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Effects of Different Concentration of Nisin, Lysozyme, and Chitosan on The Changes of Microorganism Profile in Produced Çiğ Köfte (Turkish Traditional Meat Product; Raw Meatball) During The Production Stage

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Abstract Consumption of Çiğ Köfte as an uncooked meat product has some health risks for public health. Therefore, this study aimed to determine the effects of some natural antimicrobials such as nisin, lysozyme and chitosan on the bacterial profile in Çiğ köfte and also to determine the correlation between alterations in bacteria profile and shelf-life. Çiğ Köfte samples were divided into 11 groups and individually mixed with the indicated amount of chemical agents for 2 minutes (control, 25 µg nisin/g, 50 µg nisin/g, 100 µg nisin/g, 200 µg lysozyme/g, 300 µg lysozyme/g, 100 µg nisin/g +300 µg lysozyme/g, 0.05% chitosan, 0.1% chitosan, 0.5% chitosan, 100 µg nisin/g +300 µg lysozyme/g + 0.5% chitosan). Chemical and microbiological analyses were performed in samples at 24, 36, 48, and 72nd hrs after production. There was significant decline in the numbers of Total aerob mesophile microorganisms (TAB), *E. coli*, Coliform, *Enterobacteriaceae*, *Enterococcus* spp., yeast-mould, Sulphite reducing bacteria, *Staphylococcus-micrococcus*, *Staphylococcus aureus* (*S. aureus*), *Pseudomonas* spp. in the chemical agent groups. The results of this study indicate that nisin, lysozyme, and chitosan can be used in order to provide hijyenic stability in the Çiğ köfte.

Keywords *Çiğ köfte, Hygienic quality, Nisin, Lysozyme, Chitosan.*

Çiğ Köftelere Üretim Sürecinde Farklı Yoğunluklarda Nisin, Lisozim ve Kitosan İlavesinin Bazı Mikroorganizma Profilindeki Değişim ve Bu Değişimin Raf Ömrü Üzerine Etkisi

Özet Isı işlem görmemiş biri ürün olarak çiğ köftenin tüketimi halk sağlığı açısından risklidir. Bu çalışmada nisin, lisozim ve kitosan gibi doğal bazı antimikrobiyel maddelerin kullanılarak çiğ köftenin mikrobiyel bakteri profili ve raf ömrü üzerine etkisinin belirlenilmesi amaçlandı. Çiğ köfte örnekleri 11 gruba bölünerek aşağıdaki gruplarda miktarları belirtilen ajanlar ile yaklaşık 2 dakika yoğruldu (Kontrol, 25 µg nisin/g, 50 µg nisin/g, 100 µg nisin/g, 200 µg lisozim/g, 300 µg lisozim/g, 100 µg nisin /g +300 µg lisozim/g, %0.05 kitosan, %0.1 kitosan, %0.5 kitosan, 100 µg nisin/g +300 µg lisozim/g +%0.5 kitosan). Mikrobiyel ve kimyasal analizler üretimden sonra 24.,36.,48.ve 72.saatte tekrarlandı. Total aerob mezofil bakteri (TAB), *E. coli*, Coliform, *Enterobacteriaceae*, *Enterococcus* spp., maya ve küf, Sülfit indirgeyen bakteri, *Staphylococcus-micrococcus*, *Staphylococcus aureus* (*S. aureus*) ve *Pseudomonas* spp. sayısında önemli düzeyde azalma saptandı. Bu sonuçlar, nisin, lisozim ve kitosanın çiğ köftede hijyenik stabiliteyi sağlamada başarılı olduğunu göstermektedir.

Anahtar sözcükler *Çiğ köfte, Hijyenik kalite, Nisin, Lisozim, Kitosan.*

1. INTRODUCTION

Çiğ köfte being an indigenious meat product is mainly comsumed in Turkey as well as in the the European countries where Turkish immirgants live. Besides there is no a standart about the production tecnique, microbial and chemical characteristics of Çiğ köfte. Elmali and Yaman [1] stated that ground beef meat without fat, burghol (parboiled cracked wheat), tomatoes or pepper pure, garlic, onion, parsly, olive oil, water, salt, and mixture of herbs-spices constitue of the ingredients of Çiğ köfte. Depending on preservation of the product in inconvenient temperatures and the spoilage caused high initial contamination are the important points have to be taken care attention in respect of human health risk and economic loss. The production method of traditional Çig köfte is as below: All ingredients are mixed and kneaded with bare hands as dough until having required texture. The microbiological quality of production is directly related with microbiological quality of raw meat and other ingredients, personal hygiene and any contamination during the process. Food poisoning cases can ocur related to consumption of Çiğ köfte in Turkey.

Nisin produced by *Lactococcus lactis* subsp. *lactis* is an important bacteriocisin used frequently in biotechnological studies in recent years [2]. Nisin carries out its efficiency in two different methods. The first one is destroying the stabilization of bacteria cell by forming permeable pores on cell walls, and the other one is preventing the cell wall sythesis lipid II molecular to combine with peptidoglican chain [3-5]. Nisin is used in very different foods in variaty amounts [6,7]. On the other hand, there isn't a legal regulation about Çiğ köfte in the World.

Lysozyme is an inhibitor agent commonly used in food industry in recent years like nisin. Particularly lysozyme and nisin combination has more efficiency when they are used together compared to their seperate usage [8]. Lysozyme shows its efficiency by breaking off glycosidic bonds between N-acetylglucosamine and N-acetylmuramic acid of peptidoglikan cell walls [9]. Lysozyme is more effective on Gram positive bacteria compared to Gram negative bacteria [10]. Especially lysozyme is very effective on bacteria when used combination with nisin [11]. Especially it is stated that 3:1 (one third of) lysozyme-nisin rate is a very effective dose on inhibiting the microorganisms causing meat spoiling [12].

Chitosan is a polysaccharide obtained by deasetilation of chitin in alkali environment. Specifically the chitin found in shells of marine crustaceans is the main material of chitosan [13-15]. Chitosan is an antimicrobial material on which is studied [16-18] in meat and meat products in recent years. It is stated that the usage of increasing dosages (especially 1% and over) of chitosan effects the indigineous smell and colour of product negatively. In addition to this, it is stated that the chitosan solution prepared in 1% acetic acid solution has more antimicrobial character [18].

2. MATERIALS AND METHODS

2.1. The Production of Çiğ Köfte

The Çiğ köfte samples used in the research were prepared daily in Antakya/Hatay province.

2.2. Formulation:

The ingredients; 500 gr fresh meat without nerve tissue, 1000 gr burghol, 10 gr black pepper, 50 gr onion, 30 gr salt, 200 gr green onion, 75 gr parsley, one grated tomato, 30 gr paprika paste and tomato paste.

2.3. Production technique:

Fatless meat was put in a small amounts on fine burghol (generally at the rate of 1:2) and kneaded until having a homogeneous mixture and required texture by adding different kinds of spices and the other ingredients.

Çiğ köfte samples were divided into 11 groups and kneaded for 2 minutes with chemical agents stated below. Then, small amounts of çiğ köfte mixtures were shaped in palm and were ready to serve. Samples were analysed in terms of Total aerobe mesophile bacteria, *E. coli*, Coliform, *Enterobacteriaceae*, *Enterococcus*, Yeast-mould, Sulphite reducing bacteria, *Staphycoccus-micrococcus*, *S.aureus*, and *Pseudomonas* spp. at 24^{th}, 36^{th}, 48^{th} and 72^{nd} hours respectively. (After preparing Çiğ köfte mixture initial microbial load was determined before adding antimicrobial agents.) Çiğ köfte samples were

preserved in sterilized, closed aluminum foil vessels at refrigerator temperature (+4ºC) up to 72 hours after production.

The groups and amount of antimicrobial agents are given in detail below. While nisin (VALISIN^R) and lysozyme (Sigma) were melted and prepared in water, chitosan (Aldrich) was prepared in acetic acid (Merck).

Group 1: Control (without supplementing any natural anitmicrobials)
Group 2: 25 µg nisin/g
Group 3: 50 µg nisin/g
Group 4: 100 µg nisin/g
Group 5: 200 µg lysozyme/g
Group 6: 300 µg lysozyme/g
Group 7: 100 µg nisin /g +300 µg lysozyme/g
Group 8: 0.05% chitosan
Group 9: 0.1 % chitosan
Group 10: 0.5% chitosan
Group 11: 100 µg nisin /g +300 µg lysozyme /g + 0.5 % chitosan
Eight repetitions were conducted at different times.

2.4. Microbiological analysis;

The analysis of Total aerobe mesophile bacteria, *E. coli*, Coliform, *Enterobacteriaceae*, *Enterococcus*, Yeast-mould, Sulphite reducing bacteria, *Staphycoccus-micrococcus*, *S.aureus*, and *Pseudomonas* spp. were carried out in according to Harrigan [19].

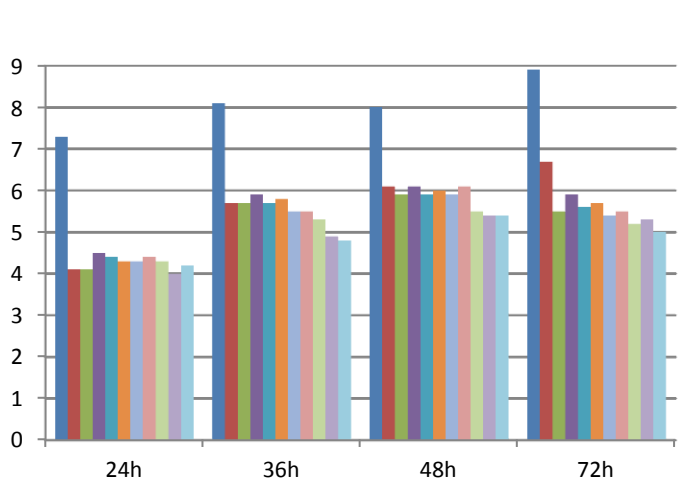
2.5. Statistical analysis;

One-way Anova test conducted for every parameter using, and Duncan test for determination of differences amoung the groups.

3. RESULTS

The results of the effects of nisin, lysozyme and chitosan individually and combination of these anti-microbial agents in terms of time and bacterial reduction in the numbers of bacteria groups in Çiğ köfte samples are summarized in Graphics 1-10.

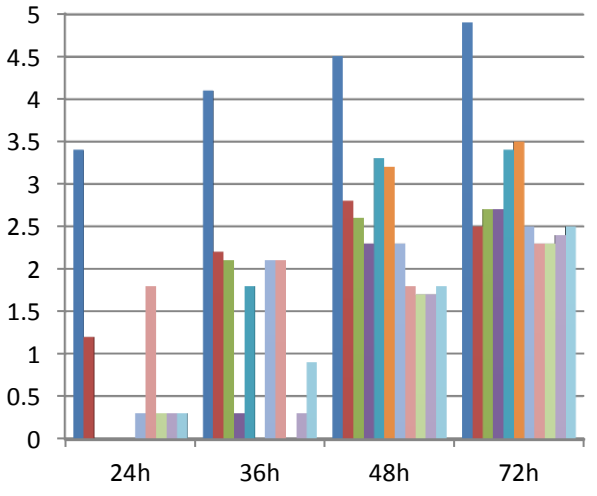
Graphic 1. Level of Total aerob mesophile bacteria (TAB) (cfu/log)



	24h	36h	48h	72h
G1; Control	7.3	8.1	8	8.9
G2;25 µg nisin /g	4.1	5.7	6.1	6.7
G3;50 µg nisin /g	4.1	5.7	5.9	5.5
G4;100 µg nisin /g	4.5	5.9	6.1	5.9
G5; 200 µg lysozyme /g	4.4	5.7	5.9	5.6
G6; 300 µg lysozyme /g	4.3	5.8	6	5.7
G7; 100 µg nisin /g +300 µg lysozyme /g	4.3	5.5	5.9	5.4
G8; 0.05% chitosan	4.4	5.5	6.1	5.5
G9;0.1% chitosan	4.3	5.3	5.5	5.2
G10; 0.5% chitosan	4	4.9	5.4	5.3
G11; 100 µg nisin /g +300 µg lysozyme /g + 0.5% chitosan	4.2	4.8	5.4	5

Significant changes were determined between groups at 24th and 72nd hours (P<0,001).

Graphic 2. Level of *E. coli* (cfu/log)

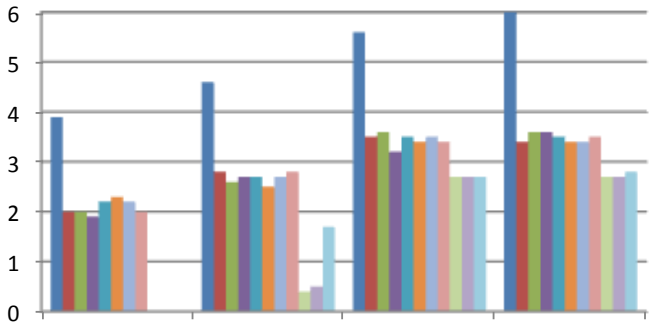


	24h	36h	48h	72h
G1; Control	3.4	4.1	4.5	4.9
G2; 25 µg nisin /g	1.2	2.2	2.8	2.5
G3; 50 µg nisin /g	0	2.1	2.6	2.7
G4; 100 µg nisin /g	0	0.3	2.3	2.7
G5; 200 µg lysozyme /g	0	1.8	3.3	3.4
G6; 300 µg lysozyme /g	0	0	3.2	3.5
G7; 100 µg nisin /g +300 µg lysozyme /g	0.3	2.1	2.3	2.5
G8; 0.05% chitosan	1.8	2.1	1.8	2.3
G9; 0.1% chitosan	0.3	0	1.7	2.3
G10; 0.5% chitosan	0.3	0.3	1.7	2.4
G11; 100 µg nisin /g +300 µg lysozyme /g + 0.5% chitosan	0.3	0.9	1.8	2.5

*O: Not Detected

Signifcant changes were determined between groups at 24th-72nd hours (P<0,001).

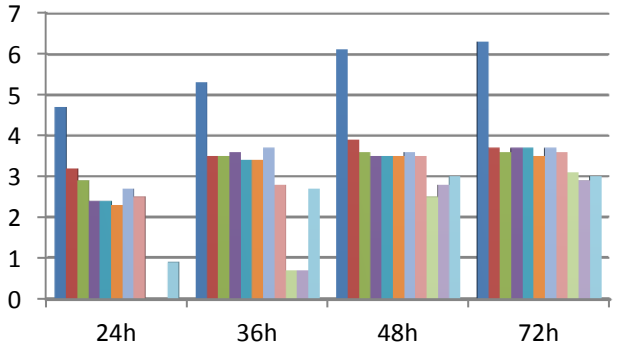
Graphic 3. Level of Coliform (cfu/log)



	24h	36h	48h	72h
G1; Control	3,9	4,6	5,6	6
G2; 25 µg nisin /g	2	2,8	3,5	3,4
G3; 50 µg nisin /g	2	2,6	3,6	3,6
G4; 100 µg nisin /g	1,9	2,7	3,2	3,6
G5; 200 µg lysozyme /g	2,2	2,7	3,5	3,5
G6; 300 µg lysozyme /g	2,3	2,5	3,4	3,4
G7; 100 µg nisin /g +300 µg lysozyme /g	2,2	2,7	3,5	3,4
G8; 0.05% chitosan	2	2,8	3,4	3,5
G9; 0.1% chitosan	0	0,4	2,7	2,7
G10; 0.5% chitosan	0	0,5	2,7	2,7
G11; 100 µg nisin /g +300 µg lysozyme /g + 0.5% chitosan	0	1,7	2,7	2,8

*O: Not Detected
Signifcant changes were determined between groups at 24th-72nd hours (P<0,001).

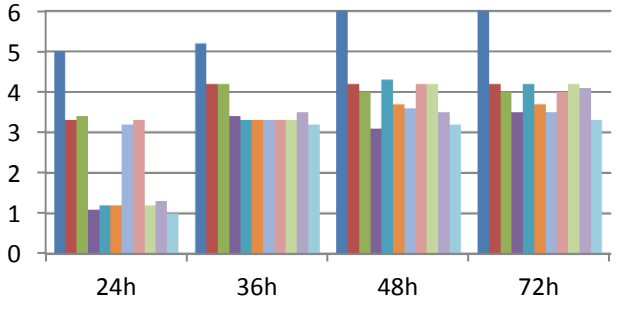
Graphic 4. Level of *Enterobacteriaceae* (cfu/log)



	24h	36h	48h	72h
G1; Control	4.7	5.3	6.1	6.3
G2; 25 µg nisin /g	3.2	3.5	3.9	3.7
G3; 50 µg nisin /g	2.9	3.5	3.6	3.6
G4; 100 µg nisin /g	2.4	3.6	3.5	3.7
G5; 200 µg lysozyme /g	2.4	3.4	3.5	3.7
G6; 300 µg lysozyme /g	2.3	3.4	3.5	3.5
G7; 100 µg nisin /g +300 µg lysozyme /g	2.7	3.7	3.6	3.7
G8; 0.05% chitosan	2.5	2.8	3.5	3.6
G9; 0.1% chitosan	0	0.7	2.5	3.1
G10; 0.5% chitosan	0	0.7	2.8	2.9
G11; 100 µg nisin /g +300 µg lysozyme /g + 0.5% chitosan	0.9	2.7	3	3

*O: Not Detected
Signifcant changes were determined between groups at 24th-72nd hours (P<0,001).

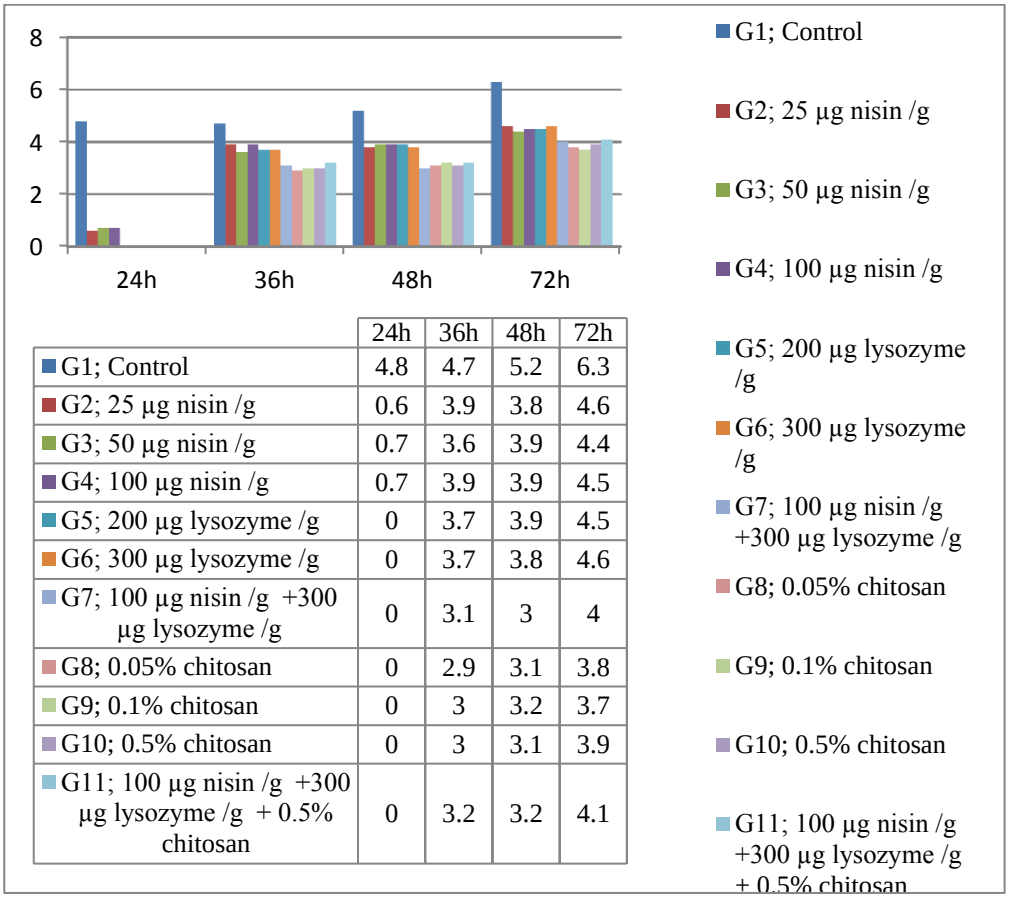
Graphic 5. Level of *Enterococcus* spp. (cfu/log)



	24h	36h	48h	72h
G1; Control	5	5.2	6	6
G2; 25 µg nisin /g	3.3	4.2	4.2	4.2
G3; 50 µg nisin /g	3.4	4.2	4	4
G4; 100 µg nisin /g	1.1	3.4	3.1	3.5
G5; 200 µg lysozyme /g	1.2	3.3	4.3	4.2
G6; 300 µg lysozyme /g	1.2	3.3	3.7	3.7
G7; 100 µg nisin /g +300 µg lysozyme /g	3.2	3.3	3.6	3.5
G8; 0.05% chitosan	3.3	3.3	4.2	4
G9; 0.1% chitosan	1.2	3.3	4.2	4.2
G10; 0.5% chitosan	1.3	3.5	3.5	4.1
G11; 100 µg nisin /g +300 µg lysozyme /g + 0.5% chitosan	1	3.2	3.2	3.3

Significant changes were determined between groups at 24th hour (P<0,001), 36th hour (P<0,05), and 48th-72nd hours (P<0,01).

Graphic 6. Level of Yeast and Mould (cfu/log)

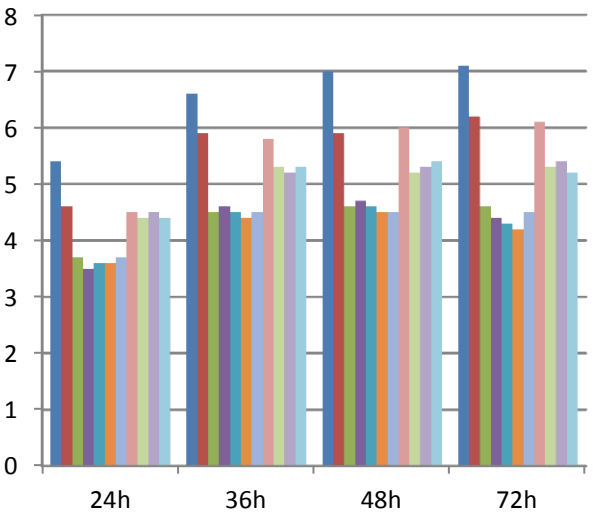


	24h	36h	48h	72h
G1; Control	4.8	4.7	5.2	6.3
G2; 25 µg nisin /g	0.6	3.9	3.8	4.6
G3; 50 µg nisin /g	0.7	3.6	3.9	4.4
G4; 100 µg nisin /g	0.7	3.9	3.9	4.5
G5; 200 µg lysozyme /g	0	3.7	3.9	4.5
G6; 300 µg lysozyme /g	0	3.7	3.8	4.6
G7; 100 µg nisin /g +300 µg lysozyme /g	0	3.1	3	4
G8; 0.05% chitosan	0	2.9	3.1	3.8
G9; 0.1% chitosan	0	3	3.2	3.7
G10; 0.5% chitosan	0	3	3.1	3.9
G11; 100 µg nisin /g +300 µg lysozyme /g + 0.5% chitosan	0	3.2	3.2	4.1

*O: Not Detected

Signifcant changes were determined between groups at 24th-72nd hours (P<0,001).

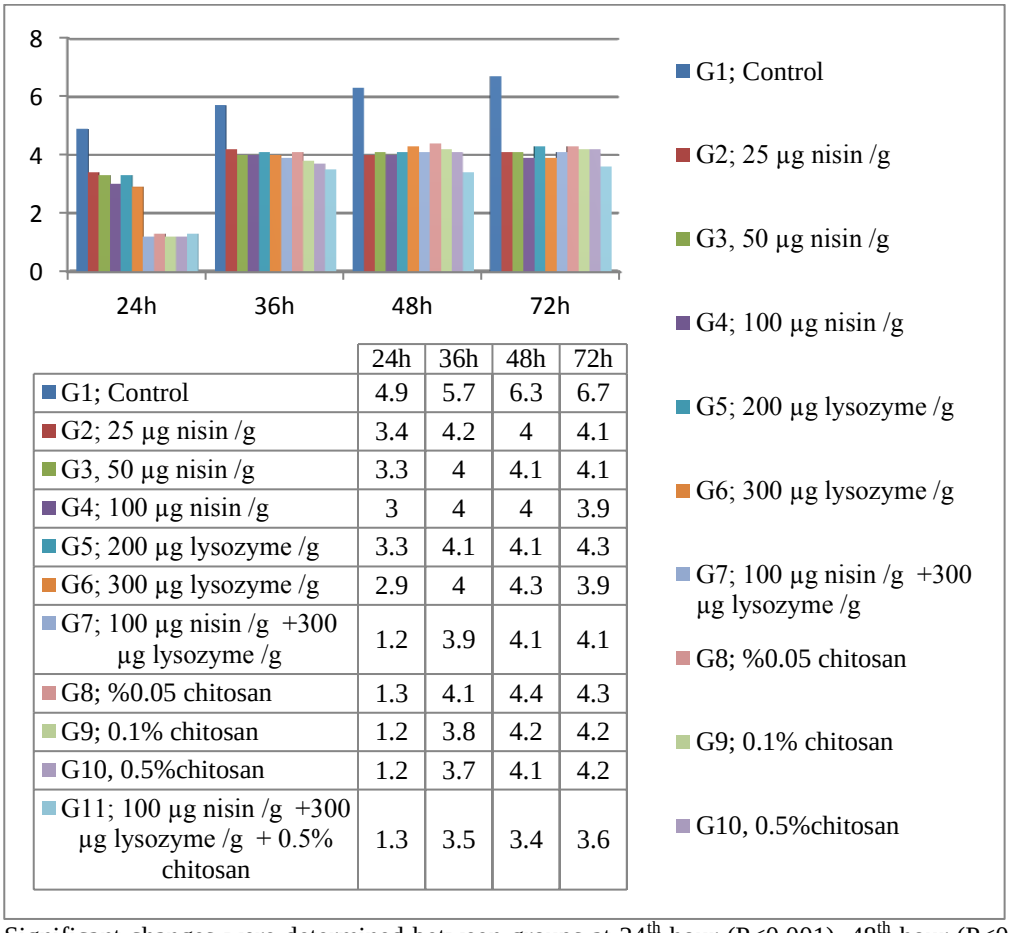
Graphic 7. Level of Sulphite reducing bacteria (cfu/log)



	24h	36h	48h	72h
G1; Control	5.4	6.6	7	7.1
G2; 25 µg nisin /g	4.6	5.9	5.9	6.2
G3; 50 µg nisin /g	3.7	4.5	4.6	4.6
G4; 100 µg nisin /g	3.5	4.6	4.7	4.4
G5; 200 µg lysozyme /g	3.6	4.5	4.6	4.3
G6; 300 µg lysozyme /g	3.6	4.4	4.5	4.2
G7; 100 µg nisin /g +300 µg lysozyme /g	3.7	4.5	4.5	4.5
G8; 0.05% chitosan	4.5	5.8	6	6.1
G9; 0.1% chitosan	4.4	5.3	5.2	5.3
G10; 0.5% chitosan	4.5	5.2	5.3	5.4
G11; 100 µg nisin /g +300 µg lysozyme /g + 0.5% chitosan	4.4	5.3	5.4	5.2

Any changes weren't determined (P>0,05) between groups at 24th-36th hours. Significant changes were determined between groups at 48th (P<0,05), and 72nd (P<0,01) hour.

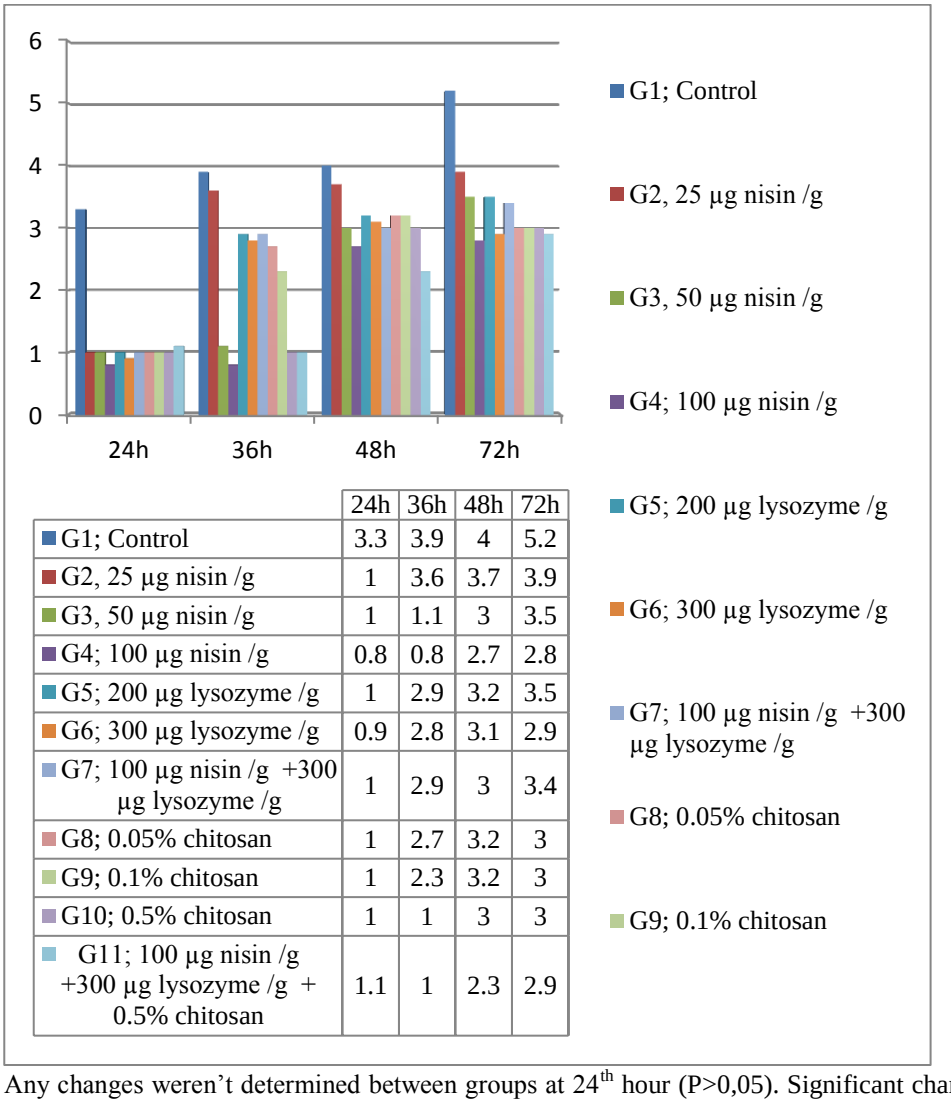
Graphic 8. Level of *Staphycoccus-micrococcus* (cfu/log)



	24h	36h	48h	72h
G1; Control	4.9	5.7	6.3	6.7
G2; 25 µg nisin /g	3.4	4.2	4	4.1
G3, 50 µg nisin /g	3.3	4	4.1	4.1
G4; 100 µg nisin /g	3	4	4	3.9
G5; 200 µg lysozyme /g	3.3	4.1	4.1	4.3
G6; 300 µg lysozyme /g	2.9	4	4.3	3.9
G7; 100 µg nisin /g +300 µg lysozyme /g	1.2	3.9	4.1	4.1
G8; %0.05 chitosan	1.3	4.1	4.4	4.3
G9; 0.1% chitosan	1.2	3.8	4.2	4.2
G10, 0.5%chitosan	1.2	3.7	4.1	4.2
G11; 100 µg nisin /g +300 µg lysozyme /g + 0.5% chitosan	1.3	3.5	3.4	3.6

Significant changes were determined between groups at 24th hour (P<0,001), 48th hour (P<0,01), and 72nd hour (P<0,001).

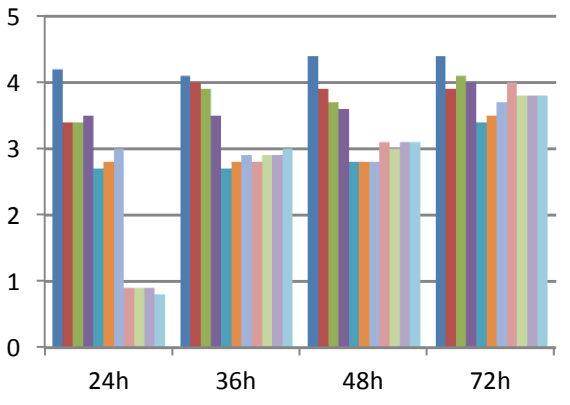
Graphic 9. Level of *S. aureus* (cfu/log)



	24h	36h	48h	72h
G1; Control	3.3	3.9	4	5.2
G2, 25 µg nisin /g	1	3.6	3.7	3.9
G3, 50 µg nisin /g	1	1.1	3	3.5
G4; 100 µg nisin /g	0.8	0.8	2.7	2.8
G5; 200 µg lysozyme /g	1	2.9	3.2	3.5
G6; 300 µg lysozyme /g	0.9	2.8	3.1	2.9
G7; 100 µg nisin /g +300 µg lysozyme /g	1	2.9	3	3.4
G8; 0.05% chitosan	1	2.7	3.2	3
G9; 0.1% chitosan	1	2.3	3.2	3
G10; 0.5% chitosan	1	1	3	3
G11; 100 µg nisin /g +300 µg lysozyme /g + 0.5% chitosan	1.1	1	2.3	2.9

Any changes weren't determined between groups at 24th hour (P>0,05). Significant changes were determined between groups at 36th hour (P<0,001), 48th hour (P <0,05), and 72nd hour (P <0,001).

Graphic 10. Level of *Pseudomanas* spp. (cfu/log)



	24h	36h	48h	72h
G1; Control	4.2	4.1	4.4	4.4
G2; 25 µg nisin /g	3.4	4	3.9	3.9
G3; 50 µg nisin /g	3.4	3.9	3.7	4.1
G4; 100 µg nisin /g	3.5	3.5	3.6	4
G5; 200 µg lysozyme /g	2.7	2.7	2.8	3.4
G6; 300 µg lysozyme /g	2.8	2.8	2.8	3.5
G7, 100 µg nisin /g +300 µg lysozyme /g	3	2.9	2.8	3.7
G8, 0.05% chitosan	0.9	2.8	3.1	4
G9; 0.1% chitosan	0.9	2.9	3	3.8
G10; 0.5% chitosan	0.9	2.9	3.1	3.8
G11; 100 µg nisin /g +300 µg lysozyme /g + 0.5% chitosan	0.8	3	3.1	3.8

Significant changes were determined between groups at 24th-36th hours (P<0,001), and 48th hour (P<0,01). Any changes weren't determined between groups at 72nd hour (P>0,05).

The initial pH value of Çiğ köfte was defined as 6,44±0,13 the average rate of 8 repetitions. pH values didn't demonstrate a significant difference among groups in the study (P>0.05). It was showed that the pH values changed between the values 6.41±0.10 and 6.99±0.11 at 24th – 72nd hours.

The initial salt content percentages of Çiğ köfte were confirmed as 3,54±0,35 the average rate of 8 repetitions. The salt percentage didn't demonstrate a remarkable difference among groups in the study (P>0.05). It was determined that the content of salt percentages changed between 3.54±0.34 and 3.68±0.37 values at 24th- 72nd hours.

4. DISCUSSION AND CONCLUSION

The studies conducted on microbial load of Cig köfte in Turkey [1,20-22] indicated that the microorganism level was very high. The studies aimed improving the hygenic quality of Ciğ köfte with different tecniques [23-25] have also drawed attention in recent years. On the other hand, Ciğ köfte is

consumed widespreadly in Turkey, however it is a main untreated production having a microorganism profile risky for human health.

In the present study, the bacterial reduction was observed mostly at the first 24th hour in G-10, at 36th hour in G-11, at 48th hour in G-10 and G-11 and at 72nd hour in G-11 with respect to TAB. In other saying, maximum bacterial reduction was seen in 24-72 hours in the group generally included in 0.5% chitosan and 100 µg nisin /g +300 µg lysozyme/g + 0.5% chitosan. Approximately, 3-4 log level differences was determined when all groups were compared with control groups for all hours. Sagoo et al. [26] reported that pig minced meat preserved at 4 °C and contained 0.3-0.6% chitosan caused 3 log reduction at TAB level at first day. They reported that TAB level of control group was higher than other groups in the preservation period lasted for 18 days. The findings of Sagoo et al. [26] showed consistency with our study findings. Darmadji and Izumimoto [14] also reported that the total aerobe mesophile microorgansim level reduces 1-2 log kob/g minced meat contain 1% chitosan after 10 days.

It was observed that bacterial reduction occured mostly in the first 24 hours in G3, G4, G5 and G6 groups, at 36th hour in G6 and G9 groups, at 48th hour in G9 and G10 groups, at 72nd hour in G8 and G9 groups with respect to *E. coli*. In other saying, it was observed that maximum bacterial reduction was carried out by the groups included in 50 µg nisin/g, 100 µg nisin/g, 200 µg lysozyme/g, 300 µg lysozyme/g, 0.05% chitosan, 0.1% chitosan and 0.5% chitosan. Gerasimenko et al. [27] reported that the chitosan having low molecular weight had showed inhibitoric effect on *E. coli.* The study findings of Gerasimenko et al. [27] showed similarity with our study. The studies [28, 29] indicated that 0,5% chitosan acetate application changes the exterior membrane of *E. coli* while it did not affect the interior membrane. In addition, the chemical composition (mixture) of production also affects the bacterial effect level of chitosan. The protein rate chitosan level, and also combination of low pH level in the environment increased the efficency of chitosan [30]. Darmadji and Izumimoto [14] reported that low concentration level of chitosan (0,2%, 0,5%) did not indicate any inhibitoric effect on bacterial growth. These findings was not resemble to our study findings. This difference may be associated with the matrix difference of the production studied on. Low molecular weight, low pH and preservation in cold environment are the important combinations increasing the efficency of chitosan in addition to the matrix of Çiğ köfte's difference.

It was observed that the bacterial reduction mostly occurred in G11 group for all time groups with respect to *Enterococcus* spp. level. G11 group contained 100 µg nisin /g +300 µg lysozyme /g + 0.5 % chitosan.

It was observed that bacterial reduction had occured in G5, G6, G7, G8, G9, G10 and G11 groups in the first at 24 hours, in G8 group at 36th hour, in G7 group at 48th hour and in G9 group at 72nd hour with respect to yeast and mold. In other way, it was observed that bacterial reduction result in the group included in 200 µg lysozyme /g, 300 µg lysozyme/g, 100 µg nisin/g +300 µg lysozyme/g, 0.05% chitosan, 0.1% chitosan, 0.5% chitosan, 100 µg nisin/g +300 µg lysozyme/g + 0.5% chitosan. Saggo et al. [26] reported that yeast-mold level of sausages applied by chitosan solution reduced 1-3 log appromaxitely. The study findings of Sagoo et al. [26] showed consistency with our study findings.

It was observed that bacterial reduction was seen in G4 group in the first 24 hours, in G6 group at 36th hour, in G9 group at 48th hour and in G6 group at 72nd hour with respect to sulfite reducing anaerob bacteria level.

It was observed that bacterial reduction occured in G4 group at 24th, 36th and 72^{n}d hours, in G11 group at 48th hour with respect to *S. aureus* level. On the other hand, it was observed the bacterial reduction in the group included in 100 µg nisin/g, 100 µg nisin/g +300 µg lysozyme/g +0.5 % chitosan. Wang [31] reported that it was needed 1%- 1,5% chitosan for the inhibition of *S. aureus.* Liu et al. [29] reported that it didn't reduce *S. aureus* level remarkably in the study they conducted.

It was observed that bacterial reduction occured in G11 group in the first 24 hours, in G5 group at 36th hour, in G5, G6, G7 groups at 48th hour and in G5 group at 72nd hour. In other way, it was observed in the group including in 200 µg lysozyme /g, 300 µg lysozyme/g, 100 µg nisin/g +300 µg lysozyme/g ve 100 µg nisin/g +300 µg lysozyme/g + 0.5 % chitosan. Darmadji and Izumimuto [14] reported that 0,5 – 1,0 % concentrations of chitosan had reduced 1-2 log in the *Pseudomonas* spp. in Çiğ köfte.

It is suggested that Çiğ köfte could be consumed at the first 24 hour and preserved at 4°C' in pursuit of production methods according to the first 24 hour part of study findings. As a result of this study, it was deternined that nisin, lysozyme and chitosan, the chemical agents, inhibited the level of some

undesirable pathogen microorganisms in the matrix of Çiğ köfte being a traditional nonheat-treated meat product. It was confirmed that inhibition of these chemical agents on some pathogen microorganisms prolong shelf life of Çiğ köfte.

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