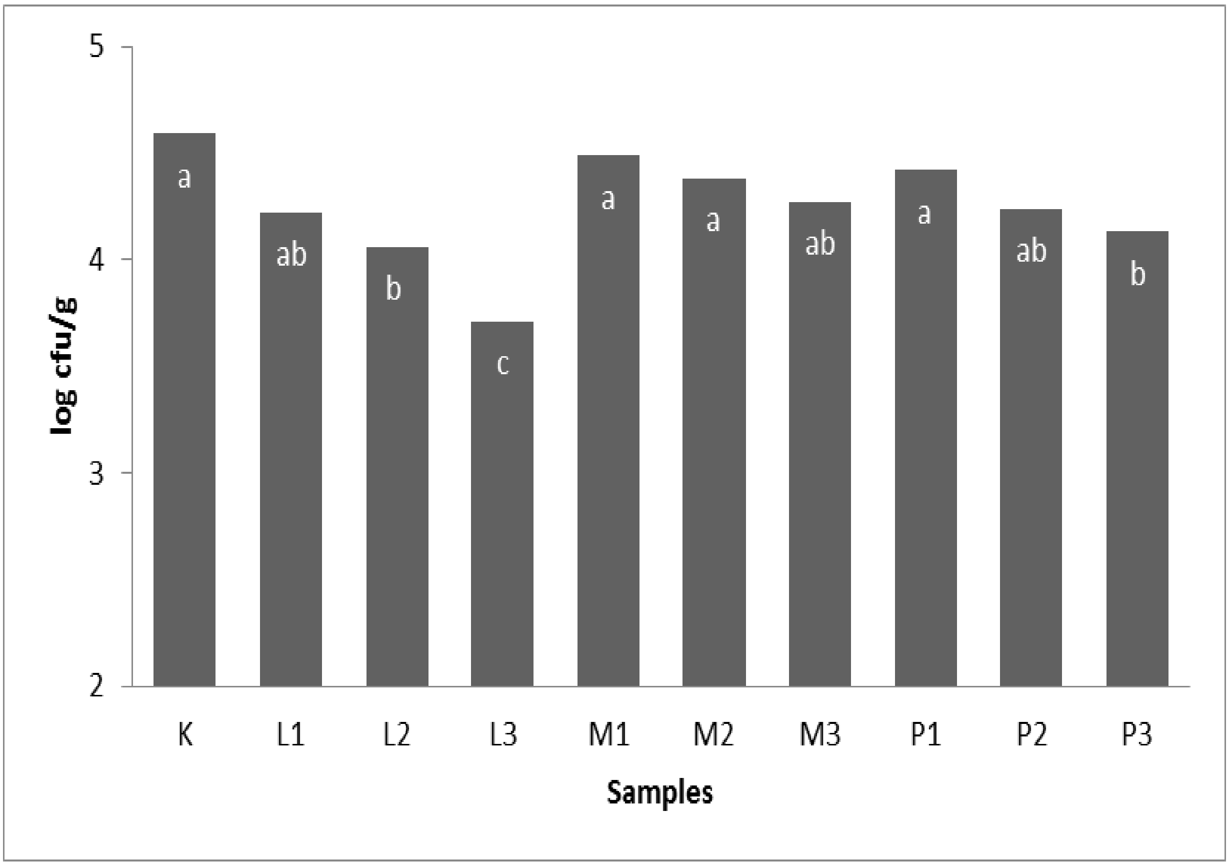

Figure 3. Yeast / mold numbers of ice cream samples (Log CFU/g).
Means within a factor with different letters are significantly different (P < 0.05).

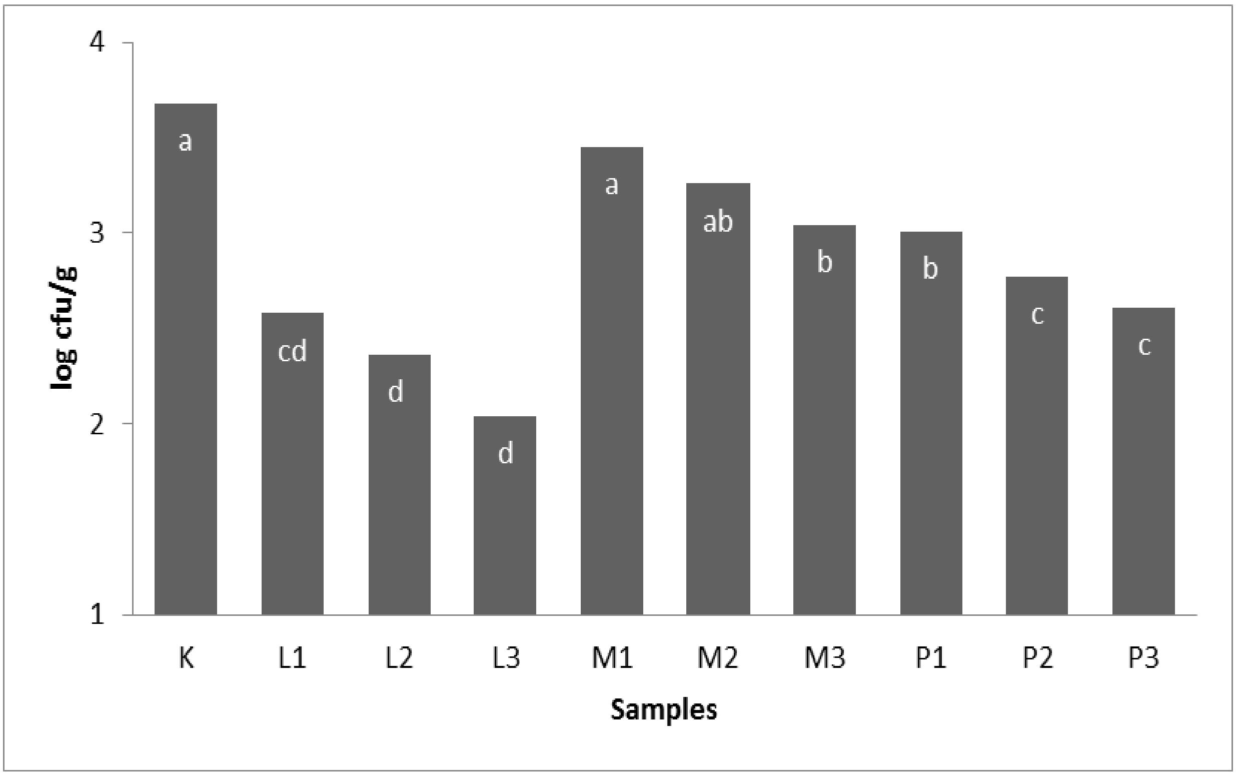

Figure 4. *Pseudomonas* spp. number of ice cream samples (Log CFU/g).
Means within a factor with different letters are significantly different (P < 0.05).

CONCLUSION

Lemon, mandarin and orange peels are the most important residues of many processes, especially fruit juice. Although the essential oils obtained from these residues are today utilized in many different areas such as cosmetics, pharmaceuticals, and chemistry, they are not much preferred in the food industry. The data obtained as a result of the study reveal that these essential oils have high antibacterial and antifungal properties. Furthermore, it has been determined that when they are added to a product which is largely consumed throughout the world such as ice cream, they do not impair the sensorial properties, on the contrary, they improve them. Along with the studies to be carried out, the usability of these oils in other fields of the food industry, especially in other dairy products, can be proven.

Nowadays, the products produced by using chemical additives have become less preferred by consumers with each passing day. Therefore, food manufacturers have been obliged to use more natural additives. It is obvious that lemon and orange peel essential oils can be used in food industry as antimicrobial preservatives and flavor enhancers.

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