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RESEARCH ARTICLE

# Analysis of Microbiological and Certain Qualitative Properties of Pastırma Marketed in Kastamonu

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**ABSTRACT**

This research implicates the physical, chemical and microbiological analysis of pastırma samples taken from eight different companies at three separate times of the year marketed in Kastamonu. Microbiological properties, the amount of free fatty acids, salt and residual nitrite were analyzed on the samples; pH and moisture properties tests were carried out. It is stated that the number of *Micrococcus/Staphylococcus* and lactic acid bacteria formed the dominant flora and the number of lactic acid bacteria and *Micrococcus/Staphylococcus* were 6.07-7.93 and 6.52-7.22 log cfu/g respectively. The number of yeast-mold and total aerobic mesophilic bacteria were detected as 4.09-6.51 and 6.81-7.76 log cfu/g respectively. Although it has been observed that the number of Enterobacteriaceae, which is an important criterion for quality in pastırma, is below $10^2$ cfu/g in general, some of the samples obtained from companies C, D, F, G, K showed values above <2 log cfu/g. In the samples, pH values were determined as 5.67-6.13 on average, moisture values were determined as 47.04-52.12% on average and salt was determined as 2.98 to 8.78% by mass. The average free fatty acid and residual nitrite values were found to be 0.031-0.118% and 0.159-10.241 ppm respectively.

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## 1. Introduction

Pastırma is a traditional meat product which the meat is obtained from certain parts of beef and then cured, dried, and covered with pasta called "çemen" (Hazar et al., 2017; Kaban, 2013; Öz & Kaya, 2019). The components used in the curing process are among the important factors that affect the quality of pastırma. Salt, nitrate and/or nitrite are used in the curing process. In addition to these ingredients, spices can also be used to enhance the flavor, smell, taste, appearance, and texture of the product during the curing process (Aksu et al., 2016; Kaban, 2013). The use of salt alone in pastırma production results in a firm texture and dark color (Hazar et al., 2017; Tekinşen & Doğruer, 2000).

Nitrite is used in meat products to prevent the formation of harmful bacteria that can cause food poisoning, inhibit the development of bacteria and their toxin production, as well as to promote color formation and prevent oxidation due to its antioxidant properties (Akköse et al., 2017; Honikel, 2008). However, nitrite also plays an important role in the formation of carcinogenic nitrosamines, which are formed as a result of the reaction between nitrogen oxide generated by the reduction of nitrite and amino groups (Sallan et al., 2019). The formation of these compounds is a complex process and depends on the

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level of nitrite used during the curing stage, production steps, microorganisms with decarboxylase activity, residual nitrite content, water activity and pH value (Sallan et al., 2020).

Salt ratio used in the curing stage of pastırma varies between 8-10% (Kaban, 2013). It plays a crucial role in reducing water activity, ensuring microbiological stability, dissolving muscle proteins that are important for texture development, and enhancing the salty taste in the final product (Hastaoğlu & Vural, 2018). On the other hand, salt has numerous negative effects on human health (Hastaoğlu & Vural, 2018). The World Health Organization (WHO) emphasizes that adults should consume less than 2 g of sodium per day (5 g of salt) (WHO, 2023).

Another important step in the production of pastırma is the covering process of meat with paste, known as "çemen". The main purpose of covering with pasta is to provide flavor, taste and texture to the product, as well as to enhance its color (Nizamlıoğlu et al., 1998). In the production of çemen, fenugreek seed flour (*Trigonella foenum graecum*), fresh garlic, red pepper and water are used (Kaban, 2013). Garlic present in çemen helps prevent the growth of various microorganisms and molds (Gökalp et al., 2012; Tekinşen & Doğruer, 2000). Kastamonu Pastırma is produced under natural conditions using traditional methods based on knowledge and skills of the craftsmen. What sets it apart from other pastırma varieties is the use of Taşköprü (Kastamonu) garlic in çemen production (Türker et al., 2019). Taşköprü garlic from Kastamonu is known for being rich in mineral substances (especially selenium) and vitamins. It is also characterized by its large size, resistance to climatic conditions and long shelf life (Doğantürk, 2016).

Within the scope of this study, samples of pastırma were collected from 8 different companies involved in the production and sale of pastırma in Kastamonu at 3 different time points. These samples were analyzed for moisture, pH, free fatty acids, residual nitrite, salt, and microbiological parameters. It is stated that the pH value of pastırma should be between 5.5 and 6 (Kaban, 2013). Additionally, according to the Turkish Food Codex Meat and Meat Products Regulation, the moisture content of pastırma should be below 50% (Tarım ve Orman Bakanlığı, 2019). Whether Kastamonu pastırma meets these criteria or not has been determined through scientific data. Furthermore, determining the microbiological characteristics of a food product is crucial for ensuring its quality and safety. This study aimed to reveal the physical, chemical and microbiological properties of pastırma in the Kastamonu market.

## 2. Materials and Methods

In the study, samples of pastırma (sırt type) were collected from eight different companies involved in the production and sale of pastırma in the Kastamonu market at three different time points. The collected pastırma samples were tested for pH, moisture content, salt, free fatty acid and residual nitrite levels, as well as microbiological parameters including *Micrococcus/Staphylococcus*, lactic acid bacteria, Enterobacteriaceae, total aerobic and mesophilic bacteria and yeast-mold.

### *2.1.* Analysis of pH

In order to determine the pH value, 10 g of pastırma sample was weighed and 100 ml of distilled water was added on each sample. The samples were homogenized for 1 minute using an Ultra-Turrax (ISOLAB, I.622.01.001, Germany). Before the pH value of the homogenized samples was measured using a pH meter (WTW, Germany), it was calibrated using suitable buffer solutions (pH 7.00 and pH 4.00).

### *2.2.* Moisture Analysis

For the purpose of determining the moisture content, 10 grams of pastırma sample was weighed into nickel containers. The samples were then dried in a drying cabinet (Megaterm, E420P) at 100-102 ºC until a constant weight was reached (18-24 hours). The amount of moisture of pastırma was determined based on the initial and final weights of the samples.

### *2.3.* Residual Nitrite and Salt Analysis

In order to specify the residual nitrite and salt content, 10 grams of samples were mixed with 10 ml of saturated borax solution. The mixture was homogenized using an Ultra-Turrax device (ISOLAB, I.622.01.001, Germany). The homogenate was then incubated in a boiling water bath (Nüve, ST30) at 100 °C for 15 minutes. After incubation, Carrez I and Carrez II solutions were put in the mixture and mixed together. The sample solution was transferred to a 200 ml measuring flask in order to get the appropriate volume. The measuring flask was stirred and kept waiting for 30 minutes at room temperature. The sample solution was then filtered twice using a filter paper (Whatman 595, diameter 150 mm, nitrate/nitrite-free). In order to determine the residual nitrite content, 10 ml of the filtrate was mixed with 10 ml of Griess reagent and incubated at room temperature in a lightless conditions for 30 minutes. After the 30-minute incubation period, the absorbance was measured at 540 nm with use of a spectrophotometer (PhotoLab7600 WTW, Germany) that was calibrated to the appropriate wavelength. The residual nitrite content was determined by taking dilution factor, sample volume and standard curve into account (Tauchmann, 1987). For salt analysis, 20 ml of the filtrate obtained from the residual nitrite analysis was taken and 10% potassium chromate solution was added on. Then, it was titrated using 0.1 N $AgNO_3$ solution in order to calculate the salt content.

### *2.4.* Free Fatty Acid

The sample of 17.5 g pastırma was taken for determining the amount of free fatty acids and 0.875 g of $Na_2SO_4$ and 35 ml

of chloroform were added on it. The mixture was then stirred for 5 minutes and filtered through Whatman No. 4 filter paper. After filtration, 25 ml of the filtrate was taken and titrated with 0.01 N potassium hydroxide-ethanol solution in the presence of phenolphthalein indicator. The amount of potassium hydroxide-ethanol solution used in the titration was benefited for result calculation and the content of free fatty acids was given as grams of oleic acid per 100 g of fat (Wang, 2001).

### *2.5.* Microbiological Analysis

MRS (De Man, Rogosa Sharpe, Merck) Agar medium was used for lactic acid bacteria enumeration in pastırma samples. Prepared from appropriate dilutions, spread method was applied on MRS Agar plates and after 2 days of anaerobic incubation at 30°C, the level of lactic acid bacteria was specified by considering catalase (-) colonies.

In order to determine the total number of aerobic mesophilic bacteria, PCA (plate count agar) plates, which were prepared previously from appropriate dilutions, were inoculated using spread method. After 2 days of incubation at 30 °C, the total number of aerobic mesophilic bacteria was determined.

RBC (Rose Bengal Chloramphenicol) Agar medium was used for yeast-mold enumeration. Petri plates were waited for 5 days at 25 °C after inoculation from appropriate dilutions using spreading method. Yeast and mold colonies developed as a result of incubation were counted and the level of yeast-molds in the samples was determined.

The number of *Micrococcus/Staphylococcus* was determined using previously prepared and sterilized MSA (Mannitol Salt Agar Oxoid) medium. The inoculated plates were incubated aerobically for 2 days at 30 °C and the level of *Micrococcus/Staphylococcus* was calculated regarding the catalase (+) cocci.

Enterobacteriaceae number in the samples was detected by spreading 0.1 mL of proper dilutions on VRBD (Violet Red Bile Dextrose) Agar plates. These plates were incubated for 2 days at 30 °C anaerobically. As a result of incubation, the level of Enterobacteriaceae was determined by counting red colonies larger than 1 mm.

### *2.6.* Statistical Analysis

In the study, pastırma from different companies were purchased at different times and analyzed. The trial was set up and carried out with 3 replications according to the randomized complete blocks trial plan. The results were applied to analysis of variance. The average of main sources of variation found to be statistically important were compared with the Duncan multiple comparison test (SPSS 20.0).

## 3. Results and Discussion

### *3.1.* pH

The pH values of pastırma samples taken from different companies at three different times are given in Table 1. It was determined that the highest pH value belonged to company E and the lowest to company C. It is thought that the reason why the pH values of the companies differ from each other is due to the differences in the production process of each company (drying time, the amount of salt used in curing, etc.) and the difference in raw materials. According to the Turkish Food Codex Communiqué on Meat, Prepared Meat Mixtures and Meat Products, it is stated that the pH value in pastırma can be at most 6.0 (Tarım ve Orman Bakanlığı, 2019). Among the pH values determined in the study, the value belonging to 4 companies was found above this upper limit.

### *3.2.* Moisture

When the average moisture values of different companies are examined, it is observed that the moisture value is highest in companies A, C and D; however, the lowest in companies B and G. According to the Turkish Food Codex Communiqué on Meat, Prepared Meat Mixtures and Meat Products, the amount of moisture in pastırma excluding fenugreek, can be up to 50% by mass (Tarım ve Orman Bakanlığı, 2019). It is thought that the moisture value varies depending on the fact that some companies do not sufficiently dry pastırma. What is more, the thickness of pastırma and their drying times may differ. Stability of meat products categorized in medium moisture food class such as pastırma can be achieved by reducing the amount of usable free water in its structures during the drying and curing stages. Otherwise, both the microbiological quality decreases and an undesirable product is obtained in terms of legislation (Işıksal et al., 2009). Aksu and Kaya (2001) determined the moisture content to be in the range of 38.92-51.81% in their study on pastırma samples obtained from Erzurum market.

### *3.3.* Residual Nitrite and Salt

Nitrite/nitrate prevents both the growth of microorganisms and pathogens that cause spoilage and delays the bitterness caused by the deterioration of fats, furthermore it forms the meat color and flavor of cured meat (Hui, 2012). However, in order for these effects to occur, nitrate must be reduced to nitrite in processes while using nitrate. Microorganisms with nitrate reductase activity have positive effect in reducing nitrate to nitrite (Akköse et al., 2017). The nitrite concentration in raw nitrate-cured meat products is quite low. Therefore, NO+ formation is very unlikely. However, nitrosamines may form in the products heated above 130 °C (Honikel, 2008). Due to the carcinogenic properties of nitrosamines, which are formed as a result of the reaction of nitrite with secondary amines in meat products, their use was restricted in the United States in the

1960s (Toldrá et al., 2009). Therefore, the level of use in food is limited by some countries (Aksu et al., 2005). According to the Turkish Food Codex Additives Regulation, the highest nitrite usage amount in non-heat-treated meat products is given as 150 ppm (Tarım ve Orman Bakanlığı, 2019). The mean residual nitrite amount was detected between 0.159-10.241 mg/kg (Table 1).

The use of salt in the production of cured meat products affects the chemical and microbiological properties of the product. It limits the growth of some pathogenic and spoilage microorganisms (Benetini et al., 2012). Salt values of pastırma samples are given in Table 1. It has been observed that the highest salt value belongs to companies B and F; however, the lowest average value belongs to company C. According to the Turkish Food Codex Communiqué on Meat, Prepared Meat Mixtures and Meat Products, the highest salt value in pastırma can be 10% (in dry matter) at most (Tarım ve Orman Bakanlığı, 2019). Among the salt values determined in the study, the values belonging to some companies were found above this upper limit. In another study on pastırma, the amount of salt was found to be in the range of 2.74-9.36% (Aksu & Kaya, 2001; Doğruer et al., 1995). In a study conducted by İhtiyar (2019) on Kastamonu pastırma, the lowest amount of salt was found to be 3.31% in Sırt Pastırma and 7.74% in average Kuşgömü Pastırma.

### *3.4.* Free Fatty Acid Value

It is stated that free fatty acids are important factors in flavor formation, physicochemical properties of fatty tissue and nutritional value of dry-cured ham, depending on their composition (Liu et al., 2019). Free fatty acid values in pastırma were found to be between 0.021 g oleic acid/100g and 0.242 g oleic acid/100g, depicted in Table 1. Similar to this study, the amount of free fatty acid in pastırma treated with different salt formulations was found to be 0.095-0.122 g oleic acid/100 g (Yalınkılıç et al., 2023).

**Table 1.** Moisture, pH, salt, free fatty acid and residual nitrite results of pastırma purchased from different companies at three different times (mean value ± standard deviation).

| Companies | pH | Moisture (%) | Salt (%) | Free Fatty Acid (g oleic acid/100 g) | Residual Nitrite (ppm) |
|---|---|---|---|---|---|
| A | 5.78±0.05d | 51.96±3.02a | 5.92±0.39b | 0.049±0.019d | 0.610±0.374d |
| B | 5.74±0.1e | 47.04±2.39c | 6.66±1.14a | 0.049±0.010d | 0.159±0.183d |
| C | 5.67±0.57f | 51.68±0.74a | 3.86±1.28f | 0.082±0.007b | 3.027±3.321c |
| D | 6.01±0.12b | 52.12±2.73a | 4.71±0.55e | 0.118±0.095a | 10.241±4.280a |
| E | 6.13±0.24a | 49.85±3.08b | 5.00±0.68de | 0.066±0.045c | 0.467±0.343d |
| F | 6.03±0.27b | 49.74±2.58b | 6.72±1.50a | 0.067±0.062c | 5.787±8.038b |
| G | 5.74±0.02de | 47.61±1.68c | 5.25±0.83cd | 0.031±0.010e | 0.175±0.074d |
| K | 5.89±0.03c | 49.96±2.71b | 5.54±1.09c | 0.096±0.061b | 0.291±0.190d |

Averages marked with different letters in the same column are different from each other.

### *3.5.* Microbiological Properties

The average values of the number of total aerobic mesophilic bacteria (TAMB) of pastırma obtained from the market are presented in Table 2. The highest number of TAMB was found in company C with an average of 7.76 log cfu/g. It was specified that the number of yeast-mold in pastırma was in the range of 3.46-7.21 log cfu/g. Kaban (2013) stated that the number of yeast and mold in pastırma increased during the drying stages and decreased in the fenugreek and final drying stages. Additionally, it has been emphasized that garlic, which constitutes 35% of the çemen mixture, protects the pastırma against mold.

Lactic acid bacteria and catalase-positive cocci are significant groups of microorganisms found in pastırma. Lactic acid bacteria contribute to the development of sensory and textural properties of the product with their low proteolytic activities and acid production. While lactic acid bacteria and *Micrococcus/Staphylococcus* bacteria are generally present in the raw material at the level of 2-3 log cfu/g, they show a significant increase during the production process (Kaban, 2013). Lactic acid bacteria, an important group in pastırma, also play a role in the development of the sensory properties of the product thanks to their low lipolytic activity (Öz et al., 2017). The highest lactic acid bacteria count in pastırma belonged to company C with the average value of 7.93 log cfu/g. Furthermore, the value of other companies did not fall below the level of $10^6$ cfu/g. Catalase positive cocci, which form the dominant flora in pastırma, have nitrate reductase activity as well as catalase activity. In addition, these microorganisms are crucial in forming color and flavor, color stability and oxidation delaying thanks to their lipolytic and proteolytic activities (Kaya & Kaban, 2010). These acid-sensitive bacterias can form the dominant flora in pastırma due to the appropriate pH (Kaban, 2013). The average number of *Micrococcus/Staphylococcus* in the samples varied between 6.50-7.52 cfu/g (Table 2).

The Enterobacteriaceae family, which includes many foodborne pathogens and unwanted microorganisms, mostly cannot survive in pastırma (Fettahoğlu et al., 2019; Hazar et al., 2017; Kaban, 2009, 2013; Öz et al., 2017). In the study, microorganisms belonging to the Enterobacteriaceace family were detected at different levels in the samples of companies C, D, F and K. In the samples of companies A, B and E, the number of Enterobacteriacea was found below the detectable limit (<2).

**Table 2.** Microbiological analysis results of pastırma purchased from different companies at three different times (cfu/g) (mean value ± standard deviation).

| Companies | TAMB | Yeast-Mold | Lactic Acid Bacteria | *Micrococcus/Staphylococcus* |
|---|---|---|---|---|
| A | 7.22±0.20bc | 6.12±0.49b | 6.78±0.34b | 7.22±0.14a |
| B | 6.87±0.62d | 6.10±0.95b | 6.07±0.99d | 6.74±0.70b |
| C | 7.76±0.45a | 4.09±0.40d | 7.93±0.60a | 6.50±0.40c |
| D | 7.27±0.35bc | 4.14±0.59d | 6.90±1.05b | 7.05±0.59a |
| E | 7.18±0.64bc | 6.51±0.50a | 6.30±0.55cd | 7.10±0.67a |
| F | 7.09±0.55c | 5.51±1.50c | 6.22±0.60d | 6.62±0.77bc |
| G | 6.81±0.52d | 6.20±0.53b | 6.50±0.53c | 6.52±0.61bc |
| K | 7.41±0.48b | 6.41±0.77a | 6.20±0.90d | 7.07±0.30a |

Averages marked with different letters in the same column are different from each other.

## 4. Conclusion

This study was focused on the analysis of characteristics of Kastamonu pastırma, taken from eight different companies at three different times in Kastamonu region. In the analysis, pastırma samples are examined in terms of moisture, pH, free fatty acids, residual nitrite, salt, and microbiological parameters. As a result, it was seen that some pH and moisture values exceeded the limits specified in the Turkish Food Codex Communiqué on Meat, Prepared Meat Mixtures and Meat Products. Manufacturers should pay more attention to pastırma drying process and take legal limits into consideration. On the other hand, it was concluded that Kastamonu pastırma is safe to consume in terms of salt and residual nitrite. Microbiologically, it has been determined that characteristics of pastırma varies according to companies and time. In addition, it has been observed that microorganisms belonging to Enterobacteriaceae family showed development in pastırma from some companies. This situation also shows that more attention should be paid to hygienic conditions.

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## Conflict of Interest

The authors declare that they have no conflict of interest.

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