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Research Article

The Effects of Different Organic Acid Treatments on Some Microflora and Pathogen *Listeria monocytogenes* of White Brine Cheese [1]

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Abstract

The aim of this study was to determine the supplementation of 1% tartaric, malic, ascorbic, fumaric, lactic, acetic and succinic acids in brine on the pH, physical and sensory properties of cheese during storage. In addition, the effect of these organic acids on growth of lactic acid bacteria, yeast/mold and possible inhibitive effect on *Listeria monocytogenes* in brine were investigated. For this purpose, these organic acids were added to 12% brine and stored at +4°C. Physical and sensory properties of cheese were defined and scored by panelists during 1st, 7th, 14th, 21st, 28th, 35th, 42th days. Cheeses ripened in tartaric, malic, fumaric, and lactic acid supplemented brine were scored as the highest points by panelists. *Lactobacillus, Lactococcus* counts significantly decreased in brine samples added with fumaric, lactic and malic acid during cheese ripening process. The highest antimicrobial activity of the organic acids was found against *L. monocytogenes*. It was also found that there was a significant decrease in *L. monocytogenes* counts in malic acid and tartaric acid groups as 2.91 log10 CFU/g and 2.95 log10 CFU/g, respectively. As a result; the supplementation of 1% tartaric acid and malic acid to the classically produced cheese brine were found effective on controlling *L. monocytogenes* which is a significant threat for public health. In addition, it was concluded that tartaric acid can be used commercially in the production of cheese due to its low inhibition effect on lactic acid bacteria, and non-destructive effect on the physical structure of the cheese.

Keywords: *Antimicrobial activity, L. monocytogenes, Organic acid, White cheese*

Beyaz Peynir Salamurasına Farklı Organik Asitlerin İlavesinin Mikroflora ve Patojen *Listeria monocytogenes* Üzerine Etkileri

Öz

Bu çalışmanın amacını laboratuvar ortamında klasik olarak üretilmiş peynirin salamura suyuna %1 oranında katılan tartarik, malik, askorbik, fumarik, laktik, asetik ve suksinik asitin depolama sürecinde peynirin fiziksel ve duyusal özellikleri ile pH değeri üzerine etkileri oluşturmuştur. Ayrıca, salamura suyuna eklenen organik asitlerin laktik asit bakterileri, küf-maya sayısı ve *Listeria monocytogenes* üzerine olası etkileri araştırılmıştır. Bu amaçla organik asitler %12'lik salamura suyuna eklenmiş ve +4°C'de depolanmıştır. Peynirin fiziksel ve duyusal özelikleri 1, 7, 14, 21, 28, 35 ve 42. günlerde belirlenmiş ve panelistler tarafından puanlanmıştır. Peynirin olgunlaşma sürecinde tartarik, malik, fumarik ve laktik asit eklenmiş salamura örnekleri panelistler tarafından en yüksek puanı almışlardır. Peynirin olgunlaşma sürecinde fumarik, laktik ve malik asit eklenmiş salamura örneklerinde *Lactobacillus, Lactococcus* sayıları istatistiksel olarak önemli derecede azalmıştır. Organik asitlerin en yüksek oranda antimikrobiyel etkinliği *L. monocytogenes* karşı tespit edilmiştir. Malik asit ve tartarik asit gruplarında *L. monocytogenes* sayılarında ortalama sırasıyla 2.91 log10 kob/g, 2.95 log10 kob/g oranında önemli derecede azalma tespit edilmiştir. Sonuç olarak; klasik yöntemle üretilen peynirin salamura suyuna katılan %1 oranında tartarik asit ve malik asit halk sağlığı için önemli bir tehdit olan *L. monocytogenes*'in kontrolünde etkili bulunmuştur. Ayrıca peynirde yapısal kusurlara neden olmaması ve laktik asit bakterilerinin üzerine düşük inhibisyon etkisinden dolayı tartarik asitin peynir üretiminde ticari olarak kullanılabileceği sonucuna varılmıştır.

Anahtar sözcükler: *Antimikrobiyel aktivite, L. monocytogenes, Organik asit, Beyaz peynir*

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INTRODUCTION

Food safety is the suitability and protectivity of food consumption, and protected against food-borne health problems. All of the hazards affect the food safety due to biological, chemical, physical contaminants and some erroneous practices in the production line [1-3]. In terms of food safety, one of the hazardous product is cheese. There are more than 130 varieties of cheese production are made in Turkey [4]. Cheese is a ready-to-eat food substance, and during production process microorganisms can easily contaminate and growth. Food pathogens such as Lipolytic *Pseudomonas* spp., *Penicillium* spp. and *Listeria* spp. cause deterioration of quality and threat the consumer health [5-7].

Listeria monocytogenes is an important bacteria in food-borne pathogens because of the psychrotrophic properties. Listeriosis causes important problems in the food industry depend on its bad aspects, besides it gives rise to abortion and meningitis in immunosuppressed adults [8,9]. The incidence of Listeriozis cases in human has increased since 2000 according to the latest data obtained from the European Union's eight countries [10]. In the analysis of data obtained from international food-borne outbreaks between 1988 and 2007 years, 337 of the 4093 outbreaks were reported to be related with dairy products, of which 6.6% were from *L. monocytogenes* [11]. Raw milk can be contaminated with *L. monocytogenes* via the using of unclean equipment during milking, mastitis infections, transport and storage problems. If the hygienic quality of the plant is not sufficient, milk or cheese samples may be contaminated with the bacteria during production process [12].

Fungal agents are the one of the important source of cheese contamination. Molds are microorganisms that have a psychotrophic character which is easily contaminated to the cheese, and can be isolated from many cheese varieties [13]. Besides, yeasts/molds cause change in colour of the cheese surface, shortening of the shelf life and rancidity due to the lipolytic enzymes leading to big economic loses [14].

Different applications are being made to ensure food safety in cheese production. Antimicrobial applications such as pasteurization, different salt concentrations, low water activity, packaging methods, high-low pH and chemical food additives are used for controlling the food safety [15,16]. Recently, consumers have increasingly inclined to natural additives that control microbial synthesis. For this purpose, the organic acids, bacteriocin and essential fatty acids (obtained from animals, plants and microorganisms) used as natural additives such as antioxidants, antimicrobials, sweeteners and colorant have been used increasingly in food production [17]. Organic acids such as lactic acid, acetic acid and citric acid have been used as preservatives in the food sector for a long time. They have antibacterial effects due to the ability of the binding to the cell membrane of the bacteria and reduce the intracellular pH values. Some of the organic acids may also occur chelate with metal ions, and reduce cell membrane permeability by causing cell disruption due to degradation of the substrate transport mechanism of the cell [18].

The present study was undertaken to evaluate the effects of different organic acids which were, added to brine, on pH, physical and sensory properties of cheese during storage. Moreover it was aimed to evaluate the supplementation of these organic acids to brine on the count of lactic acid bacteria, yeast/mold, and also inhibition potential of *L. monocytogenes* during storage days.

MATERIAL and METHODS

Raw milk samples (120 L for stage 1 and 2, separately) were analyzed by using Bentley IBCm and Kombi FTS 600 (USA) analysers. Then, classical cheese was produced from these pasteurized milk samples under the laboratory conditions.

The study was performed in two stages.

Stage 1: Organic acids (1%) were added to the brine (12%) of the clasically produced cheeses. Cheese samples were divided into eight groups as follows: one control and tartaric acid, malic acid, ascorbic acid, fumaric acid, lactic acid, acetic acid and succinic acid groups. All of the samples were put into three kg of sterile tin cans. Sensory and physical characteristics of the cheese samples were detected during the 1st, 7th, 14th, 21st, 28th, 35th, 42th storage days (+4°C). These parameteres were evaluated blindly according to the appearance, smell, taste and intensity within the range from 1 to 5 (1: worse, 2: bad, 3: moderate, 4: good, 5: better) by 10 panelists who taken the course of the cheese technology.

Stage 2: At the end of the storage period, 12% brine samples which include 1% tartaric acid, malic acid, fumaric acid or lactic acid were found suitable for stage 2 because of the sensory-physical structures were preserved and scored above 12 points by 10 panelists. In order to reveal the antimicrobial effect of suitable brine samples with organic acid, *Lactobacillus* spp., *Lactococcus* spp., mold-yeast counts were analyzed at +4°C during the storage conditions on 1st, 7th, 14th, 21st, 28th, 35th and 42th days. Besides, the effects of organic acids were investigated in cheese samples that were inoculated with *L. monocytogenes* (ATCC13932), obtained a final cell density of approximately 10^6 CFU/mL, in the fermentation stage.

Microbial Analysis

***L. monocytogenes inoculation in cheese-milk*:** The raw milk was analysed for the presence of *L. monocytogenes* by using ISO11290-1, 2:2017 method [19]. After the confirmation,

L. monocytogenes ATCC13932 was inoculated to the milk to obtain a final concentration of 6 log10 CFU/g. The white cheese was produced by standard tecnhnique with *L. monocytogenes* inoculated to raw milk. Cheese samples were divided into the five equal parts, and 1% tartaric, fumaric, lactic, malic acid were added to the brine except control group (brine without any supplement). All of the *L. monocytogenes* inoculated cheese groups were stored at +4ºC, and test samples were taken at 1st, 7th, 14th, 21st, 28th, 35th, 42th days.

Microbial analysis of cheese samples: Control and brine cheese samples which include 1% organic acid were taken 10 g portions for counting lactic acid bacteria, mold-yeast and *L. monocytogenes*. These 10 g portions were homogenized in stomacher (Seward stomacher, model 400 circulator) in 90 mL of 0.1% buffered peptone water for 2 min. Homogenated serial dilutions (10^{-1}-10^{-8}) were prepared. Dilutions were cultured on MRS Agar (Merck 1,10660) for *Lactobacillus* spp. isolation by using spreading plate technique. Plates were anaerobically incubated (Merck Anaerogen Kit) at 42°C for 48 h and light yellow colored colonies that grown on MRS agar were evaluated as *Lactobacillus* spp. colonies at the end of the incubation period [20].

Lactococcus spp. were cultured on M17 (Merck1.15108) Agar according to the spreading plate technique from the dilutions. Then, plates were (Merck Anaerogen Kit) incubated at 42°C for 24 h, anaerobically. Light yellow colored colonies that grown on M17 agar were evaluated as *Lactococcus* spp. colonies at the end of the incubation period [21]. Yeast- mold counts were cultured from the dilution of YGC agar (Merck 116000) according to spreading plate technique. After the application, plates were incubated at 25°C for 5 days and grown colonies were counted at the end of this period [22].

Listeria monocytogenes was cultured on Palcam Agar and Oxford Agar (Oxoid, CM 856) according to spreading plate technique from the dilutions and then incubated at 37°C for 48 h. At the end of this period, colonies which brown, black and green colours and centrally sunken were counted and evaluated [23].

Statistical Analysis

Analysis of data was made with IBM Statistical Package for Social Sciences (SPSS) software version 20.0 for Windows (SPSS Inc., Chicago, IL, USA). Comparison of 5 independent groups in terms of quantitative variables was performed by Duncan test. Confidence interval was 95% and differences associated with a P value less than 0.05 were considered as statistically significant.

RESULTS

Quality parameters of raw milk (120 L) were determined as dry substance (11.19), fat (3.25%), lactose (4.37%), protein (2.71%), freezing point (-0.519ºC), pH (6.8), somatic cell (123.000), total number of bacteria (5.08 log10 CFU/g) in present study. Physical, sensory and pH analysis results of the brine cheese solutions (12% (w/v) salt), which are included 1% (v/v) tartaric, malic, ascorbic, fumaric, lactic, acetic and succinic acids during the storage, shown in *Table 1*. The mean average pH values of cheese brines were also determined as 5.54, 5.25, 5.60, 5.21, 5.42, 5.38, 5.22 and 5.89 for control, tartaric, malic, ascorbic, fumaric, lactic, acetic and succinic acids groups during the storage days, respectively. The highest sensory scores were taken from the tartaric, fumaric, lactic and malic acids' brine solution. One percent of tartaric, fumaric, lactic and malic acid included brine cheese samples were chosen for the microbial analysis (stage 2) because of the highest sensory and good physical scores.

The difference between *Lactococcus* spp. count was not found significant (P>0.05) in tartaric acid added groups during the storage days. When compare the *Lactococcus* spp. counts in fumaric, lactic and malic acid included groups with the control group, average 0.5 log10 CFU/g decrease was found in mentioned groups. Fumaric and malic acids showed higher antimicrobial activities against *Lactococcus* spp. than the lactic acid (P<0.05) which shown in *Table 2*.

Table 1. *Sensory, physical and chemical analysis results (average of three different measurement results)*

Organic Acid Groups	pH Changes of the Organic Acid Groups During the Ripening Period							The Average Scores of the Sensory and Physical Properties Given by the Panelists
	1. day	7. day	14. day	21. day	28. day	35. day	42. day	
Control	6	5.11	5.72	5.59	5.49	5.48	5.43	16.9
Tartaric acid	4.24	5.28	5.64	5.52	5.42	5.38	5.27	14
Malic acid	5.74	5.23	5.83	5.72	5.65	5.59	5.49	13.7
Ascorbic acid	3.75	5.24	5.65	5.55	5.43	5.45	5.43	7.6
Fumaric acid	4.84	4.78	5.79	5.72	5.68	5.64	5.49	15.3
Lactic acid	4.71	4.94	5.73	5.63	5.62	5.57	5.51	16.5
Acetic acid	4.25	4.84	5.69	5.6	5.42	5.41	5.39	10.4
Succinic acid	6.84	5.89	5.89	5.79	5.67	5.61	5.58	9.9

Table 2. *Microorganism counts in the treatment samples during the storage*

Organic Acid Groups	*Changes of Laktococcus* spp., *Lactobacillus* spp. and Mold-yeast Counts of the Organic Acid Groups During the Ripening Period								The Average Statistical Results (D)	
	0	1	7	14	21	28	35	42		
Control	7.54±0.19^{a}	7.85±0.10^{a}	6.76±0.09^{bc}	6.74±0.07^{bc}	7.04±0.08^{b}	6.52±0.08^{c}	5.53±0.034^{d}	5.64±0.36^{d}	6.70±0.19^{a}	
Tartaric acid	7.54±0.19^{b}	8±0.15^{a}	6.77±0.06^{d}	7.20±0.33^{c}	6.59±0.14^{d}	6.48±0.18^{d}	5.85±0.11^{e}	4.71±0.14^{f}	6.64±0.09^{a}	
Fumaric acid	7.54±0.19^{a}	7.12±0.09^{b}	6.63±010^{c}	5.97±0.06^{d}	6.13±0.13^{d}	5.98±0.09^{d}	5.30±0.07^{e}	4.67±0.32^{f}	6.16±0.91^{c}	A
Lactic acid	7.54±0.19^{a}	729±0.04^{a}	7.24±0.27^{a}	6.78±0.2^{b}	6.25±0.14^{c}	6.36±0.16^{c}	4.88±0.10^{d}	4.22±0.09^{e}	6.32±0.14^{b}	
Malic acid	7.54±0.19^{a}	7.30±0.19^{b}	7.11±0.07^{b}	6.06±0.07^{c}	6.01±0.11^{c}	5.94±0.08^{c}	5.09±0.14^{d}	4.20±0.17^{e}	6.16±0.09^{c}	
Control	6.58±0.03^{a}	6.34±0.08^{b}	6.33±0.2^{b}	6.20±0.06^{b}	5.59±0.25^{c}	5.23±0.08^{d}	4.93±0.04^{e}	3.61±0.07^{f}	5.60±0.95^{a}	
Tartaric acid	6.58±0.03^{a}	6.74±0.23^{a}	6.41±0.21^{a}	5.96±0.08^{b}	5.54±0.47^{c}	5.37±0.28^{c}	4.69±0.14^{d}	2.92±0.11^{e}	5.53±0.21^{a}	
Fumaric acid	6.58±0.35^{a}	6±0.18^{b}	5.58±0.19^{c}	5.14±0.07^{d}	5.14±0.12^{d}	4.95±0.09^{d}	4.66±0.13^{e}	3.08±0.14^{f}	5.14±0.99^{b}	B
Lactic acid	6.58±0.03^{a}	6.20±0.06^{b}	6.23±0.2^{b}	5.34±0.12^{c}	5.17±0.14^{cd}	5.02±0.08^{de}	4.75±0.09^{e}	2.77±0.35^{f}	5.26±0.15^{b}	
Malic acid	6.58±0.03^{a}	6.24±0.11^{b}	5.98±0.04^{b}	5.37±0.15^{c}	5.33±0.34^{cd}	5.07±0.13^{d}	4.53±0.11^{e}	3.99±0.00^{f}	5.26±0.08^{c}	
Control	5.08±0.42^{a}	4.81±0.13^{ab}	4.34±0.35^{bc}	4.79±0.32^{ab}	4.74±0.05^{ab}	4.24±017^{c}	4.35±0.07^{bc}	4.45±0.16^{bc}	4.60±0.35^{a}	
Tartaric acid	5.08±0.42^{a}	4.60±0.32^{ab}	4.55±0.07^{ab}	4.36±0.46^{ab}	4.35±0.06^{ab}	3.23±0.18^{c}	3.89±0.57^{bc}	4.17±0.16^{ab}	4.28±0.59^{c}	
Fumaric acid	5.08±0.42^{a}	5.05±0.06^{ab}	4.53±0.83^{ab}	5.01±0.14^{ab}	4.55±0.12^{ab}	4.39±0.12^{b}	4.38±0.11^{b}	4.39±0.15^{b}	4.67±0.42^{a}	C
Lactic acid	5.08±0.42^{a}	5.08±0.09^{a}	4.54±0.15^{b}	4.45±0.41^{b}	4.45±0.20^{b}	4.15±0.12^{b}	4.24±0.06^{b}	4.36±0.08^{b}	4.54±0.39^{ab}	
Malic acid	5.08±0.42^{a}	5.07±0.13^{a}	4.40±0.06^{bc}	4.77±0.26^{ab}	4.30±0.69^{bc}	3.64±0.30^{d}	3.91±0.06^{cd}	4.07±0.14^{cd}	4.40±0.57^{bc}	

a. b. c. d. e. f P<0.05 is important in the same line; A: Laktococcus spp. B: Lactobacillus spp. C: Mold-yeast count D: Statistical data represent the significant importances according to the organic acid treatments

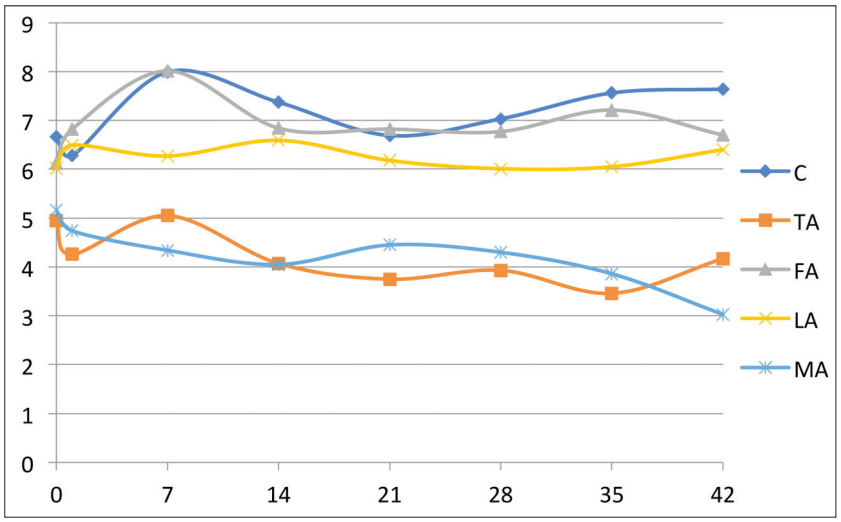

Fig 1. Antimicrobial effects of organic acids on *L. monocytogenes* during storage (C: Control. TA: Tartaric acid. FA: Fumaric acid. LA: Lactic acid. MA: Malic acid)

Lactococcus spp. counts decreased from 7.29 log10 CFU/g to 4.22 log10 CFU/g in lactic acid, from 7.12 log10 CFU/g to 4.67 log10 CFU/g in fumaric acid and from 7.30 log10 CFU/g to 4.20 log10 CFU/g in malic acid added samples at the end of the storage day (42th day), respectively.

The effects of organic acids on *Lactobacillus* spp. count were determined and there was not found significant difference (P>0.05) between the tartaric acid and control groups which shown in *Table 2*. Besides, averagely 0.4 log10 CFU/g decreasing were found in *Lactobacillus* spp. counts in fumaric acid, lactic acid and malic acid including groups. *Lactobacillus* spp. counts decreased from 6.20 log10 CFU/g to 2.77 log10 CFU/g in lactic acid, from 6.24 log10 CFU/g to 3.99 log10 CFU/g in malic acid and from 6.00 log10 CFU/g to 3.08 in fumaric acid included groups at the end of the storage (42th day).

The influence of organic acids on mold-yeast count were determined, and there also was not detected any significant difference between the fumaric acid and control groups (P>0.05). Averagely 0.5 log10 CFU/g decreasing were found in mold-yeast counts in tartaric acid, lactic acid and malic acid included groups which shown in *Table 2*. Mold-yeast counts decreased from 4.60 log10 CFU/g to 4.17 log10 CFU/g in tartaric acid, from 5.07 log10 CFU/g to

4.07 log10 CFU/g in malic acid and from 5.08 log10 CFU/g to 4.36 log10 CFU/g in lactic acid included group at the end of the storage (42nd day).

Listeria monocytogenes counts were found significant between the control and the organic acid brine samples (fumaric, tartaric, lactic and malic acid) during the storage days ($P<0.05$) which presented in *Fig 1*. When compared the *L. monocytogenes* counts between control and organic acid added brine sample groups during the storage days, decreased *L. monocytogenes* counts was detected as 0.22 log10 CFU/g, 0.90 log10 CFU/g, 2.91 log10 CFU/g, 2.95 log10 CFU/g in fumaric acid, lactic acid, malic acid and tartaric acid included brine samples, respectively.

DISCUSSION

Organic acids have been used as preservatives in the food sector for a long time because of the antibacterial properties. These organic acides, diffuse across the bacterial cell membranes, dissociate in the cell cytoplasm, reduce the intracellular pH and lead to cessation of growth or cell death [24]. Some organic acids can be produced by using lactic acid bacteria in fermented foods. Among those acetic acid has synergistic effect with lactic acid on the prevention of fungal growth [25]. Although acetic acid is described as more potent because of its higher pKa value, and level of dissociation inside bacteria cell, acetic, ascorbic and succinic acids disrupted the physical structure of the cheese samples in present study. Besides, sensory characteristics of the brine cheese samples which included acetic, ascorbic and succinic acid were evaluated by 10 panalists, and they did not get enough points for the second stage of the study. Furthermore, the lowest score was detected as 7.6 in ascorbic acid group by panalists. Interestingly, brine samples which contained succinic acid had higher pH levels (pH: 6.84) when compared to control group (pH: 6). There was not found any literature about the addition of organic acids into the brine cheese samples. The decomposition of the physical and sensory structure of the brine samples could be due to quicker decrease in the pH levels of the brine (3.75) in the first experiment day.

The count and the ratio of lactic acid bacteria is an important factor in the fermented food because it forms quality of fermentation, and effets the flavor and aroma of cheese samples. At the beginning of the fermentation, cheese samples, which included organic acids, the *Lactococcus* spp. and *Lactobacillus* spp. counts were determined as 7.54 log10 CFU/g and 6.58 log10 CFU/g, respectively. While the *Lactococcus* spp. counts were similar between the control and the organic acid brine sample groups, an important decrease was observed in the *Lactobacillus* spp. counts during the storage days. It was not found in the literature about counts or ratio of bacteria population in brine cheese samples processed with organic acids. It was suggested that antimicrobial effects of organic acids may be more efficient on *Lactobacillus* spp. than *Lactococcus* spp. because of having different membrane permeability or resistance [26].

Another important factor of cheese production and preservation is mold-yeast count. Shokri et al.[27] evaluated antifungal activity of organic acid treatments on *Trichophyton mentagrophytes* var. mentagrophytes, *Candida albicans*, *Aspergillus fumigatus* and *Malassezia furfur*. The results of the study showed that citric acid has more fungistatic and fungicidal activities than tartaric acid. Besides, the antifungal activity of the acid mixture was similar with citric acid but higher than tartaric acid alone. In contrary, tartaric acid had higher antifungal activity than the other groups ($P<0.05$) in our study. The main target of organic acids and its relatives are cell wall and membrane proteins. The hyphae wall of filamentous fungi contains less protein than the cell wall of yeast. For this purpose, the researcher explained this may be related to the different structures of the fungal cell walls [27].

Recently, there have been performed some scientific researches about the disinfectant or inhibition potentials of organic acids in various areas. One of those is the use of organic acids against food pathogens. Yıldırım et al.[28] showed that lactic acid bacteria, probiotic bacteria and their metabolites can inhibit *L. monocytogenes* in Turkish white cheese at maturation period. In bacterial fermentation due to the production of organic acids, such as lactic, acetic or propionic acids which play a role in the biofermentation of fermented food. Organic acids also include a large spectrum of compounds and many of them are known to be effective antifungal metabolites [29]. In another study, Swaranandam et al.[30] also researched the effectiveness of some organic acids (such as malic acid) in nisin-incorporated soy protein film against *L. monocytogenes*, *E. coli* O157:H7, *Salmonella gaminara*. Incorporated soy protein film was found the fewest survivors of *L. monocytogenes*, *S. gaminara*, and *E. coli* O157:H7 (5.5, 3.0 and 6.8 log10 CFU/mL, respectively). Pintado et al.[31] also defined the effects of nisin, natamycin and malic acids which were incorporated with whey protein films, on inhibitory activity against *L. monocytogenes*, *Penicillium commune* and *P. chrysogenum*. Another study was performed about using of organic acids for decreasing of *L. monocytogenes* counts in biofilms. It was determined that counts of *L. monocytogenes* decreased due to lactic acid washing solution on PVC and stainless steel surfaces. In addition, *S.* Typhimurium, *L.monocytogenes* and *E. coli* O157:H7 were also reduced to the below detection limit (1.48 log10) by using lactic acid (2%) and steam applications [32]. Brown et al.[33] (2018) also investigated the inhibitory effect of acetic acid, citric acid and lactic acid supplementation to the brine of cheese samples aganist *L. monocytogenes* (6 log CFU/m) during the storage period similar with the present study. This result revealed rapid inhibition against *L. monocytogenes* in acetic acid brine samples, and it required non-practical volumes. In

current study, inhibition time was found too long in citric acid added samples compared to lactic acid against *L. monocytogenes* in normal volumes. However, there are very limited numbers of study about the using of organic acids in a dairy product, *L. monocytogenes*. Counts in brine cheese sample groups were affected from organic acids, significantly ($P<0.05$) in the present study *(Fig. 1)*. Initial *L. monocytogenes* counts averagely decreased from 6.67 log10 CFU/g to 0.22 log10 CFU/g, 0.90 log10 CFU/g, 2.91 log10 CFU/g and 2.95 log10 CFU/g in fumaric, lactic, malic and tartaric acid included brine cheese compared with control group, respectively. Besides, tartaric which is the one of the important acid to suppress *L. monocytogenes* counts, and to decrease the *L. monocytogenes* counts from 6.67 log10 CFU/g to 4.95 log10 CFU/g at the 0th day and to 4.17 log10 CFU/g on the 42nd day. Our study also demonstrated the suppressing effects of organic acids on *L. monocytogenes* counts in brine cheese samples, and current results are in aggrement with previous studies. Although, succinic, acetic and formic acids caused structural defects in the brine cheese samples, tartaric, malic, lactic and fumaric acid did not cause physical and sensory deficits. It is also thought that the addition of malic and tartaric acid in brine cheese samples could be used effectively in the control of *L. monocytogenes*.

In conclusion, this research demonstrated useful effects of some organic acids against moulds, yeasts and *L. monocytogenes* contaminations. Morever, supplementation of organic acids in brine solutions has a novel method to enhance the microbial safety and quality of white cheese.

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