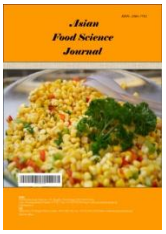

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Determination of Hydroxyproline Amount of Fermented Sausages by HPLC and Revealing the Relationship between Quality Parameters

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

Introduction: Fermented sausage is the food that has the largest share in consumption among other fermented meat products in Turkey. Although it is difficult to achieve the production standard in fermented sausage production, it is necessary not to exceed the legally determined reference values for quality production. Determining and controlling quality parameters in macro and micronutrients in the composition of fermented sausage, compliance with production standards, storage conditions, etc. has become a necessity.
Purpose: It is aimed to determine whether there is adulteration and imitation in the production of fermented sausages, to determine the structural defects during and after production, to evaluate their compliance with legal regulations and their nutritional quality.
Study Design: The study was mainly designed on the determination of hydroxyproline amounts with a High-Performance Liquid Chromatography device.
Study Place and Duration: The samples used in the study were collected within the borders of Burdur province. All experimental studies were carried out at Burdur Mehmet Akif Ersoy University (MAKU), Veterinary Faculty, Department of Food Hygiene and Technology; MAKU Scientific and

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Technology Application and Research Center and Uşak University, Engineering Faculty, Department of Food Engineering Laboratories. The study covers a period of 3 months between October 2020 and December 2020.
Methodology: A total of 30 fermented sausage samples, which are offered for sale by butchers and markets, were collected, brought to the laboratory by cold chain and subjected to analysis. After the samples were analyzed in terms of hydroxyproline, physicochemical and textural aspects, they were evaluated statistically.
Results: The pH values of the examined fermented sausages were 4.82-5.93, fat ratios were 12.50-38.50%, protein amounts were 12.24-24.49%, total ash values were 2.37-4.58%, salt values were determined as 1.97-4.05%, humidity values as 20.22-50.36%, water activity (a_w) values as 0.80-0.97, hydroxyproline amounts as 2.01-14.85 g/kg. In the textural analysis, elasticity was found between 0.51-0.91 mm, cohesiveness values between 0.37-0.85 and hardness in the range of 94.50-2564.13 mm.
Conclusion: The absence of adulteration in fermented sausage production, compliance with legal reference values and increasing the relevant inspections are of great importance in terms of the quality and nutritional value of the product.

Keywords: Collagen; connective tissue; fermented sausage; hydroxyproline; HPLC.

1. INTRODUCTION

Meat and meat products are important for human health and development with their macro and micronutrients and high bioavailability. For this reason, it is important to include meat and meat products in a balanced diet [1].

Meat and meat products can easily deteriorate if suitable storage conditions are not provided, allowing microorganisms to reproduce easily depending on the change in water activity (a_w) and pH values. Thus, traditional processes such as fermentation, curing, smoking and drying have been used to extend the preservation period of meat, and these processes have led to the emergence of meat products with different qualities such as colour, flavour, and texture [2,3].

In Turkey, beef, buffalo, goat, sheep and poultry meat products are produced as meat products. Distribution of meat products by species in 2019, poultry meat with 64.66%, beef with 31.64%, mutton with 3.22%, goat meat with 0.49% and buffalo meat with a production index of 0.00%, which decreased by 77% in the 2015-2019 period. [4]. Fermented sausage is one of the foods that have the largest production and consumption share among fermented meat products produced in Turkey. Sausage is defined as the meat product that is obtained as a result of certain fermentation and drying processes, by chopping bovine and/or ovine carcass meats and fats in a meat grinder, mixing them with various flavourings, then filling them in artificial or natural casings. The putrefaction test should be negative in the sausages and there should be no viscera or equine meat [5,6,7].

The quality of meat is determined by factors such as the animal's breed, age, chemical composition, structure, and connective tissue ratio in its composition. The high level of connective tissue in the composition of ready-to-eat meat products such as sausage and salami cause significant losses in the nutritive value of the meat by ensuring that the total protein amount comes mainly from the connective tissue. Collagen, which is among the connective tissue proteins, is the only basic building material that contains the amino acid hydroxyproline in its structure. Therefore, hydroxyproline analysis is necessary to determine the connective tissue ratio and total protein quality [8,9,10].

There are many methods to determine the amount of hydroxyproline, which is an indicator of the protein value of meat. In addition to histological and physical methods, the most commonly used methods in these analyzes are High-Performance Liquid Chromatography (HPLC), which has recently made rapid progress between colorimetric and chromatographic methods [10].

The HPLC method is based on the principle of separating the dissolved components in a column under high pressure, often between the stationary phase and the mobile phase on a solid support, leaving the column at different times and speeds. The HPLC method, as a widely used method, is characterized by its sensitivity, being easily adaptable to quantitative determinations,

non-volatile, etc. It has become indispensable in many areas with its suitability for the separation of compounds. HPLC is also suitable for the separation and analysis of compounds that are not suitable for other chromatographic methods [10,11].

Recently, with the developing technology, products that can be prepared more practically have started to be preferred, and in response to this demand, some manufacturers have turned to producing adulteration products that threaten public health in order to gain more income. For this reason, it has become important to measure the connective tissue ratio and hydroxyproline amounts of meat and meat products and to determine their protein qualities [12].

This study was carried out to describe the effects of hydroxyproline on the nutritional quality, bioavailability rates, chemical and textural properties of fermented sausages.

2. MATERIALS AND METHODS

2.1 Material Supply

In this study, 30 fermented sausages produced from 100% beef were used as material. Fermented sausages were obtained commercially from butchers and markets in Burdur. The samples were brought to Burdur Mehmet Akif Ersoy University, Faculty of Veterinary Medicine, Department of Food Hygiene and Technology, and subject to the following analyzes under the cold chain. All analyzes were performed in parallel.

2.2 Determination of Hydroxyproline

Hydroxyproline amounts in sausage samples were determined by the High-Performance Liquid Chromatography (HPLC) method. The method of Köse et al. was used in HPLC analysis by modifying it [13].

Sample preparation; 20 ml of 6 N HCl was added to 5 g sample and kept for 24 hours at 110 °C for hydrolysis. Then the volume was completed to 25 ml with deionized water and evaporated to remove the effect of HCl taken from the hydrolyzate. 100 µL of 2 N NaOH, 150 µL of saturated sodium bicarbonate and 1 ml of dansyl chloride were placed on the residue, and the resulting mixture was incubated at 40 ºC for 45 minutes and then left at room temperature for 10 minutes. 50 µL of 25% NH_3 was added to the mixture and waited for another 30 minutes at room temperature, and then 5 ml of ammonium acetate:acetonitrile (1:1 v/v) was added. The final mixture was passed through a 45 µm filter and injected into the HPLC system.

Chromatographic conditions;

Used Device and System Features: Shimadzu Prominence Brand HPLC CBM: 20ACBM
Detector: DAD (SPD-M20A) Column Furnace: CTO-10ASVp Pump: LC20 AT
Autosampler: SIL 20ACHT Computer Program: LC Solution
Mobile Phase: A: 0.1 M Ammonium acetate B: Acetonitrile

The gradient program and operating conditions of the HPLC method, which is used to determine the amount of connective tissue in sausage samples, are as follows:

Gradient program: 1 minute; Ammonium acetate (80%) – Acetonitrile (20%)
25 minutes; Ammonium acetate (50%) – Acetonitrile (50%)
40 minutes; Ammonium acetate (20%) – Acetonitrile (80%)
Column: ACE5 C-18 (250 x 4.6 mm, 5 µm)
Column Temperature: 40 °C
Flow rate: 1 ml/min
Injection volume: 50 µL
Wavelength; 254 nm
Recovery Values: 83%

2.3 Physicochemical Analysis

Fermented sausage samples were analyzed for pH, fat, protein, humidity, total ash and salt determinations.

2.3.1 pH

The pH value of the sausage samples was determined according to TS 3136 ISO 2917 [14]. 10 g samples were taken from each sausage and mixed in 100 ml distilled water until it became homogeneous, and pH measurement of the samples was completed with the calibrated Mettler Toledo pH meter.

2.3.2 Fat determination

Fat determination of the samples was carried out by acid hydrolysis method according to the Turkish Standards Institute (TSE) meat and meat products total fat determination standard [15].

2.3.3 Protein determination

Protein determination of sausage samples was made according to the Kjeldahl method (Velp Scientifica, UDK 139). First, the % nitrogen (N) amounts were determined by applying combustion, distillation and titration processes. Then, the amount of N obtained was multiplied by the coefficient of 6.25 to determine the % protein level [16].

2.3.4 Determination of total ash

Total ash determination analysis of sausage samples was made according to TS 1746 ISO 936, which is TSE's meat and meat products total ash determination method. 3-5 g of the homogenized sample was taken and left in the crucible, 1 ml of 10% magnesium acetate was added, and pre-combustion was performed. Then, the ash percentage was calculated by burning in the muffle furnace at 600 °C until a constant weight was obtained [17].

2.3.5 Salt determination

Salt value of sausage samples was determined according to TS 1747-1 ISO 1841-1 [18]. 0.1 N 5% potassium chromate (K_2CrO_4) required for salt analysis was prepared. After 10 g of sausage sample was homogenized and diluted with 100 ml of distilled water in bottles, 10 ml of filtrate was obtained by filtering through filter paper. After adding 1 ml of potassium chromate solution, which is used as an indicator, to the obtained filtrate, titration was carried out with 0.1 N silver nitrate ($AgNO_3$) until a stable brick red colour was obtained. The amount of $AgNO_3$ spent was recorded and the % salt content of the samples was calculated.

2.3.6 Determination of humidity

The moisture content of the sausage samples was determined according to TS 1743 ISO 1442 [19]. Empty drying cups were dried in an oven at 105 °C for 2 hours, then cooled in a desiccator and weighed on a precision balance. 5 g of sausage samples were placed in drying cups and weighed, and drying at the same temperature was applied and cooled in a desiccator at the end of the process. Then, their weights were calculated and the dry matter percentages were determined. The humidity ratios of the samples were calculated according to the formula given below;

Moisture amount %: 100 - Dry matter %

2.3.7 Determination of water activity

The water activity of the sausage samples was determined with the Testo 650 (Germany) water activity device. The water activity values of the samples placed in the sample cabinet were read and recorded approximately 20 minutes after the device was closed [20].

2.4 Textural Analysis

Elasticity, firmness and cohesiveness analyzes of fermented sausages were performed in triplicate using a tissue analyzer (Brookfield, CT3, Middleboro, MA, USA). The conditions stated in Table 1 were applied for the analyses.

After the sausage samples were cut into slices, data were obtained using the TA4/1000 probe for their analysis [21,22,23].

2.5 Statistical Analysis

Statistical analyzes of the obtained data were performed using Pearson correlation analysis with the help of SPSS for Windows Package program. The relationship between the textural and physicochemical analyzes was analyzed statistically.

3. RESULTS AND DISCUSSION

3.1 Hydroxyproline Results

As a result of the analyzes performed by applying the HPLC method, it was determined that the hydroxyproline contents of the sausage samples were at the lowest level of 2.01 g/kg, the highest 14.85 g/kg, and the average was 4.49 g/kg (Table 2). With these results, the connective tissue ratios in the total meat protein of sausages were calculated as the lowest 11.73%, the highest 73.12%, and the average was calculated as 25.32%. According to the data obtained, the connective tissue ratios in the total meat protein of the analyzed sausages are as seen in Fig. 1. It was determined that 19 of the examined sausage samples did not comply with the maximum 20% collagen connective tissue value in the total meat protein specified in the Turkish Food Codex Communique on Meat, Prepared Meat Mixtures and Meat Products in terms of hydroxyproline values [6]. Accordingly, it was concluded that 63.33% of the samples were outside of the maximum 20% collagen connective tissue limit value in the total meat protein.

Table 1. Textural analysis test conditions

Test type:	TPA	Recovery time:	0 sec
Aim:	20.0 %	Same Trigger:	True
Keep time:	0 sec	Pre-test Speed:	1.50 mm/sec
Trigger load:	5.0 g	Data rate:	100.00 points/sec
Speed test:	1.00 mm/sec	Prob:	TA4 / 1000; D
Rotation speed:	1.0 mm/sec	Fixture:	TA-RT-KIT
Number of Cycles:	2.0	Load Cell:	4500 g
Target Type:	Deformation %		

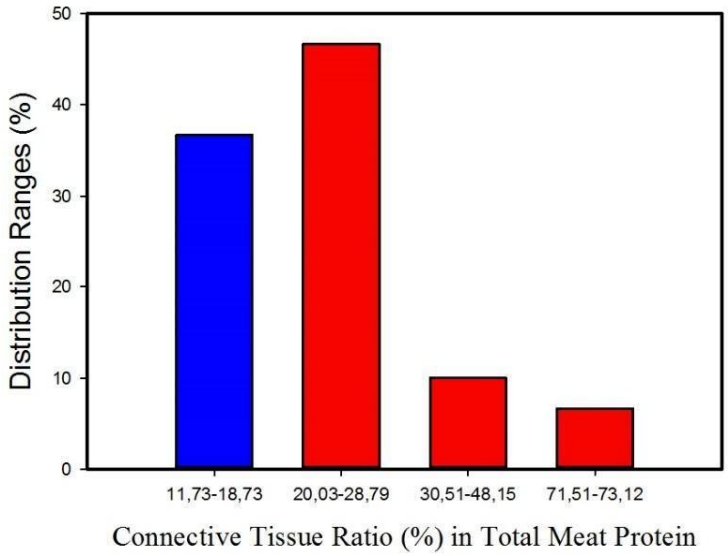

Fig. 1. Distribution of connective tissue ratio in total meat protein of fermented sausage samples (%)

The standard chromatogram, sample chromatogram and standard calibration graph used in the analysis are given in Figs 2, 3 and 4, respectively.

The data acquisition and processing computer, which is included in the composition of the HPLC method, create a chromatogram with the peaks of the concentration profiles of the substances coming out of the column. The chromatograms of the sausage samples created by the data collection and processing computer used in the study gave results compatible with the standard chromatogram. The obtained results show the importance and accuracy of the HPLC method for the determination of the amount of hydroxyproline in sausages.

3.2 Physicochemical Results

The results of the analysis on the physicochemical properties of 30 fermented sausages collected from different butchers and markets are as seen in Table 2.

The pH values of the fermented sausage samples were found to be between 4.82 and 5.93, with an average of 5.41. According to these results, it was determined that 46.6% of the samples did not comply with the phrase 5.4 the highest pH value specified in the Turkish Food Codex Communique on Meat, Prepared Meat Mixtures and Meat Products [6]. The fat values of the samples were between 12.50% and 38.50%, and the average was calculated as 25.68%. According to the data, it was determined that the samples did not exceed the 40% fat ratio, which is the legal reference value in the TS 1070 Turkish Sausage Standard [5]. In addition, it was determined that 10% of the samples had a maximum fat/total protein ratio above the 2.5 limit specified in the Turkish Food Codex Communiqué on Meat, Prepared Meat Mixtures and Meat Products. [6]. The protein values of the sausage samples were found to be between 12.24% and 22.49%, and the average was 17.23%. When these values were examined, it was determined that 40% of the samples were below the protein ratio, which should be at least

16% by mass, which was determined in the Turkish Food Codex Communiqué on Meat, Prepared Meat Mixtures and Meat Products [6]. As a result of the analysis, the total ash values of the sausage samples were found to be between 2.37% and 4.58%, with an average of 3.44%. While the salt values of the sausage samples varied between 1.97% and 4.05%, the average was determined as 2.53%. All of the analyzed samples comply with the statement that they should not exceed 5% salt rate stated in the TS 1070 Turkish Sausage Standard [5]. The moisture content of the sausage samples, calculated from the % dry matter results, was determined as the lowest 20.22%, the highest 50.36% and the average 39.02%. It was determined that 40% of the sausages did not comply with the statement that the ratio of moisture content to total protein should be below 2.5 in the Turkish Food Codex Communiqué on Meat, Prepared Meat Mixtures and Meat Products [6]. In addition, 56.67% of the 30 fermented sausages in the study do not meet the requirement that the moisture content be below 40% in the TS 1070 Turkish Sausage Standard [5]. The water activity values of the sausage samples varied between 0.80 and 0.97, with an average of 0.90. According to the findings, it was determined that 36.67% of the samples were above the limit values.

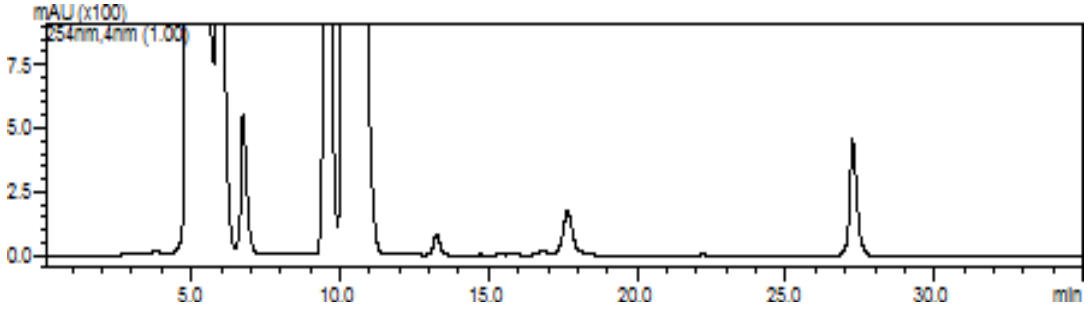

Fig. 2. Standard chromatogram

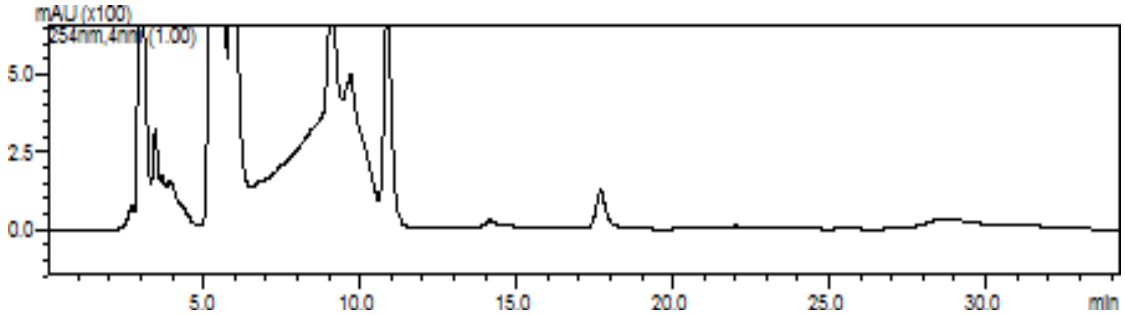

Fig. 3. Sample chromatogram

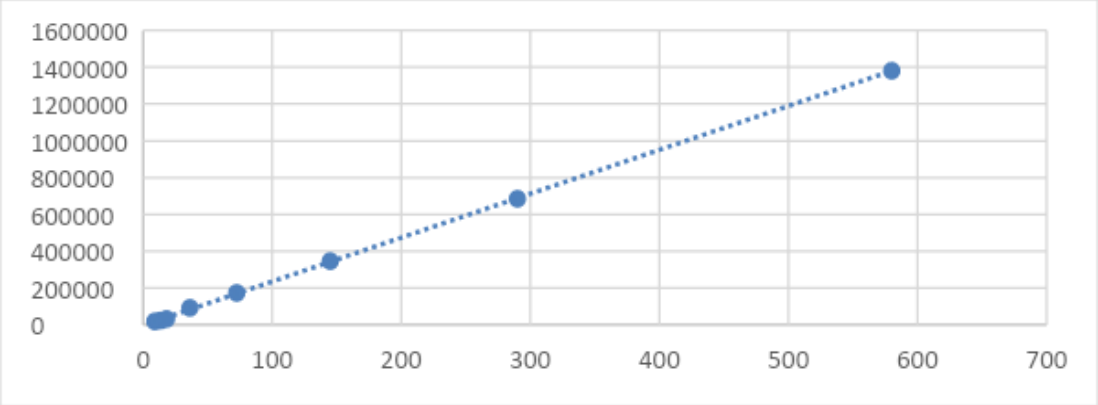

Fig. 4. Standard calibration graph

Table 2. Physicochemical analysis results of fermented sausages

Samples no	Ash%	Humidity%	Salt %	pH	a_w	Fat%	Protein%	Hydroxyproline(g/kg)
1	2.98	43.67	2.49	5.41	0.90	24.30	14,83	3,20
2	4.58	27.93	3.06	5.32	0.84	29.80	18,46	3,93
3	3.23	44.01	2.30	5.76	0.92	19.00	19,42	3,89
4	3.11	35.64	2.16	5.03	0.82	30.00	17,93	5,47
5	3.79	50.19	2.37	5.60	0.95	28.30	13,96	3,30
6	2.43	44.05	2.06	5.63	0.97	23.50	15,81	3,56
7	2.99	37.93	2.30	5.18	0.95	28.50	16,18	3,03
8	3.04	40.85	2.22	5.19	0.82	21.90	20,71	4,47
9	2.54	38.98	1.97	4.82	0.92	17.20	20,78	5,07
10	3.07	40.20	2.05	5.13	0.97	23.50	17,94	3,76
11	3.02	50.36	2.11	5.69	0.96	19.50	22,49	10,83
12	3.81	43.39	2.00	5.37	0.94	19.20	20,31	14,85
13	4.18	40.87	2.93	5.53	0.94	23.40	18,30	6,13
14	4.38	34.60	2.59	5.38	0.83	23.70	19,75	3,24
15	4.25	39.48	2.63	5.55	0.95	34.40	13,53	2,84
16	3.85	44.11	2.50	5.42	0.89	28.60	21,74	2,55
17	3.65	34.50	2.65	5.28	0.90	33.80	17,02	4,09
18	3.50	37.06	2.76	5.35	0.87	24.10	16,54	2,01
19	3.04	39.77	2.59	5.35	0.92	29.50	14,82	2,04
20	3.86	25.27	2.93	4.98	0.86	27.60	21,46	3,30
21	4.58	26.58	4.05	5.17	0.90	12.50	23,70	3,95
22	3.93	20.22	2.60	5.24	0.80	22.80	20,91	5,92
23	3.15	43.07	3.50	5.60	0.86	23.00	15,53	4,33
24	3.51	41.42	2.41	5.12	0.91	30.60	15,63	3,41
25	3.61	45.27	2.28	5.93	0.90	31.50	14,17	2,51
26	3.39	40.94	2.33	5.88	0.86	38.50	15,00	2,74
27	3.58	41.53	3.36	5.76	0.91	33.10	12,24	3,50
28	3.31	41.48	2.24	5.00	0.91	27.30	16,99	12,15
29	2.52	40.28	2.59	5.91	0.91	17.40	13,75	2,40
30	2.37	37.03	2.00	5.86	0.90	23.90	13,37	2,27
Avg.	3,44	39,02	2,53	5,41	0,90	25,68	17,44	4,49

Although there are legal obligations to be complied with within the production of sausage, both the composition and chemical properties of sausage produced by traditional methods differ according to the regions where they are produced. The results obtained in this study revealed that 14, 3, 12 and 11 samples of the sausages examined in terms of pH, fat, protein and aw values, respectively, did not comply with the reference values in TS 1070 Turkish Sausage Standard. When the results are evaluated in terms of moisture values, it has been shown that 12 fermented sausages according to the Turkish Food Codex and 17 according to the TS 1070 Turkish Sausage Standard are outside the legal limit values. It was determined that the legal reference values were exceeded in 26 out of 30 samples. These results reveal that the producers do not largely comply with the production standards in the production of fermented sausage with traditional methods.

According to the phrase specified in the TS 1070 Turkish Sausage Standard, sausages with a protein ratio of at least 16% are in the class 1 category, and those with at least 18% are in the extra class category. According to this statement, when the protein results of the sausage samples are examined, 6 of them are in the class 1 and 12 of them are in the extra class category. [5].

The variations in the results obtained in terms of moisture content, aw and pH values are closely related to the differences in the production season and duration of the sausage. Factors such as production conditions, storage time and storage conditions are the main reasons for these differences. a_w values are especially important in terms of technology and durability of meat products. In this regard, water activity and pH values of some German meat products were determined and a grouping was made related to durability times. These groups are named 'Easily

Perishable', 'Perishable' and 'Resistant' and are given in Table 3 [24].

The association of 30 fermented sausages used in the study with the endurance groups according to the values specified in Table 3 is as in Table 4. According to this table, only 3 fermented sausages are included in the perishable group, 12 fermented sausages in the perishable group, and 15 fermented sausages in the durable group, based on the a_w values alone. According to the pH values alone, 21 fermented sausages are included in the perishable group, 7 fermented sausages in the perishable group, and 2 fermented sausages in the durable group. Among the durability groups, only 8 fermented sausages that met the aw and pH criteria were included in the resistant category out of 30 samples. Findings obtained from fermented sausages offered for sale in different butchers and markets indicate that producers have problems in meeting water activity and pH criteria. Failure to meet these criteria may lead to the formation of an environment suitable for the growth of mould and bacteria.

According to the textural analysis results of the samples, the elasticity values were between 0.51-0.91 mm, the hardness values between 94.50-2564.13 mm, and the cohesiveness values between 0.37-0.85. The statistical analysis results of the textural characteristics of the sausage samples are as seen in Table 5. According to these results, the relationship between the hardness, cohesiveness and elasticity analysis results of the sausage samples used in the study was statistically significant (P<.01).

Table 3. Grouping of meat products according to water activity and pH values and storage conditions Endurance groups Criteria Storage temperature

Endurance groups	Criteria	Storage temperature
Group A	a_w value > 0,95	≤ + 5°C
“Easily Perishables”	pH value > 5,2	
Group B	a_w value ≤ 0,95 ≥ 0,91	≤ + 10°C
“Perishables”	pH value ≤ 5,2 ≤ 5,0	
Group C	aw value ≤ 0,95 and pH value ≤ 5,2 or	Not necessary to store in cold.
“Resistant”	only a_w value < 0,91 oronly pH value < 5,0	

Table 4. The distribution of durability groups of fermented sausages used in the study

Endurance groups	Only a_w value	Only pH value	a_w and pH values
Group A “Easily Perishables”	6, 10, 11	1, 2, 3, 5, 6, 11, 12, 13, 14, 15, 16, 17, 18, 19, 22, 23, 25, 26, 27, 29, 30	6, 11
Group B “Perishables”	3, 5, 7, 9, 12, 13, 15, 19, 24, 27, 28, 29	5, 7, 8, 10, 21, 24, 28	3, 5, 7, 24, 28
Group C “Resistant”	1, 2, 4, 8, 14, 16, 17, 18, 20, 21, 22, 23, 25, 26, 30	9, 20	4, 7, 8, 9, 20, 21, 24, 28

Table 5. Correlation coefficients of elasticity, hardness and cohesiveness values

Correlations		Elasticity	Hardness	Cohesiveness
Elasticity	Pearson Correlation	1	,690**	,739**
	Sig. (2-tailed)		,000	,000
	N	30	30	30
Hardness	Pearson Correlation	,690**	1	,461*
	Sig. (2-tailed)	,000		,010
	N	30	30	30
Cohesiveness	Pearson Correlation	,739**	,461*	1
	Sig. (2-tailed)	,000	,010	
	N	30	30	30

**Correlation is significant at the 0.01 level (2-tailed); *Correlation is significant at the 0.05 level (2-tailed).*

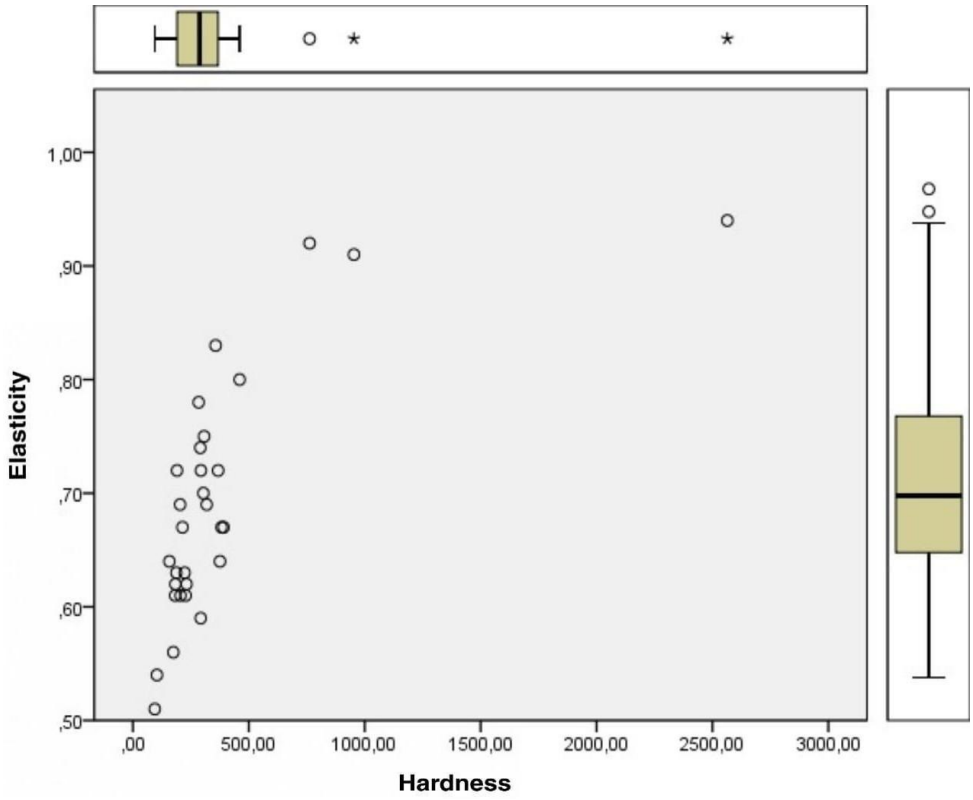

Fig. 5. The relationship between elasticity and hardness

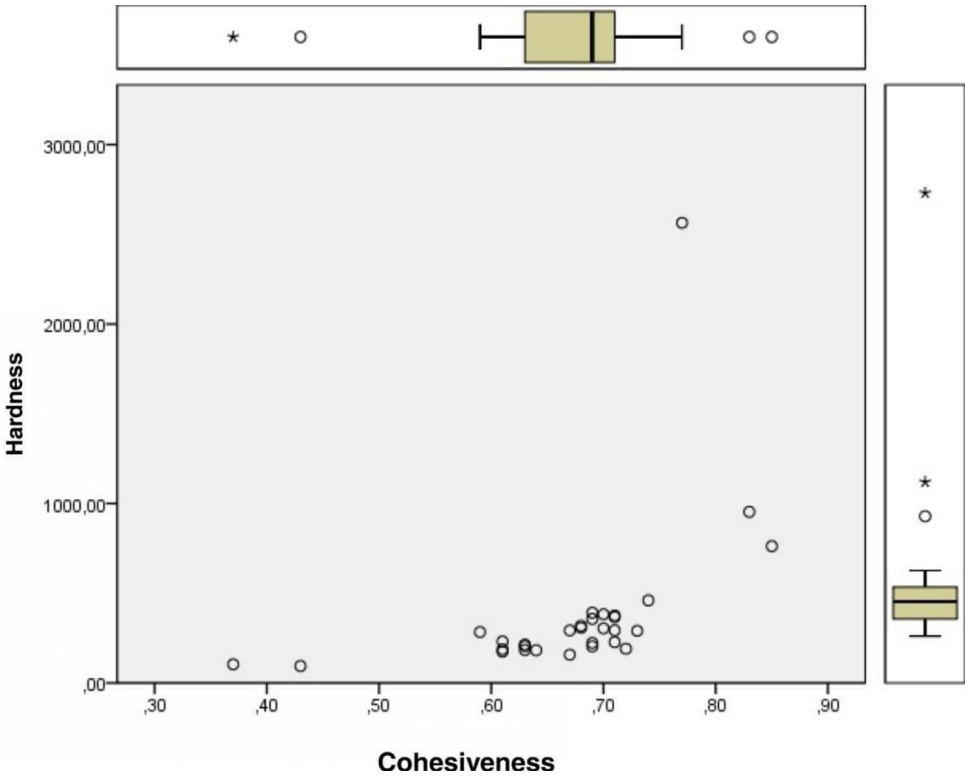

Fig. 6. Relationship between hardness and cohesiveness

Table 6. Correlation coefficients of elasticity, moisture, pH, water activity, fat variables

	Correlations	Elasticity	Moisture	pH	Water activity	Fat
Elasticity	Pearson Correlation	1	-,911**	-,328	-,688**	,099
	Sig. (2-tailed)		,000	,077	,000	,602
	N	30	30	30	30	30
Moisture	Pearson Correlation	-,911**	1	,451*	,588**	,060
	Sig. (2-tailed)	,000		,012	,001	,751
	N	30	30	30	30	30
pH	Pearson Correlation	-,328	,451*	1	,199	,132
	Sig. (2-tailed)	,077	,012		,291	,487
	N	30	30	30	30	30
Water activity	Pearson Correlation	-,688**	,588**	,199	1	-,103
	Sig. (2-tailed)	,000	,001	,291		,587
	N	30	30	30	30	30
Fat	Pearson Correlation	,099	,060	,132	-,103	1
	Sig. (2-tailed)	,602	,751	,487	,587	
	N	30	30	30	30	30

***Correlation is significant at the 0.01 level (2-tailed);*. Correlation is significant at the 0.05 level (2-tailed).*

Table 7. Correlation coefficients of hardness, moisture, pH, water activity, fat variables

	Correlations	Hardness	Moisture	pH	Water activity	Fat
Hardness	Pearson Correlation	1	-,756**	-,263	-,566**	-,039
	Sig. (2-tailed)		,000	,161	,001	,836
	N	30	30	30	30	30
Moisture	Pearson Correlation	-,756**	1	,451*	,588**	,060
	Sig. (2-tailed)	,000		,012	,001	,751
	N	30	30	30	30	30
pH	Pearson Correlation	-,263	,451*	1	,199	,132
	Sig. (2-tailed)	,161	,012		,291	,487
	N	30	30	30	30	30
Water	Pearson Correlation	-,566**	,588**	,199	1	-,103
activity	Sig. (2-tailed)	,001	,001	,291		,587
	N	30	30	30	30	30
Fat	Pearson Correlation	-,039	,060	,132	-,103	1
	Sig. (2-tailed)	,836	,751	,487	,587	
	N	30	30	30	30	30

***Correlation is significant at the 0.01 level (2-tailed);*. Correlation is significant at the 0.05 level (2-tailed).*

The correlation coefficient of elasticity and hardness variables is 0.690, and there is a strong positive linear relationship between them. Since the *P* value of the correlation between elasticity and hardness variables was 0.000, the relationship between them was statistically significant (*P*<.01) (Fig. 5).

The correlation coefficient between hardness and cohesiveness values was 0.461, and it was determined that there was a positive linear relationship between them. Since the *P* value of the correlation between these variables was 0.010, the relationship between the two variables was statistically significant (Fig. 6).

The correlation coefficient between elasticity and cohesiveness variables was found to be 0.739, and it was determined that there was a strong positive linear relationship between them. In addition, since the *P* value of the correlation between elasticity and cohesiveness values was 0.000, the relationship between the two variables was statistically significant (*P*<.01) (Fig. 7).

Table 8. Correlation coefficients of cohesiveness, moisture, pH, water activity, fat variables

	Correlation	Cohesiveness	Moisture	pH	Water activity	Fat
Cohesiveness	Pearson Correlation	1	-,740**	-,465**	-,512**	,263
	Sig. (2-tailed)		,000	,010	,004	,161
	N	30	30	30	30	30
Moisture	Pearson Correlation	-,740**	1	,451*	,588**	,060
	Sig. (2-tailed)	,000		,012	,001	,751
	N	30	30	30	30	30
pH	Pearson	-,465**	,451*	1	,199	,132
	Sig. (2-tailed)	,010	,012		,291	,487
	N	30	30	30	30	30
Water activity	Pearson Correlation	-,512**	,588**	,199	1	-,103
	Sig. (2-tailed)	,004	,001	,291		,587
	N	30	30	30	30	30
Fat	Pearson	,263	,060	,132	-,103	1
	Sig. (2-tailed)	,161	,751	,487	,587	
	N	30	30	30	30	30

***Correlation is significant at the 0.01 level (2-tailed); *. Correlation is significant at the 0.05 level (2-tailed).*

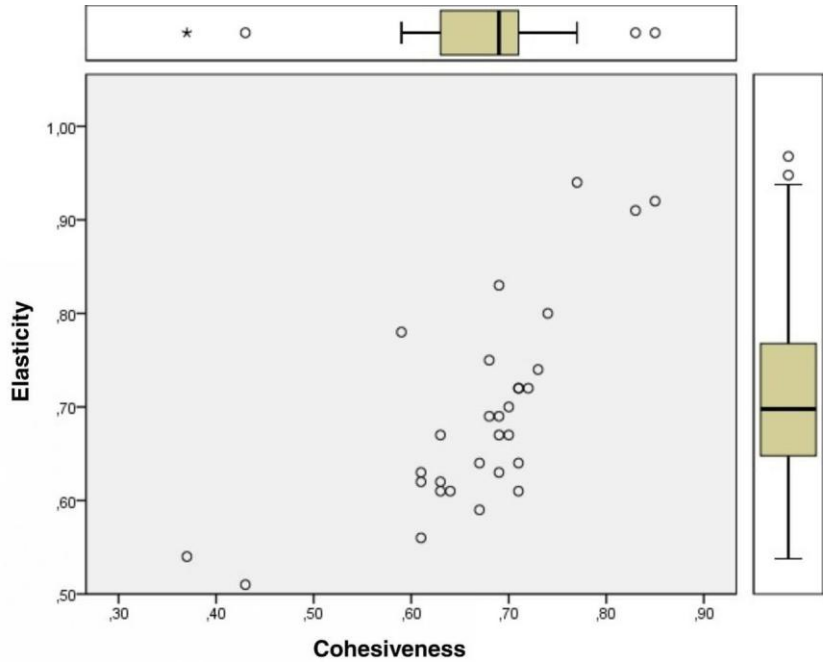

Fig. 7. The relationship between elasticity and cohesiveness

The statistical relationships of elasticity, hardness and cohesiveness values in the textural analysis part of the study with humidity, pH, water activity and fat variables are presented in Tables 6, 7 and 8, respectively. The results showed that the elasticity values of 30 fermented sausages used in this study were affected by moisture and water activity, hardness values were affected by oil, moisture and water activity, and adhesiveness values were affected by moisture, pH and water activity.

4. CONCLUSION

In this study, besides the determination of the amount of hydroxyproline by HPLC method of 30 fermented sausages offered for sale in different butchers and markets, physicochemical

properties such as pH, fat, protein, total ash, salt, humidity, water activity, and textural properties such as elasticity, hardness and stickiness were determined. It is based on the determination of the relations between these values. The fact that hydroxyproline levels were outside the legal limits in most of the sausages revealed that the products were adulterated by adding connective tissue-containing viscera and offal-like substances to the products. This situation both negatively affects the quality of the product and causes deception of consumers. In addition, the physicochemical analyzes carried out showed that some of the samples were outside the legal limits in terms of these qualities. It has been revealed in this study that the unsuitable values of sausage and similar products in terms of physicochemical properties also negatively affect the textural properties of the product. All these results revealed that there is a need for more inspections in the production of meat products and that the production of high quality and nutritious products can be realized depending on these inspections.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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