EVALUATION OF THE ANTIMICROBIAL EFFECT OF SALICYLIC ACID ON MEATBALLS

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ABSTRACT

Salicylic acid application prevents deterioration of some foods and also has positive effects on human health. The objective of this study was to the investigation of microbial quality changes during the refrigerator storage of the meatballs after dipping off the meatballs in sterile distilled water and salicylic acid solutions. In this research, the effects of salicylic acid on the microbial loads of the meatballs were investigated. For this purpose, total aerobic mesophilic and total aerobic psychrophilic bacteria counts, total anaerobic mesophilic bacteria counts, and total coliform bacteria counts were analyzed by the classical culture methods on Plate Count Agar, Reinforced Clostridial Agar, and Violet Red Bile Agar, respectively. The bacterial analysis was performed daily until the total aerobic mesophilic bacteria counts of control group reached to the microbiological limit of 7 log CFU g^{-1}. It was found that salicylic acid containing groups had lower total aerobic mesophilic and total anaerobic mesophilic bacteria counts when compared to the control group. As a result, salicylic acid-containing groups generally have approximately 2 logarithmic lower bacterial loads according to the control group.

Key Words: Food, food safety, meatball, microbial quality, salicylic acid

SALİSİLİK ASİDİN KÖFTELER ÜZERİNDEKİ ANTİMİKROBİYAL ETKİSİNİN DEĞERLENDİRİLMESİ

ÖZET

Salisilik asit uygulaması bazı gıdalarda bozulmayı engellediği gibi aynı zamanda insan sağlığına olumlu etkileri de bulunmaktadır. Bu çalışmanın amacı köftelerin steril distile su ve salisilik asit solüsyonuna daldırılmasından sonra buzdolabı sıcaklığında köftelerin muhafazası esnasında mikrobiyal kalite değişimlerinin araştırılmasıdır. Bu çalışmada salisilik asidin köftelerin mikrobiyal yükü üzerindeki etkileri araştırılmıştır. Bu amaçla Plate Count Agarda toplam mezofil bakteri sayısı ve toplam psikrofil bakteri sayısı, Reinforced Clostridial Agarda toplam anaerobik bakteri ve Violet Red Bile Agarda toplam koliform bakteri sayıları klasik kültür yöntemi ile araştırılmıştır. Kontrol grubunda bozulma sınırı olarak kabul edilen 7 log kob/g düzeyine kadar analizler günlük olarak yapılmıştır. Sonuç olarak, salisilik asit içeren grupların genel olarak kontrol grubuna göre yaklaşık 2 logaritmik daha düşük bakteri yüküne sahip olduğu görülmüştür.

Anahtar Kelimeler: Gıda, gıda güvenliği, köfte, mikrobiyolojik kalite, salisilik asit

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Geliş tarihi/Received: 04.07.2019
Kabul tarihi/Accepted: 31.12.2019

INTRODUCTION

Meat could be evaluated as an essential source of the animal protein for human growth and development (1, 2). Generally, meat consist of several nutrients like high nutritional value protein as well as micronutrients such as iron, selenium, zinc and vitamin B_{12} (2-8). Meat proteins are also important in point of its essential amino acids content (2, 7). Essential amino acids are not synthesized by the humans, therefore, they must be taken by the diet (2).

Meat is a complex ecosystem and its chemical and physical structure allows for growth and colonization of the wide variety of organism. Many factors affect the presence of several microorganism groups in meat. Post-slaughter carcass contamination originates from animals such as skin, feces, and intestine as well as environments such as air, water, soil, staff, and process. Bacterial load and type of the carcass could be changed during the storage and sign of the deterioration may be felt. Therefore the shelf life of the meat depends on various conditions such as initial bacterial load and type, storage condition, temperature, pH and gaseous atmosphere. Depending on the metabolism of microorganism, a sign of the deterioration occurred as an odor, taste disorders and discoloration (9).

The chemical composition of meat is a source of the microbial growth and this may cause spoilage of the meat. Initial bacterial load of the meat depends on several factors such as the physiological condition of the animal in a slaughterhouse, contamination prevalence at the slaughterhouse and process steps, temperature and storage conditions (10). Higher water content (aw 0.99), optimal pH range for the microbial growth (pH 5.5-6.5), the presence of the energy-yielding nutrients (e.g. glucose, ribose, amino acids and nucleotides), vitamin and minerals affect the shelf life of meat and meat products. Recently, microbial spoilage is defined as increasing the number of microorganisms and the occurrence of the biochemical changes. These changes are related to the dominant microflora of the meat (11).

Antioxidants and preservatives are generally added to the foods alone or joined in order to extend the shelf life of foods or improve the food products (12). Salicylic acid is used as an antimicrobial agent in the foods and pharmaceutical preparations (13). Salicylic acid is a strong antioxidant, and it is used effectively in order to prevent the blood clot in the obstructed artery and reduce cholesterol. The lower amount of salicylic acid is used as a preservative in foods and also as an antiseptic (14). Salicylic acid is an organic acid and has keratolytic activity. This keratolytic activity provides removal of bacteria which attached to the skin owing to remove the dead layer of skin (15). Acetylsalicylic acid or 2-acetoxy benzoic acid is the formal name of the drug which is known as aspirin. Aspirin is prophylactically effective against coronary heart disease at a lower dose such as 30mg/day (16). Salicylic acid, which has benefits for human health, has lower oral LD 50 value as 891 mg/kg in rats (17). Salicylic acid is a seconder metabolite and is produced naturally by plants. In low concentrations, salicylic acid is safe for human health (18).

The objective of this study was to the investigation of microbial quality changes during the refrigerator storage of the meatballs after dipping off the meatballs in

sterile distilled water and salicylic acid solutions.

MATERIALS AND METHODS

Experimental Model

Minced beef meat was purchased from the local market in Ankara, and transferred to the laboratory in a cold chain condition. After the transfer of the minced beef meat, 30 gram of meatballs formed aseptically in order to create a homogeneous sample. Meatballs were divided into three groups; the first group was a control group, and the second and third group were dipped into 10% and 20% salicylic acid solution for 1 minute. Meatballs were placed into the polypropylene packaging and covered with stretch film and then stored in the dark at 4°C, and analyzed at day 0 and at one-day intervals until the microbial load of the samples reached >7 log Colony Forming Unit (CFU) g^{-1}, which was considered microbiological spoilage limit in meat and meat products (19).

Sampling and Analysis

Plate Count Agar (PCA, Merck 105463), Reinforced Clostridial Agar (RCA, Merck 105410), Violet Red Bile Agar (VRB, Merck 101406), Maximum Recovery Diluent (MRD, Merck 112535), stomacher (Bagmixer-400, France), distilled water, and salicylic acid (Merck, 54-21-7) were used.

A 10 g sample of each meatball was homogenized for 1 min in a stomacher with 90 ml of Maximum Recovery Diluent (MRD, Merck 112535) and the sample was serially diluted (10^1 to 10^7) with the MRD. Total aerobic mesophilic bacteria (TAMB) counts were determined on Plate Count Agar (Merck 105463) at 28°C for 48h (20). In the standard PCA practice, general hygiene and operational sanitation can be controlled with an incubation temperature of 28 ° C and an incubation time of 48 hours (21).

Total coliform bacteria were grown on double layer Violet Red Bile Agar (Merck 101406) at 32°C for 24h (20, 21). Coliform bacteria are members of the *Enterobacteriaceae* family; It contains *Escherichia*, *Enterobacter*, *Citrobacter* and *Klebsiella* and is considered as a general hygiene marker in foods (21).

Total anaerobic mesophilic bacteria were grown on the Reinforced Clostridial Agar (RCA, Merck 105410) anaerobically at 37 °C for 48 h.

Total aerobic psychrophilic bacteria counts were determined on Plate Count Agar (Merck 105463) at 4°C for 4-5 days (21).

Statistical Analysis

Statistical evaluation of the data was conducted by SPSS statistical programme. Factors influencing the change occurred in quality criteria were determined as the sample group and storage period. As a result of the one-way analysis of variance in repeated measurements, Duncan's significant difference method was used (22).

RESULTS AND DISCUSSION

Meat and meat products are the perfect nutrient source for the microorganisms due to its rich chemical composition and high water content (23). Initial total aerobic bacteria count was 5.0 log CFU g^{-1}, and TAMB count of the control group increased during the storage ($p<0.05$) (Figure 1). The decrease of TAMB counts of 10 % and 20 % salicylic acid containing groups was observed according to the first day of storage. The difference of the TAMB counts between salicylic acid containing groups and control group were statistically significant after the 2^{nd} days of storage ($p<0.05$). Salicylic acid containing

groups had lower TAMB counts when compared to the control group. Approximately 2.7 logarithmic reductions between salicylic acid added groups and control group were determined at the 7th day of storage. After the seventh days of storage, PCA counts of control samples exceeded 7 log CFU g^{-1}, which was considered as an acceptable upper limit of bacteria in meat spoilage (19).

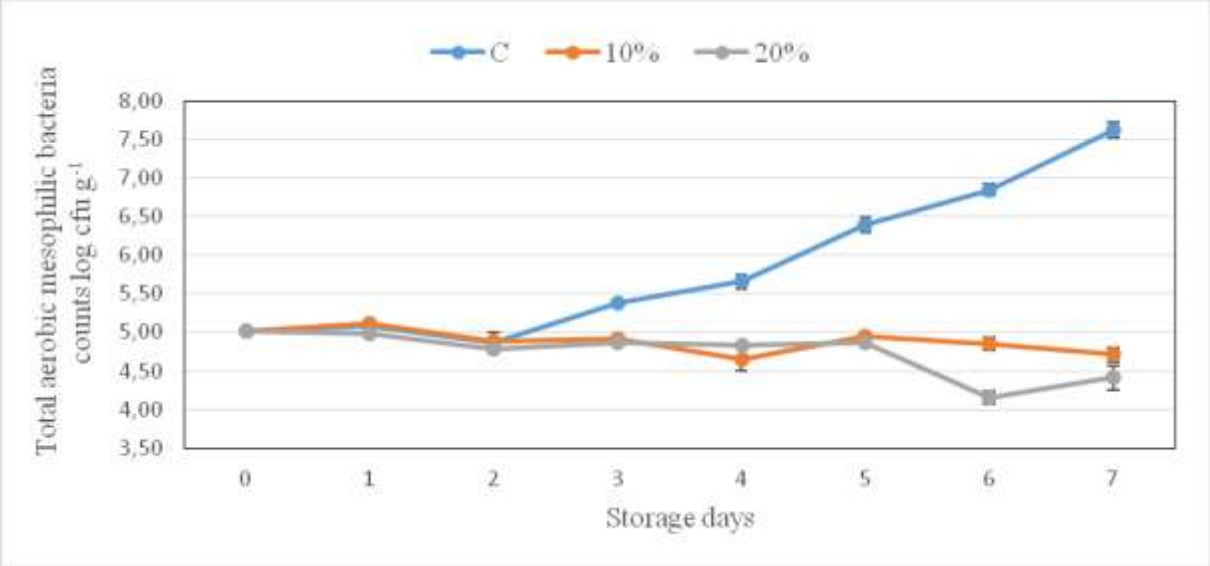

Figure 1. Changes in total aerobic mesophilic bacteria counts (log CFU g^{-1}) of meatballs during refrigerated storage for 7 days. All data points represent the mean ± standard error. Standard errors are given by error bars. C: Control group, 10%: 10% salicylic acid group, 20%: 20% salicylic acid group

Initial psychrophilic bacteria count of raw material was found as 4.9 log CFU g^{-1}. During the storage period, there was a significant increase in the psychrophilic bacteria counts of salicylic acid containing groups ($p<0.05$) (Figure 2). First three days of storage the difference between salicylic acid containing groups and the control group was significant ($p<0.05$). After the 3rd days of storage, psychrophilic bacteria count of the control group was too many to count. Total mesophilic psychrophilic bacteria count of the control group was found as 1.5 log unit higher than salicylic acid containing groups at the 3rd days of storage. Only 5th days of storage, a difference of psychrophilic bacteria count between 10% and 20% salicylic acid groups was statistically significant ($p<0.05$). Bacteria exposed to acid shock in a low pH environment may develop an acid tolerance responses and adapt to acid stress to protect themselves (24). Increase of the total psychrophilic bacteria counts of 10% and 20% salicylic acid groups observed from 3rd days to the end of the storage could be explained by the acid tolerance response of the psychrophilic bacteria.

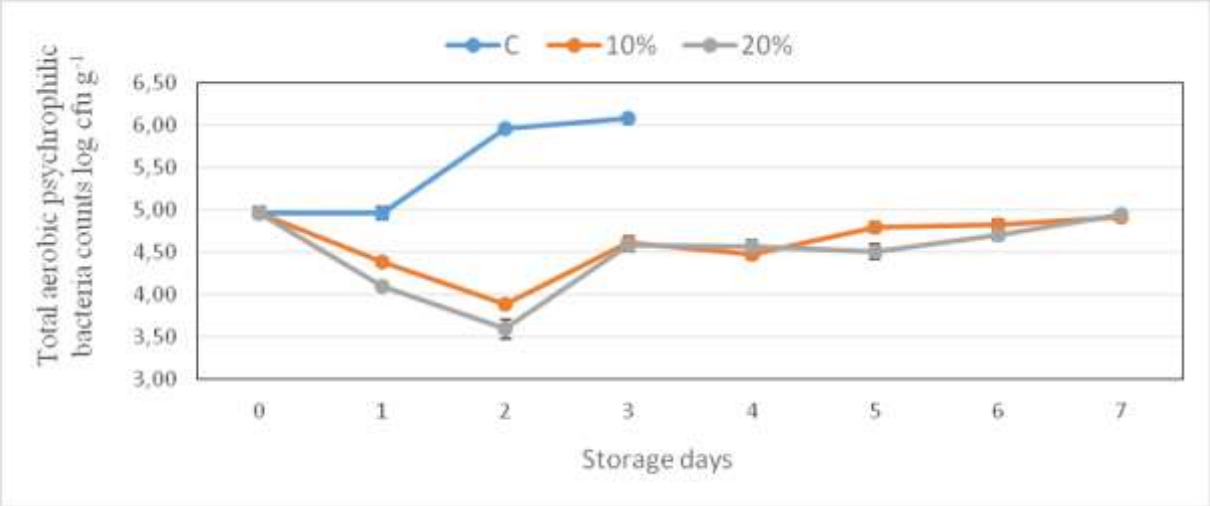

Figure 2. Changes in total aerobic psychrophilic bacteria counts (log CFU g^{-1}) of meatballs during refrigerated storage for 7 days. All data points represent the mean ± standard error. Standard errors are given by error bars. C: Control group, 10%: 10% salicylic acid group, 20%: 20% salicylic acid group

Initial total anaerobic mesophilic bacteria count was 4.8 log CFU g^{-1}. Total anaerobic mesophilic bacteria count of the control group increased during the storage ($p<0.05$) (Figure 3). Total anaerobic mesophilic bacteria counts of salicylic acid containing groups variable during the storage. The difference between salicylic acid containing groups and control group was statistically significant after the 2nd days of storage ($p<0.05$). Salicylic acid containing groups had lower total mesophilic anaerobic bacteria counts compared to the control group. At the end of the storage, total anaerobic mesophilic bacteria count of the control group was higher as 2.3 log unit compared to the salicylic acid containing groups.

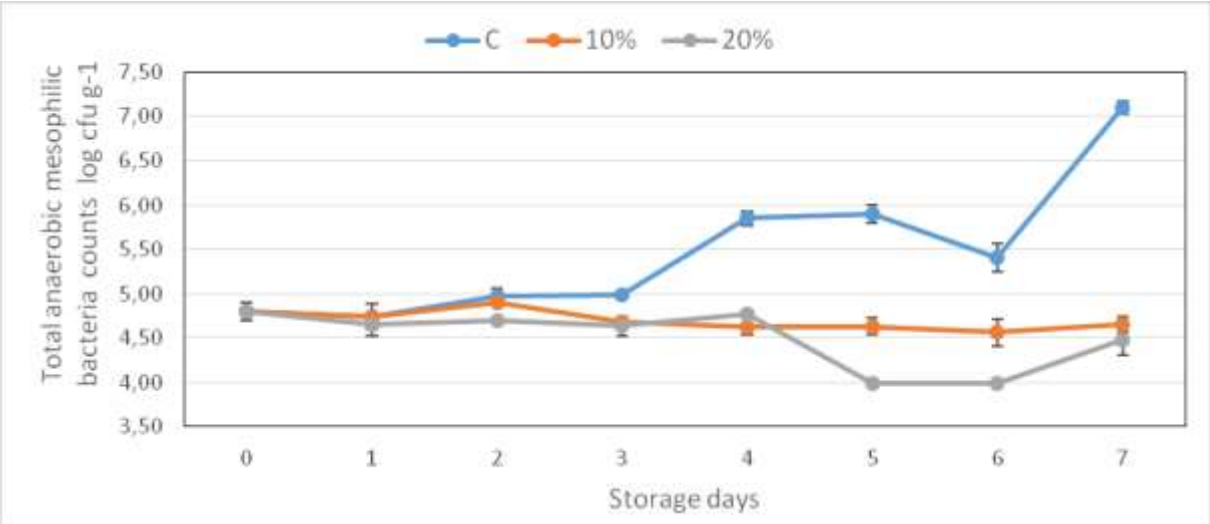

Figure 3. Changes in total anaerobic mesophilic bacteria counts (log CFU g^{-1}) of meatballs during refrigerated storage for 7 days. All data points represent the mean ± standard error. Standard errors are given by error bars. C: Control group, 10%: 10% salicylic acid group, 20%: 20% salicylic acid group

The initial total coliform count of raw material was 3.4 log CFU g^{-1}. Total coliform counts of control and salicylic acid containing groups were given in the Table 1. Total coliform bacteria of the control group was determined only as 2.93, 2.30 and 3.42 log CFU g^{-1} at 1st, 4th and 7th days of storage, respectively. Total coliform bacteria of the 20% salicylic acid containing group was determined as 2.3 log CFU g^{-1} at the only first day of the storage.

Table 1. Changes is total coliform counts (log CFU g^{-1}) of meatballs during refrigerated storage

	Storage days							
	0	**1**	**2**	**3**	**4**	**5**	**6**	**7**
Control	3.4	2.93	<2	<2	2.30	<2	<2	3.42
10%	3.4	<2	<2	<2	<2	<2	<2	<2
20%	3.4	2.30	<2	<2	<2	<2	<2	<2

When taking into account the TAMB counts which is the deterioration marker of the foods, it was determined that the control group decayed 7th days of the storage. Salicylic acid containing groups had 2.3 log units lower TAMB counts at the 7th days of storage compared to the control group, consequently, salicylic acid seems to be effective in the preservation of the meatballs. Considering the anaerobic bacteria and psychrophilic bacteria counts, salicylic acid containing groups had lower bacterial load compared to the control group.

Salicylic acid is the antimicrobial additives in order to use for extending the shelf life and reducing the bacterial contamination risk in cosmetic products. Salicylic acid has not only antimicrobial effects on the gram-negative microorganism but also have an antifungal effect and beneficial effect on the keratocyte. Therefore, it could be inhibited the growth of some gram-negative microorganisms and fungus. Salicylic acid which is the natural products may be synthesized by the lactic acid bacteria and plants (25).

Hinton and Cason (15) investigated the effects of salicylic acid on bacterial flora in processed broiler skins and reported a significant reduction in plate count agar after washing the skin with 20% salicylic acid. They reported that washing the broiler skin with salicylic acid reduces the number of bacteria. Ivanov (26) investigated the effects of organic acids on broiler litter and they reported that broiler litter containing 1.5% salicylic acid had lower microbial load than the control group. They also stated that 1.5% salicylic acid was effectively reduced the microbial growth in the broiler litter. Considering similar studies in other foodstuffs, Satraj et al (27) investigated the effects of different concentrations of salicylic acid on apricot and they found that the control group had the maximum microorganism counts when compared to salicylic acid contained

group. They also stated that the number of microorganisms decreased depending on the salicylic acid concentration increased. Shelf life is connected with the qualitative and quantitative initial bacterial load of the products and is also affected largely by storage conditions. When considered as a whole, initial bacterial load indicate the hygienic processing steps at the slaughterhouse and transport unit (28).

CONCLUSION

Meat especially minced meatballs are perishable food due to its higher water content and important nutrients. The increase in the number of spoilage microorganism could affect the shelf life of food products. Signs of deteriorations may vary depending on the alteration in the nutrient content of food products (29-31). This study shows that the application of 10% and 20% salicylic acid in minced meatballs could inhibit the microbial load when compared to the control group. When looking at the results, the salicylic acid application has a positive effect on the microbial load of the meat products.

REFERENCES

1. Dave D, Ghaly AE. Meat spoilage mechanisms and preservation techniques: A critical review. Am J Agric Biol Sci. 2011; 6(4): 486-510.
2. Pereira PMCC, Vicente AFRB. Meat nutritional composition and nutritive role in the human diet. Meat Sci. 2013; 93: 586–592.
3. Valsta LM, Tapanainen H, Mannisto S. Meat fats in nutrition. Meat Sci. 2005; 70: 525–530
4. Williamson CS, Foster RK, Stanner SA, Buttriss JL. Red meat in the diet. Nutr Bull. 2005; 30: 323–355.
5. Williams P. Nutritional composition of red meat. Nutr Diet. 2007; 64(4): S113–S119.
6. Zhang W, Xiao S, Samaraweera H, Lee EJ, Ahn DU. Improving functional value of meat products. Meat Sci. 2010; 86: 15–31.
7. Wyness L, Weichselbaum E, O'Connor A, Williams EB, Benelam B, Riley H, Stanner S. Red meat in the diet: an update. Nutr Bull. 2011; 36: 34–77.
8. De Smet S, Vossen E. Meat: The balance between nutrition and health. Meat Sci. 2016; 120: 145–156.
9. Russo F, Ercolini D, Mauriello G, Villani F. Behaviour of *Brochothrix thermosphacta* in presence of other meat spoilage microbial groups. Food Microbiol. 2006; 23: 797–802.
10. Doulgeraki AI, Ercolini D, Villani F, Nychas GJE. Spoilage microbiota associated to the storage of raw meat in different conditions. Int J Food Microbiol. 2012; 157: 130–141.
11. Pothakos V, Devlieghere F, Villani F, Björkroth J, Ercolini D. Lactic acid bacteria and their controversial role in fresh meat spoilage. Meat Sci. 2015; 109: 66–74.
12. Boyce MC. Simultaneous determination of antioxidants, preservatives and sweeteners permitted as additives in food by mixed micellar electrokinetic chromatography. J Chromatogr A. 1999; 847: 369–375.
13. Fung YS, Luk SF. Determination of salicylic acid in pharmaceutical formulations and foods by differential-pulsevoltammetry using a glassy carbon electrode. Anlst. 1989; 114: 943-945.
14. Pavlović A, Mitić S, Tošić S, Micić R, Rašić I, Mitić M, et al. The determination of salicylic acid in wines using a ligand-exchange reaction. Advanced Technologies. 2014; 3(1): 11-15.
15. Hinton Jr. A, Cason JA. Changes in the bacterial flora of skin of processed broiler chickens washed in solutions of salicyclic acid. Int J Poult Sci. 2007; 6(12): 960-966.
16. Venema DP, Hollman PCH, Janssen KPLTM, Katan MB. Determination of acetylsalicylic acid and salicylic acid in foods, using HPLC with fluorescence detection. J Agric Food Chem. 1996; 44: 1762-1767.
17. Wei Y, Liu Z, Su Y, Liu D, Ye X. Effect of salicylic acid treatment on postharvest quality, antioxidant activities, and free polyamines of asparagus. J Food Sci. 2011; 76(2): S126-S132.
18. Zhou D, Li L, Wu Y, Fan J, Ouyang J. Salicylic acid inhibits enzymatic browning of fresh-cut Chinese chestnut (*Castanea mollissima*) by competitively inhibiting polyphenol oxidase. Food Chem. 2015; 171: 19–25.
19. ICMSF. International commission on microbiological specifications for foods, second edition. Sampling plans for fish and shellfish. "Microorganisms in foods 2. Sampling for microbiological analysis. Principles and spesific applications". 1986, p: 181-197.

20. Anonymous. *Gıda Mikrobiyolojisi ve Uygulamaları*. Ankara Üniversitesi Ziraat Fakültesi Gıda Mühendisliği Bölümü, Ankara 2000.
21. Halkman AK. *Merck Applications of Food Microbiology*. Başak Matbaacılık ve Tanıtım Hizmetleri Ltd. Şti., Ankara 2005, s: 358.
22. Daniel NW. Bioistatistic: a foundation for analysis in the health sciences, published by Wiley, New York 1991 p: 5.
23. Kovacs T, Lootz K, Dorn A, Andrieu J, Horvath M, Maty A, et al. Potential of small-scale jar systems to extend the shelf life of raw meats, and hinder the proliferation of *Campylobacter jejuni* and Enterohemorrhagic *Escherichia coli*. LWT - Food Sci Technol. 2017; 79: 525-533.
24. Ricke SC. Perspectives on the use of organic acids and short chain fatty acids as antimicrobials. Poult Sci. 2003; 82: 632–639.
25. Li J, Chaytor JL, Findlay B, McMullen LM, Smith DC, Vederas JC. Identification of didecyldimethylammonium salts and salicylic acid as antimicrobial compounds in commercial fermented Radish Kimchi. J Agric Food Chem. 2015; 63: 3053-3058.
26. Ivanov IE. Treatment of broiler litter with organic acids. Res Vet Sci 2001; **70:** 169–173.
27. Satraj A, Masud T, Abbasi KS, Mahmood T, Ali A. Effect of different concentrations of salicylic acid on keeping quality of apricot cv. Habi at ambient storage. Journal of Biological and Food Science Research. 2013; 2: 66–78.
28. Raimondi S, Nappi MR, Sirangelo TM, Leonardi A, Amaretti A, Ulrici A, et al. Bacterial community of industrial raw sausage packaged in modified atmosphere throughout the shelf life. Int J Food Microbiol. 2018; 280: 78–86.
29. Marklinder I, Eriksson MK. Best-before date–food storage temperatures recorded by Swedish students. Br Food J. 2015; 117(6): 1764-1776.
30. Van Haute S, Raes K, Van der Meeren P, Sampers I. The effect of cinnamon, oregano and thyme essential oils in marinade on the microbial shelf life of fish and meat products. Food Control. 2016; 68: 30-39.
31. Susanti S, Setiani BE, Rizqiati H, Febriandi DR, Bintoro VP. Inhibitory activity of cashew apple (*Anacardium occidentale*) extract marinade on the meat total bacterias. Curr Res Nutr Food Sci. 2018; 6(1): 106-112.