Ankara Üniv Vet Fak Derg, **66**, 281-288, 2019
DOI: 10.33988/auvfd.475104

Presence of *Listeria* species in ready-made meatballs offered by sale under freezing or cooling preservation

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Received date: 26.10.2018- Accepted date: 26.04.2019

Abstract: This study was conducted to detect contamination level of *Listeria* species in ready-made meatballs kinds that are stored under frozen or cooled conditions. In isolations and identifications of *Listeria* species from the samples, method approved and suggested by USDA/FSIS (United States Department of Agriculture/Food Safety and Inspection Service) was used. The strains that were identified to be *Listeria monocytogenes* with biochemical tests was verified as species through Real Time PCR method by using a primary pair specific to *hly A* gene location. In this study, a total number of 290 different type ready-made meatball samples were analysed. As a result of examining all samples was isolated *L. monocytogenes* in 32 (11.04%) samples, *L. ivanovii* in 9 (3.10%) samples, *L. innocua* in 22 (7.59%) samples, *L. welchimerii* in 8 (2.76%) samples and also *L. seeligeri* in 4 (1.38%) samples. In the serotyping of the 32 *L. monocytogenes* strains isolated from the samples; 15 isolated are found to be Type 1, where 3 strains are found to be Type 4, 11 strains to be type Poly and the rest 3 strains could not typified. The Mean pH and water activity values for the samples were found to be 6.62±0.56 and 0.985±0.007 respectively. In the result of the study, identifying *Listeria* species especially *L. monocytogenes* in cooled and frozen ready-made meatball samples studied suggest that such products whose consumption increased in the recent years pose important risk in terms of public health.

Keywords: *Listeria monocytogenes*, *Listeria* species, meatball, PCR.

Dondurma veya soğutma ile muhafaza edilerek satışa sunulan hazır köftelerde *Listeria* türlerinin varlığı

Özet: Bu çalışma, kasap, şarküteri ve süpermarketlerde dondurulmuş veya soğutulmuş olarak muhafaza edilen ve satışa sunulan hazır köfte çeşitlerinde *Listeria* türlerinin kontaminasyon düzeyini ortaya koymak amacıyla yapılmıştır. Örneklerden *Listeria* türlerinin izolasyon ve identifikasyonunda USDA/FSIS (United States Department of Agriculture/Food Safety and Inspection Service) tarafından önerilen yöntem kullanılmıştır. Biyokimyasal testlerle *L. monocytogenes* olarak identifiye edilen suşların *hly A* gen bölgesine spesifik bir primer çifti kullanılarak Real Time PCR yöntemiyle tür olarak doğrulaması yapılmıştır. Çalışmada toplam 290 adet hazır köfte örneği analize alınmıştır. Tüm örneklerin 32 (%11.04) tanesinden *L. monocytogenes*, 9 (%3.10) tanesinden *L. ivanovii*, 22 (%7.59) tanesinden *L. innocua*, 8 (%2.76) tanesinden *L. welchimeri* ve 4 (%1.38) tanesinden de *L. seeligeri* izole edilmiştir. Yapılan serotiplendirmede izole edilen 32 adet *L. monocytogenes* türünden 15 tanesi Tip 1, 3 tanesi Tip 4 ve 11 tanesi de Tip Poli olarak tanımlanmış, 3 izolat ise tiplendirilememiştir. Tüm örneklerde ortalama pH değeri 6.62±0.56 olarak bulunurken, su aktivitesi değeri ise ortalama 0.985±0.007 olarak saptanmıştır. Çalışma sonucunda incelenen örneklerde önemli oranlarda *Listeria* türlerinin, özellikle de *L. monocytogenes*'in tespit edilmesi, son yıllarda tüketimi gittikçe artan bu ürünlerin halk sağlığı açısından önemli bir risk oluşturabileceğini göstermektedir.

Anahtar sözcükler: Köfte, *Listeria monocytogenes*, *Listeria* türleri, PCR.

Introduction

Ready-made meatballs produced from red meat and poultry meat have become an ever increasing significant group of food in the recent years in parallel to the developments made in ready-made and semi-ready food industry. If these meatballs where various production techniques and different ingredients are used in production process are not going to be consumed immediately, they should be subjected to cold chain or commonly stored under frozen conditions (1, 19, 26).

Ready-made meatballs are often contaminated with microorganisms due to hygiene-related mistakes made during their production. Even if the meatballs are preserved by cooling or freezing once they are produced,

these pathogens, especially the ones with psychrophilic properties, are able to sustain their lives. If the cold chain is broken before the time the product is consumed, it may lead to a rapid multiplication of such pathogens, causing significant risks for the public health (8, 18, 19). Preservation by cooling and freezing are effective methods to limit the reproduction of pathogenic and non-pathogenic microorganisms in food. That being said, some psychrophilic microorganisms like *L. monocytogenes* are still able to multiply in cold-stored food and may cause infections (16, 19).

Various sources like additives, tools, equipment, personnel, water, environment, cross-contamination, and refrigerators may play a role in contamination of ready-made meatballs with *Listeria* species (11, 12, 18). Many studies in the past have revealed the fact that refrigerated or frozen ready-made meatballs offered to market are contaminated with substantial amounts of pathogenic and non-pathogenic microorganisms (8, 19, 24, 28, 29). This study aims to reveal the presence and prevalence of *Listeria* species especially that of *L. monocytogenes* in the refrigerated or frozen ready-made meatballs put up for sale in butchers, charcuteries and supermarkets in Van city (Turkey).

Materials and Methods

In this study, a total number of 290 ready-made meatball samples were analysed namely as follows; 50 pieces of Inegol kofte, 40 pieces of hamburger kofte; and 20 pieces each of; Tekirdag kofte, Akcaabat kofte, Adana kofte, Tire kofte, Izmir kofte, kasap kofte, cızbız kofte, odun kofte, satır kofte, and ızgara kofte (Kofte is a word in Turkish language and it is the counterpart of meatball in English language. Sample names were kept in their original marketed names). The samples were selected and collected by using simple randomly sampling method from the refrigerated or frozen ready-made meatballs offered by sale in butchers, charcuteries and supermarkets in Van city (Turkey) during January 2015-August 2015. All samples taken for analysis were consisted of the products of companies producing and selling throughout the country. The samples were collected 200 grams minimum in weight (about 10 samples each week), and they were transported to the laboratory within 2 hours max after collection in cold chain (+4 ºC), either in their original packaging or in aseptic sterile jars. Production dates, expiry dates and product batch numbers of samples of the same brand or same type were checked to ensure that they did not belong to the same production group.

Physicochemical analyses: The pH values of the samples were measured using calibrated pH-meters (Hanna® PH 221, Hanna Instruments, Italy), while their water activity was measured using a calibrated a_w device (Novasina® MS 1 Set, Switzerland).

Isolation and identification of Listeria species: For this purpose, pre-enrichment of the samples was conducted in *Listeria* Primary Selective Enrichment Broth (Oxoid CM863+SR142) where their selective enrichment was conducted in *Listeria* Secondary Selective Enrichment Broth (Oxoid CM863+SR143). From both of the enrichment broths, inoculation by a colony each is made by streaking method from *Listeria* Selective Agar (LSA) (Oxoid CM856+SR140). Typical colonies in 1-3 mm diameter with grey-brown colour surrounded by black colour are evaluated as *Listeria*-suspicious (9, 15).

The colonies growing in the LSA were purified on the Tryptone Soy Agar (containing 0.6% Yeast Extract) and identification of purified colonies were made by subjected to Henry's Oblique Illumination Test and other conventional tests of identification (Gram staining, catalase, oxidase, urea, motility, methyl red/Voges-Proskauer, β-hemolysis, CAMP, nitrate and glucose, sorbitol, D-mannitol, L-rhamnose, D-xylose fermentation) (14, 20, 22).

Confirmation of identified *L. monocytogenes* species with PCR: In order to confirm *L. monocytogenes* species with PCR, the modified method developed by Aznar and Alarcón's (6) from Border et al. (7) was used. In this purpose, in order to detect *hly A* gene in the samples, specific primary pair to this zone (LMF: CCTAAGACGCCAATCGAA; LMR: AAGCGCTTG CAACTGCTC) and instant commercial master mix (GeneAll®, Real. Amp™ SYBR qPCR Master Mix, Korea) was used. From the colonies identified as *L. monocytogenes*, DNA extraction was made by using commercial kits (GeneAll®, Exgene™ Cell SV). PCR tubes prepared separately for each *Listeria*-suspicious DNA extract are placed in real-time PCR device (Rotor GeneTM 6000 Corbett Research, Australia) and upon denaturation phase for 30 sec in 94 ºC, 45 sec of bonding in 55.5 ºC, 45 sec of extension in 72 ºC and 5 min of final extension in 72 ºC phases, totally 35 cycle of PCR amplification is applied. In the results of the application, locations of positive controls and negative control samples in graph were investigated.

Serological tests: In order to determine the serotypes of *L. monocytogenes* strains, was made aglutination test with commercial antiseras (BD Difco *Listeria* O Antisera Type 1, 4 and Poly) (4).

***Reference strains*:** The *L. monocytogenes*, *Staphylococcus aureus,* and *Rhodococcus equii* strains used in the research were obtained from Etlik Central Veterinary Control & Research Institute (Ankara, Turkey).

Statistical analysis: In this study, samples were selected and collected by using simple randomly sampling method. As the mean pH values determined in 12 different ready-made meatballs varieties showed normal

distribution in Kolmogorov-Smirnov test, one-way analysis of variance was applied for detection of statistically relation of between mean pH values of different kofte groups. The Duncan multiple comparison test was used to determine the significant differences as a result of the variance analysis. Since the a_w values determined in 12 different meatball groups did not show normal distribution as a result of Kolmogorov-Smirnov test, Kruskal-Wallis and Mann-Whitney tests were applied. Pearson correlation analysis was used to determine the relationship between pH and water activity (a_w) in each type of meatballs. The statistical analysis was performed using SPSS 23 statistical software program (2).

Results

Listeria species isolated from ready-made meatball samples and PCR verification with serotype distribution of *L. monocytogenes* isolated are given in Table 1 and Figure 1. Taking all the samples into consideration, *L. monocytogenes* is found to have the highest isolation rate (11.04%) and it is followed by *L. innocua* (7.59%), *L. ivanovii* (3.10%), *L. welchimeri* (2.76%) and *L. seeligeri* (1.38%) respectively. Table 2, Table 3 and Table 4 represents the levels of statistical significances of the ready-made meatball sample groups' and sub-groups' pH and water activity differences.

Table 1. The distribution of *Listeria* species in the samples and the serotyping of *L. monocytogenes* isolates.
Tablo 1. Örneklerde belirlenen *Listeria* türlerinin dağılımı ve *L. monocytogenes*'in serotiplendirilmesi.

Sample type	Meat species	n	Numbers of positive samples						Serotypes of *L. monocytogenes*			
			Listeria spp. positive samples	*L. monocytogenes*	*L. ivanovii*	*L. innocua*	*L. welchimeri*	*L. seeligeri*	Tip 1	Tip 4	Tip poli	Not typed
Inegol kofte	Chicken (F)	10	3 (30%)	2 (20%)	ND	**1** (10%)	**1** (10%)	ND	1 (10%)	-	1 (10%)	-
	Turkey (F)	10	3 (30%)	1 (10%)	1 (10%)	ND	1 (10%)	ND	1 (10%)	-	-	-
	Beef (C)	10	3 (30%)	ND	1 (10%)	2 (20%)	ND	ND	-	-	-	-
	Beef (F)	10	0 (0%)	ND	ND	ND	ND	ND	-	-	-	-
	Beef+Lamb (F)	10	2 (20%)	1 (10%)	ND	1 (10%)	ND	ND	-	-	1 (10%)	-
	Total samples	50	11 (22%)	4 (8%)	2 (4%)	4 (8%)	2 (4%)	0 (0%)	2 (4%)	0 (0%)	1 (2%)	0 (0%)
Tekirdag kofte	Beef (F)	20	5 (25%)	3 (15%)	**1** (5%)	**2** (10%)	ND	ND	1 (5%)	-	1 (5%)	1 (5%)
Akcaabat kofte	Beef (F)	20	4 (20%)	2 (10%)	1 (5%)	1 (5%)	ND	ND	2 (10%)	-	-	-
Adana kofte	Beef (F)	20	3(15%)	1 (5%)	ND	ND	2 (10%)	ND	-	-	1 (5%)	-
Tire kofte	Beef (F)	20	7 (35%)	3 (15%)	2 (10%)	**2** (10%)	**1** (5%)	ND	2 (10%)	1 (5%)	-	-
Izmir kofte	Beef (F)	20	6(30%)	3 (15%)	2 (10%)	ND	ND	1 (5%)	1 (5%)	-	2 (10%)	-
Hamburger kofte	Beef (F)	10	1 (10%)	1 (10%)	ND	ND	ND	ND	1 (10%)	-	-	-
	Beef (C)	10	2 (20%)	1 (10%)	ND	1 (10%)	ND	ND	1 (10%)	-	-	-
	Chicken (F)	10	1 (10%)	ND	ND	**1** (10%)	ND	**1** (10%)	-	-	-	-
	Chicken (C)	10	3 (30%)	2 (20%)	ND	1 (10%)	ND	ND	-	1 (10%)	-	1 (10%)
	Total samples	40	7 (17.5%)	4 (10%)	0 (0%)	3 (7.5%)	0 (0%)	1 (2.5%)	2 (5%)	1 (2.5%)	0 (0%)	1 (2.5%)
Butcher kofte	Beef (F)	10	2 (20%)	1 (10%)	1 (10%)	ND	ND	ND	1 (10%)	-	-	
	Beef (C)	10	3 (30%)	**1** (10%)	ND	**3** (30%)	ND	ND	-	-	1 (10%)	
	Total samples	20	5 (25%)	2 (10%)	1 (5%)	3 (15%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (5%)	0 (0%)
Cızbız kofte	Beef+Lamb (C)	10	3 (30%)	1 (10%)	ND	2 (20%)	ND	ND	-	-	1 (10%)	-
	Beef (F)	10	4 (40%)	1 (10%)	ND	2 (20%)	1 (10%)	ND	1 (10%)	-	-	-
	Total samples	20	7 (35%)	2 (10%)	0 (0%)	4 (20%)	1 (5%)	0 (0%)	1 (5%)	0 (0%)	1 (5%)	0 (0%)
Odun kofte	Beef (F)	20	5 (25%)	4 (20%)	ND	ND	ND	1 (5%)	1 (5%)	1 (5%)	1 (5%)	1 (5%)
Satır kofte	Beef (F)	20	2 (10%)	**2** (10%)	ND	ND	**2** (10%)	ND	-	-	2 (10%)	-
Izgara kofte	Beef (F)	20	5 (25%)	**2** (10%)	ND	3 (15%)	ND	**1** (5%)	2 (10%)	-	-	-
Total positive samples		**290**	**97 (33.45%)**	**32 (11.04%)**	**9 (3.10%)**	**22 (7.59%)**	**8 (2.76%)**	**4 (1.38%)**	**15 (5.17%)**	**3 (1.04%)**	**11 (3.79%)**	**3 (1.04%)**

F: Frozen, C: Cooled, ND: Not Detected, The bold numbers in the same column indicate that two different species were isolated from a single sample

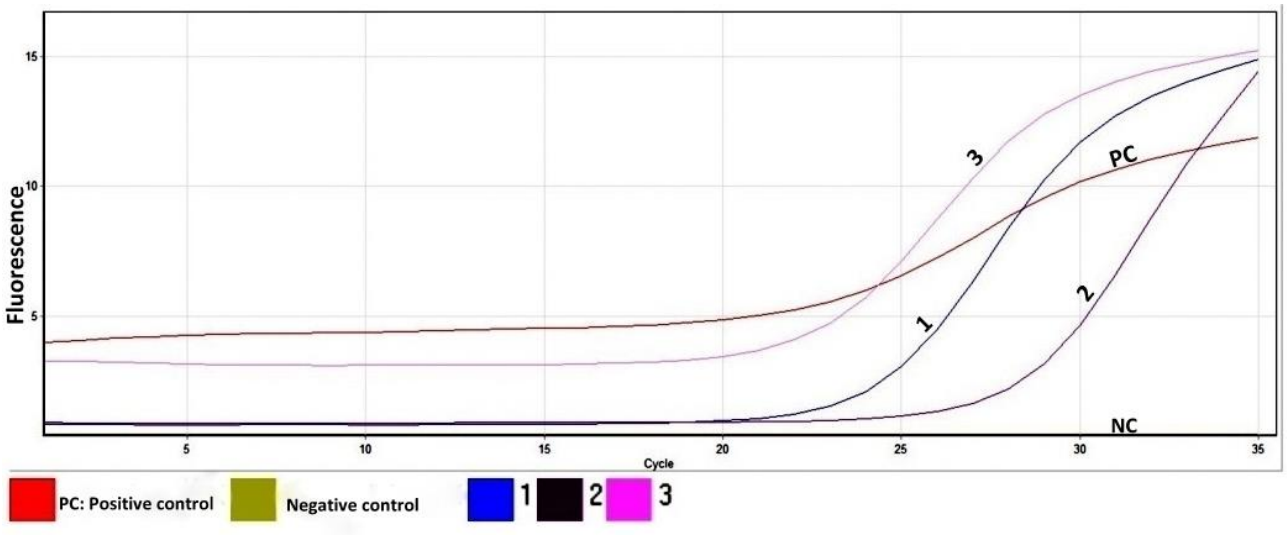

Figure 1. The graphics of PCR amplification of DNA extracts obtained from positive control, negative control, and some *L. monocytogenes* strains isolated from samples.
Şekil 1. Örneklerden izole edilen bazı *L. monocytogenes* suşları ile pozitif ve negative kontrolden elde edilen DNA ekstraktlarının PCR amplifikasyon grafikleri.

Table 2. The mean pH values of sample groups and the statistical significance of the differences in the between of mean pH value of the different types samples within the same sample groups and between the different sample groups.
Tablo 2. Örnek gruplarında belirlenen ortalama pH değerleri ve farklı örnek grupları arasındaki ve örnek grupları içindeki farklı tip hazır köfte örneklerinin ortalama pH değeri farklılıklarının istatistiksel önem dereceleri

Sample name	Meat and preservation type	n	pH
			Mean±SE
Inegol kofte	Chicken/Freezing	10	6.761 ± 0.047^{C}
	Turkey/Freezing	10	7.076 ± 0.093^{B}
	Beef/Cooling	10	6.564 ± 0.120^{xC}
	Beef/Freezing	10	7.426 ± 0.052^{aA}
	Beef +Lamb/Freezing	10	7.118 ± 0.089^{B}
Tekirdag kofte	Beef/Freezing	20	7.123 ± 0.079^{b}
Akcaabat kofte	Beef/Freezing	20	6.077 ± 0.046^{e}
Adana kofte	Beef/Freezing	20	6.471 ± 0.072^{d}
Tire kofte	Beef/Freezing	20	6.723 ± 0.061^{c}
Izmir kofte	Beef/Freezing	20	7.094 ± 0.052^{b}
Hamburger kofte	Beef/Freezing	10	6.013 ± 0.117^{efA}
	Beef/Cooling	10	6.206 ± 0.071^{yA}
	Chicken/Freezing	10	6.241 ± 0.087^{A}
	Chicken/Cooling	10	6.213 ± 0.099^{A}
Kasap kofte	Beef/Freezing	10	6.343 ± 0.128^{dA}
	Beef/Cooling	10	6.366 ± 0.099^{yA}
Cızbız kofte	Beef +Lamb/Cooling	10	6.149 ± 0.115^{A}
	Beef/Freezing	10	6.108 ± 0.126^{eA}
Odun kofte	Beef/Freezing	20	7.078 ± 0.052^{b}
Satır kofte	Beef/Freezing	20	5.840 ± 0.069^{f}
Izgara kofte	Beef/Freezing	20	7.262 ± 0.037^{ab}

The difference between the means marked with different small letters in the same column are statistically significant (P<0.05) (for the groups of frozen beef meatball). The difference between the means marked with different capital letters in the same column are statistically significant (P<0.05) (for different types of samples within Inegol kofte, hamburger kofte, kasap kofte and cızbız kofte groups).
The difference between the means marked with different x, y, z letters in the same column are statistically significant (P<0.05) (for the groups of cooled beef meatball).

Table 3. The statistical significance of the differences in the mean rank a_w values of ready-made beef meatballs groups offered by sale under cooling or freezing preservation.
Tablo 3. Soğukta veya dondurarak muhafaza altında satışa sunulan hazır sığır eti kofte örneği gruplarının ortalama rank a_w değerlerinin farklarının istatistiksel önem dereceleri.

Sample name	Meat and preservation type	n	a_w
			Mean rank
Inegol kofte	Beef/Cooling	10	16.450^{xy}
	Beef/Freezing	10	38.350^{e}
Tekirdag kofte	Beef/Freezing	20	63.300^{ed}
Akcaabat kofte	Beef/Freezing	20	46.800^{ed}
Adana kofte	Beef/Freezing	20	75.280^{cd}
Tire kofte	Beef/Freezing	20	119.850^{c}
Izmir kofte	Beef/Freezing	20	80.880^{bc}
Hamburger kofte	Beef/Freezing	10	153.550^{ab}
	Beef/Cooling	10	10.050^{y}
Kasap kofte	Beef/Freezing	10	145.550^{ab}
	Beef/Cooling	10	20.000^{x}
Cızbız kofte	Beef/Freezing	10	63.950^{ab}
Odun kofte	Beef/Freezing	20	110.130^{bc}
Satır kofte	Beef/Freezing	20	164.030^{a}
Izgara kofte	Beef/Freezing	20	144.050^{ab}

The difference between the mean ranks marked with different small letters in the same column are statistically significant (P<0.05) (for the groups of frozen beef meatball). The difference between the mean ranks marked with different x, y, z letters in the same column are statistically significant (P<0.05) (for the groups of cooled beef meatball).

Table 4. The statistical significance of the differences of mean rank a_w values within the group in the ready-made meatball sample groups offered for sale under cooling or freezing preservation.
Tablo 4. Soğukta veya dondurarak muhafaza altında satışa sunulan hazır kofte örneği gruplarında, grup içi ortalama rank a_w değerlerinin farklarının istatistiksel önem dereceleri

Sample name	Meat and preservation type	n	a_w
			Mean rank
Inegol kofte	Chicken/Freezing	10	20.450^{c}
	Turkey/Freezing	10	38.400^{a}
	Beef/Cooling	10	29.250^{b}
	Beef/Freezing	10	11.500^{d}
	Beef +Lamb/Freezing	10	27.900^{b}
Hamburger kofte	Beef/Freezing	10	32.400^{a}
	Beef/Cooling	10	21.000^{b}
	Chicken/Freezing	10	16.650^{bc}
	Chicken/Cooling	10	11.950^{c}
Kasap kofte	Beef/Freezing	10	12.220^{a}
	Beef/Cooling	10	8.800^{a}
Cızbız kofte	Beef +Lamb/Cooling	10	8.400^{a}
	Beef/Freezing	10	12.400^{a}

The difference between the mean ranks marked with different small letters in the same column are statistically significant (P<0.05).

Discussion and Conclusion

Meatballs represent an important group of ready-made foods. They are produced using minced meat, oil, various spices and filling materials, and are offered for sale either in raw form or after undergoing thermal semi-processing in oil. *Listeria* species, and especially the pathogenic species of *L. monocytogenes*, are readily encountered in meatballs, and cooling or freezing process doesn't affect their presence in foods drastically (16, 18, 19, 21, 27).

Many studies conducted in Turkey have displayed that hygienic quality in ready-made meatballs and other

similar products show variability and that these products can be contaminated with various pathogenic microorganisms (18, 24, 29).

Of the 290 meatball samples in this study, *Listeria* spp. was isolated from 97 (33.45%) of them, while *L. monocytogenes* was isolated from 32 (11.04%) of those (Table 1). These results are concordant to other studies in the literature which report very low hygienic quality for meatballs (8, 18, 24, 29).

When the results are inspected, it can be seen that *Listeria* spp. was isolated from all the samples, except for the Inegol kofte made from beef which were preserved by freezing. It can also be seen that *L. monocytogenes* was isolated from all the samples except for the Inegol kofte made out of beef, and hamburgers made out of chicken meat and preserved by freezing. The results indicate that there are no significant differences in terms of *L. monocytogenes* isolation based on the meat type and preservation method. This result implies that preservation by cooling or freezing the meatballs is not very effective. The factors effective in *L. monocytogenes* contaminations are mostly environmental contaminations and cross-contamination through the equipment used during the production stage (16, 17, 21, 28, 29). On the other hand, the fact that antibacterial properties of some spices like clove, thyme, onion, and garlic used in the production of meatballs having a negative effect on the *L. monocytogenes* count and vitality should also be kept in mind (5, 13).

The *L. monocytogenes* isolation prevalence of some samples was lower in our study compared to the literature (23, 28, 29) while others were higher (11). Such variations may be based on the differences between the production and preservation methods employed by the production companies, the inhibition effect of some of the additives and spices used during the production of the meatballs or the competing microflora, or due to the differences in analysis methods used (5, 13).

The equipment used, and the preferred hygiene protocols like HACCP and GM used by the production plants, also play an important part in the contamination of meat and meat products by *Listeria* spp. In the establishments that produce ready-made meatballs, the *Listeria* spp. that have spread to the equipment used can keep contaminating the products continuously, which may be a contributor to the high isolation rates revealed by the studies (12, 28, 29).

The species that was isolated the most in this study was *L. monoctogenes*, followed by *L. innocua*. Sharif and Tunail (23) isolated *L. monocytogenes* the most from more than 200 meat product samples they tested consisting of frozen cooked, half-cooked, thermally pre-processed, and raw products, and also found it was followed by *L. innocua*. Sireli et al. (28), on the other hand, found *L. innocua* was the predominant species for minced meat, meatball and burger samples, which was followed by *L. monocytogenes.*

In our study, the pH value for the meatball samples was found to vary between 5.16 and 7.75, with mean of 6.62±0.56. The pH range detected in our study is wider compared to the study of Soyutemiz (25) (which detected a pH range of 5.85-7.32 in ready-made meatballs), which may be due to the difference in regions from which the samples were collected from. Some researchers report that a high initial pH value in Inegol kofte's stored in +4 ºC is connected to the bicarbonate used in the production, and the drop at the 7th day is due to the acids produced by the micro-flora (27). The pH values in our study were found to be of alkali range for Tekirdag kofte, Izmir kofte, odun kofte, ızgara kofte, and some of the Inegol kofte samples, while it was found to be below pH 7.0 for the others (Table 2).

In the statistical analysis, some significant differences ($P<0.05$) were found in terms of pH value among the frozen ready-made beef meatball groups (Table 2). Among the groups of cooled ready-made beef meatballs a significant difference ($P<0.05$) was found only between Inegol kofte group and hamburger kofte/kasap kofte groups (Table 2). In groups contains more than one different types of meatballs, the differences between the pH values of some types of meatballs were found to be significant ($P<0.05$) only in Inegol kofte group (Table 2). No significant difference was found between meatball types in hamburger kofte, kasap kofte and cızbız kofte groups in terms of pH values (Table 2). These variations may be related to the difference of raw materials, additives, and micro-flora of the samples, to the preservation method employed, or to variances in the storage period length. Soyutemiz (25) has found that hamburgers had a lower pH value on average compared to other meatball types. While the pH values found in our study for the hamburgers were lower compared to the study of Soyutemiz (25) and similar to the study of Yörük (29), our study also has revealed that hamburger meatballs had a comparatively lower pH nonetheless.

The water activities of the samples were found to vary between 0.975±0.015 and 0.992±0.003, with mean of 0.985±0.007. In the statistical analysis, some significant differences ($P<0.05$) were found in terms of a_w value among the frozen ready-made beef meatball groups (Table 3). Also, among the groups of cooled ready-made beef meatballs a significant difference ($P<0.05$) was found only between a_w values of hamburger kofte group and kasap kofte group (Table 3). In addition, in the statistical analysis of the a_w values of the meatballs types in the groups, while there was no significant difference between the types of meatballs in the kasap kofte group and the cızbız kofte group, there were some significant differences

between the types of meatballs in the Inegol kofte group and hamburger kofte group (Table 4). When the water activity of the products are considered, some statistically significant differences ($P<0.05$) between both the groups and sub-groups can be observed (Table 3 and Table 4). It can be thought that this situation is due to the raw material, production and preservation differences for all product groups.

In the correlation analysis performed to determine whether there is a relationship between the pH values and a_w values of meatball groups, only in hamburger kofte samples were found to have a negative correlation ($P<0.05$) between pH and water activity values. This finding may be due to fact that half of the hamburger kofte samples were stored by cooling, where some psychrophilic microorganisms may have multiplied in the storage temperature, causing acidity (5, 26).

The Microbiological Criteria Code of the Turkish Food Codex mandates zero *L. monocytogenes* in 25 g of the sample for ready-made meatballs (3). Evaluated in this perspective, it is evident that 11.04% of the samples inspected in our study are in violation of the legal regulations. This situation suggests that these products may pose a serious public health hazard, in situation such as cooking with inadequate heat treatment and cross-contamination with uncooked foods.

As a result of this study, it has been revealed that meatballs preserved by cooling or freezing in the sale points are contaminated with *Listeria* species, especially with *L. monocytogenes*, and this contamination level is capable for forming a significant risk for the public health. This risk may further increase with inadequate cooking time or methods before consumption (10). The results clearly indicate that trusting the products preserved by cooling or freezing to be perfect in terms of food safety is a faulty perspective. To prevent these kinds of pathogenic risks, the ready-made meatballs have to be produced by paying the utmost attention to hygiene rules and must be delivered to the final consumer without breaking the cold chain.

Acknowledgement

This study was partially supported by the Van YYU Directorate of Scientific Research Projects under project number 2008-VF-B059.

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