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Microbiological Quality Assessment of Meatball Samples Collected from Businesses with Different Pricing Strategies

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ABSTRACT
Objective: The purpose of this study was to evaluate the microbiological quality of meatball samples collected from establishments employing different pricing strategies. **Materials and Methods:** Samples were obtained from low-priced establishments (Business I) and high-priced establishments (Business II) and were analyzed for total aerobic mesophilic bacteria (TAMB), yeasts and molds, coliform bacteria, *Staphylococcus aureus*, *Escherichia coli*, *Salmonella* spp., and *Listeria monocytogenes*. **Results:** Both groups of samples complied with Turkish Food Codex standards for TAMB and *S. aureus*, while yeast and mold count in Business I samples exceeded the permissible limits. *Salmonella* spp. was detected in 3.3% of the samples from Business I and 6.6% from Business II, whereas *L. monocytogenes* was not detected in any of the samples. **Conclusion:** The study findings showed that there was no significant difference in microbiological quality between the two groups, except for yeast and mold counts. It was also concluded that there was no direct relationship between product price and hygienic quality.
Keywords: Food Pricing Strategies, Hygienic Quality, Meatball, Microbiological Quality.

Farklı Fiyatlandırma Stratejilerine Sahip İşletmelerden Alınan Köfte Örneklerinin Mikrobiyolojik Kalite Değerlendirmesi

ÖZ
Amaç: Bu çalışmanın amacı farklı fiyatlandırma stratejileri uygulayan işletmelerden toplanan köfte örneklerinin mikrobiyolojik kalitesinin değerlendirilmesidir. **Gereç ve Yöntem:** Düşük fiyatlı işletmelerden (İşletme I) ve yüksek fiyatlı işletmelerden (İşletme II) alınan örnekler, toplam aerobik mezofilik bakteri (TAMB), maya ve küf, koliform bakteriler, *Staphylococcus aureus*, *Escherichia coli* ve *Salmonella* spp. ve *Listeria monocytogenes* varlığı açısından analiz edilmiştir. **Bulgular:** Her iki grup örnek de TAMB ve *S. aureus* açısından Türk Gıda Kodeksi standartlarını karşılarken, İşletme I örneklerindeki maya ve küf sayıları limitleri aşmıştır. *Salmonella* spp. pozitifliği İşletme I örneklerinin %3,3'ünde ve İşletme II örneklerinin %6,6'sında tespit edilmiştir. Hiçbir örnekte *L. monocytogenes* tespit edilmemiştir. **Sonuç:** Çalışma bulguları maya ve küf sayıları dışında, iki grup arasında mikrobiyolojik kalitede anlamlı bir fark olmadığını gösterdi. Ayrıca, ürün fiyatı ile hijyenik kalite arasında doğrudan bir ilişki olmadığı sonucuna varılmıştır.
Anahtar Kelimeler: Gıda Fiyatlandırma Stratejileri, Hijyenik Kalite, Köfte, Mikrobiyolojik Kalite.

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INTRODUCTION

Red meat is among the food sources that pose the highest risk in terms of food safety and foodborne diseases. Due to its physicochemical properties, such as slightly acidic pH levels (5.5–6.0), high water activity (0.99 aw), and rich nutritional content, meat provides an ideal environment for the growth and activity of many microorganisms (Altan & İşleyici, 2012; Güldemir et al., 2022). Ground meat, which is used in meatball production, has a very short shelf life and typically needs to be consumed within 1–3 days (Lizcano-Prada et al., 2024; Karasu & Özdemir, 2022). In addition to the issue of short shelf life, factors such as the health status of the animal, processing methods during slaughter, workers, tools, and equipment can lead to the contamination of red meat with pathogenic microorganisms. The consumption of meat products made from fresh ground meat has been increasing in both developed and developing countries due to technological advancements and changing dietary habits. In particular, ready-to-cook meatballs made from fresh ground meat are among the most preferred meat products (Bostan, 2002; Yıldız et al., 2004). Meatballs are defined as mixtures of raw or cooked red or poultry meat prepared from carcass meat of cattle, sheep, goats, or poultry, alone or in combination, along with the addition of one or more food ingredients such as animal fats, flavorings, and other food ingredients, prepared in accordance with the relevant communiqué (Meat Communiqué, 2018/52). Bacteria present on the surface of meat spread throughout the product during the grinding and mixing processes. These types of products, marketed raw, are prone to spoilage during storage and may harbor numerous pathogenic microorganisms from various sources, posing a significant threat to consumer health (Bostan, 2002; Kamumu et al., 2021).

The primary public health risks associated with meatballs are foodborne pathogens such as *Salmonella* spp., *L. monocytogenes*, and *Escherichia coli* O157. According to the EFSA 2021 report, these pathogens are among the most concerning microorganisms regarding food safety in Europe. Their animal origins, presence in many different foods, and the large number of foodborne illnesses they cause each year keep these pathogens a constant concern (Lizcano-Prada et al., 2024; Güldemir et al., 2022). While some consumers are aware of these risks and pay attention to maintaining hygienic conditions during food preparation, understanding consumer behavior is crucial. Understanding consumer behavior helps businesses offer the right products or services, identify factors influencing purchasing decisions, and analyze the purchasing process, thereby enhancing sustainability and consumer satisfaction (Bekar & Gövce, 2015).

Consumers often focus on the quality-price relationship of products and believe that branded products are higher quality. Factors such as social class and income level influence brand preferences (Kızılaslan & Kızılaslan, 2008). Price variations are a critical criterion for determining food prices, particularly at the provincial level and among different income levels. In addition to social class, the residential area also significantly affects price differences. In this context, the aim of our study was to compare the hygienic quality of meatballs sold at economical prices in low- to middle-income regions with those sold at higher prices in high-income regions.

MATERIALS AND METHODS

Sample collection

Meatball samples were obtained from businesses with two different pricing strategies in this study. Business I represents butcher shops and restaurants selling raw meatballs at economical prices. In this group, a total of 30 raw meatball samples, each weighing 50 grams, were collected from different locations in sterile packaging. Business II represents butcher shops and restaurants selling raw meatballs at higher prices. Similarly, a total of 30 raw meatball samples, each weighing 50 grams, were collected from different locations in sterile packaging. All collected samples were transported under a cold chain to the XXX University Department of Food Hygiene and Technology laboratory, where the analyses were performed.

Microbial analysis

From each meatball sample, 10 grams were weighed and placed in stomacher bags, and 90 mL of Maximum Recovery Diluent (MRD, Merck 1.12535) was added. The samples were then homogenized for 2–3 minutes in the stomacher, and 1/10 dilutions were prepared. Subsequently, serial dilutions were made using tubes containing 9 mL of MRD.

Total aerobic mesophilic bacteria (TAMB) counts were performed using the pour plate method on PCA (Plate Count Agar, Merck). The samples were incubated at 37°C for 48 hours, and petri plates containing 30–300 colonies were evaluated (ISO 4833-2:2013).

Yeast and mold counts were determined using the spread plate method on DRBC Agar (Dichloran Rose Bengal Chloramphenicol Agar, Merck). The samples were incubated at 25°C for 5 days, after which colony counting was performed (ISO 21527-2:2008).

Coliform counts were determined using the double-layer pour plate method on VRBA (Violet Red Bile Agar, Merck). The samples were incubated at 30°C for 24 hours, and colony evaluation was performed afterward (ISO 4832:1996).

Staphylococcus/*Micrococcus* counts were determined using the spread plate method on BPA (Baird-Parker Agar, Merck) supplemented with egg yolk–tellurite emulsion (Merck). After 48 hours of incubation at 37°C, black colonies were identified as *Staphylococcus*/*Micrococcus* counts.

Coagulase tests were performed on zoned and atypical colonies to determine the *S. aureus* count (ISO 6888-1:2003).

E. coli counts were determined using the spread plate method on TBX (Tryptone Bile X-Glucuronide, Oxoid) Agar. The samples were incubated initially at 30°C for 4 hours and then at 44°C for 20 hours, after which the colonies were evaluated (ISO 16649-2:2001).

For isolation of *Salmonella*, 25 grams of meatball samples were weighed and placed in stomacher bags, and 225 mL of Buffered Peptone Water (BPW, Merck) was added. The samples were homogenized in the stomacher for 2–3 minutes and incubated at 37°C for 24 hours for pre-enrichment. Following pre-enrichment, 0.1 mL of the sample was transferred into tubes containing 10 mL of Rappaport-Vassiliadis Soya Peptone Broth (RVS, Oxoid) and incubated at 42°C for 24 hours for selective enrichment. After selective enrichment, an inoculation loopful of the liquid medium was streaked onto XLT4 (Xylose Lysine Tergitol-4) Agar plates, which were then incubated at 37°C for 24–48 hours. Black colonies grown on XLT4 Agar were dissolved in 0.5–1 mL of sterile water for biochemical tests and inoculated with a needle loop into Lysine Iron Agar (LIA, LAB 054) and Triple Sugar Iron Agar (TSIA, Oxoid CM 277). These media were incubated at 37°C for 24 hours. Colonies showing yellow-black at the bottom, red at the surface, and gas holes in TSIA medium were considered *Salmonella* spp. suspects (FDA, 2011). The *Salmonella spp.* suspect colonies were subsequently preserved in Tryptic Soy Broth (TSB, Merck) for PCR analysis.

For isolation of *L. monocytogenes*, 25 grams of meatball samples were placed in stomacher bags, and 225 mL of Half-Fraiser Broth (HF, Oxoid) was added. The samples were homogenized in the stomacher for 2–3 minutes and incubated at 30°C for 24 hours for pre-enrichment. Following pre-enrichment, 0.1 mL of the sample was transferred into tubes containing 10 mL of Fraiser Broth (FB, Oxoid) and incubated at 35°C for 24 hours for selective enrichment. After selective enrichment, an inoculation loopful of the liquid medium was streaked onto Oxford Agar (Merck) plates using the streaking method, and the plates were incubated at 35°C for 48 hours (EN-ISO 11290-1:2017). At the end of the incubation period, colonies suspected of being *L. monocytogenes* were preserved in Tryptic Soy Broth (TSB) medium for PCR analysis.

Polymerase chain reaction (PCR)

The primers used for the identification of *L. monocytogenes* and *Salmonella spp.* are shown in Table 1. Gene amplification was performed using a Thermal Cycler (Thermo Scientific, Finland). The total reaction volume was set to 25 µL, consisting of 12.5 µL Master Mix, 0.25 µM of each primer, 1 µL DNA, and 11 µL DNase-free water. PCR conditions were as follows: for *L. monocytogenes*, 94°C for 30 seconds denaturation, 68°C for 60 seconds primer annealing, and 72°C for 90 seconds final extension; for *Salmonella* spp., 95°C for 30 seconds denaturation, 51°C for 30 seconds primer annealing, and 72°C for 30 seconds final extension, each carried out for 35 cycles. PCR products were stained with ethidium bromide (0.5 µg/mL), run on a 1.5% agarose gel, visualized under UV light using a Vilber Lourmat (Quantum ST4 1100/26MX Xpress UV Table, France) imaging system, and the resulting bands were examined.

Table 1. Primers for Salmonella spp. and L. monocytogenes.

Gen	Primer	PCR (bp)	Bacteria	Reference
hlyA	F: 5'-ATCATCGACGGCAACCTCGGAGAC-3' R: 5'-ACCATTCCCAAGCTAAACCAGTGC-3'	404	*L. monocytogenes*	Wu and ark., 2004
iroB	F: 5'-GCAGAAGCTGGGTTGGTGGTATTT-3' R: 5'-AGAAGACGCTTGCGATCAGGTGTA-3'	500	*Salmonella* spp.	Nair and ark., 2015

Statistical analysis

The SPSS 30 statistical software was used to compare the meatball samples. T-test was applied to determine the differences between the samples. The significance level was considered at $p<0.05$ for all analyzes.

Ethical considerations

This study does not require ethics committee approval as it does not involve data collection from human or animal subjects.

RESULTS

The microbiological analysis results of the meatball samples are presented in Table 2 and Table 3. In Business I, the average counts for TAMB, yeast-mold, coliforms, and *Staphylococcus/Micrococcus* were found to be 5.83 log CFU/g, 4.46 log CFU/g, 3.50 log CFU/g, and 3.97 log CFU/g, respectively. In Business II, these values were determined as 5.81 log CFU/g,

4.97 log CFU/g, 3.27 log CFU/g, and 3.51 log CFU/g, respectively.

When Table 2 is examined, a statistically significant difference was found only in yeast-mold counts among the microbiological analysis results ($p<0.05$).

In the analyses conducted on meatball samples, the *S. aureus* count was determined to be an average of 2.12 log CFU/g in Business I and 1.96 log CFU/g in Business II. The *E. coli* counts were found to be 3.26 log CFU/g in Business I and 3.31 log CFU/g in Business II. No statistically significant difference was observed between the business types in terms of *S. aureus* and *E. coli* counts ($p>0.05$).

Table 2. Microbiological analysis of meatball samples I (log CFU/g±SD).

Business	TAMB	Yeast/Mold	Coliform	*Staph/Micrococcus*
Business I	5.83±0.18	4.46±0.16[a]	3.50±0.22	3.97±0.19
Business II	5.81±0.19	4.97±0.19[b]	3.27±0.32	3.51±0.17

TAMB: Total aerobic mesophilic bacteria count

[a]-[b]: Different superscripts in the same column indicate statistically significant differences ($p<0.05$).

The *Salmonella* positive rate was determined to be 3.3% in the samples from Business I and 6.6% in the samples from Business II. However, *L. monocytogenes* was not detected in meatball samples from either business type (Table 3).

Table 3. Microbiological analysis of meatball samples ii.

Business	*S. aureus* (log CFU/g±SD)	*E. coli* (log CFU/g±SD)	Business I and II positive samples (%)	
			Salmonella spp.	*L. monocytogenes*
Business I	2.12±0.19	3.26±0.17	1 (%3.3)	-
Business II	1.96±0.23	3.31±0.26	2 (%6.6)	-

SD: Standard deviation.

DISCUSSION

Microbiological analyses applied to food are critical indicators for assessing the suitability of raw materials, water, personnel, tools, equipment, storage conditions, and building hygiene used in food production (Bayizit et al., 2003). In this study, microbiological analyses of meatball samples collected from 60 businesses operating in two different locations were conducted to evaluate the hygienic conditions of production environments and raw material selection. Based on the analysis, the compliance level of the samples with the Turkish Food Codex (TFC) "Raw Red Meat and Prepared Red Meat Mixtures Communiqué" (Communiqué No: 2006/31) was assessed. Accordingly, TAMB (m: 5×10^5; M: 5×10^6) and *S. aureus* (m: 5×10^2; M: 5×10^3) values were found to be compliant with the communiqué. However, the yeast and mold counts exceeded the limits set by the TFC Communiqué No: 2006/31 (m: 10^3; M: 10^4).

In terms of *Salmonella* spp. positivity rates, 1 meatball sample (3.3%) from Business I and 2 meatball samples (6.6%) from Business II tested positive. These results indicate non-compliance with the criteria of the relevant communiqué in the TFC (Communiqué No: 2006/31). However, no presence of *L. monocytogenes* was detected in the meatball samples from either location (Table 3). The TAMB, *Staphylococcus/Micrococcus*, coliform, and *S. aureus* values obtained in this study were found to be lower than the microbiological analysis results reported by Kök et al. (2007) and Yıldız et al. (2004), while the yeast and mold counts were higher than those reported in these studies. In both studies (Kök et al., 2007; Yıldız et al., 2004), *Salmonella* spp. positivity rates were reported as 18% and 5.4%, respectively, which are higher than the findings of the current study. Additionally, the microbiological analysis results reported by Yilmaz et al. (2002) for meatball samples (TAMB, yeast-mold, staphylococci-micrococci, and coliform) were higher than those of our study (6.02×10^6 CFU/g, 2.4×10^5 CFU/g, 3.3×10^2 CFU/g, 1.1×10^5 CFU/g, respectively), while *E. coli* and *S. aureus* values were lower (1.0×10^2 CFU/g, 85

CFU/g). These differences, along with the dates of the studies, suggest that hygienic quality in meatball production may have improved to some extent over the years.

The primary aim of our study was to determine whether there was a difference in hygienic quality between meatball samples collected from businesses with low- and high-pricing policies. The findings revealed that, except for yeast and mold counts, there was no statistically significant difference in microbiological quality between the meatball samples from businesses following two different pricing strategies. This indicates that there is no direct relationship between product price and hygienic quality.

Under free-market conditions, the selling price of a product is generally determined based on the purchasing tendencies of the individuals (consumers) offering the product (Karasu & Özdemir, 2022). While many factors influence consumers' purchasing decisions (Özsungur & Güven, 2016), one of these factors is the perceived quality of the product. A product with fixed costs sold at different prices is often attributed to quality differences. The quality of a product like meatballs can vary depending on factors such as its microbiological flora and chemical composition (Atlan & İşleyici, 2012).

The most critical factor determining the cost of meatballs is the quality of red meat (Güldemir et al., 2022). The price of red meat, in turn, varies depending on the characteristics of the animal carcass (age, gender, health status), trimmings obtained during carcass processing, or, in worse scenarios, raw materials obtained from illegal or dead-slaughtered animals (Tosun & Demirbaş, 2012). The most fundamental indicator of the hygienic quality of this raw material is microbiological analysis results.

However, price differences among businesses operating in the same location are not solely attributable to raw material quality. One possible reason for this could be customer dependency. In consumer behavior, dependency refers to loyalty to a specific product, brand, or business and resistance to change (Espejel et al., 2011). Additionally, factors beyond price may also influence consumers' purchasing tendencies. For instance, a study (Lizcano-Prada et al., 2024) examining food purchasing preferences in Turkey, Spain, and Colombia found that Turkish consumers prioritize healthy and high-quality food more than price compared to consumers in other countries. The same study highlighted that Turkish consumers' preference for healthy and high-quality food products increases their tendency to choose higher-priced products (Terkan, 2014). This supports the perception that lower-priced products are often associated with being inferior or unhealthy. In the current study, the ability of high-priced meatball businesses to sustain their presence in a limited location may also be linked to this perception.

CONCLUSION

This study compared the microbiological quality of meatball samples collected from businesses applying low- and high-pricing policies. The results demonstrated that total aerobic mesophilic bacteria (TAMB) and *Staphylococcus aureus* values in both pricing groups complied with the Turkish Food Codex. However, it was found that yeast and mold counts in samples from low-priced businesses exceeded the specified limits, while *Salmonella spp.* positivity rates were 3.3% in low-priced businesses and 6.6% in high-priced businesses. *L. monocytogenes* was not detected in samples from either group. The most notable finding of the study is that, apart from yeast and mold counts, there was no statistically significant difference in microbiological quality between the meatball samples from businesses applying different pricing policies. This finding suggests that product price is not a direct determinant of hygienic quality and that consumer perceptions in this regard can be misleading. In conclusion, while microbiological quality alone may not be sufficient to evaluate the overall quality of a product, this study provides valuable insights into the relationship between food pricing and hygienic quality. It underscores the need for a more comprehensive examination of how pricing policies influence perceptions of safety and hygiene, both for consumers and producers.

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Conflict of Interest

The authors declare no potential conflicts of interest with respect to the research, authorship and/or publication of this article.

Author Contributions

Plan, design: TEG, HT; **Material, methods and data collection:** TEG; **Data analysis and comments:** TEG, HT; **Writing and corrections:** TEG, HT.

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Ethical Approval

Non-applicable.

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