Research Article

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Free amino acids profile and quantities of 'sırt', 'bohca' and 'sekerpare' pastirma, dry cured meat products

Sinan Ceylan and Muhammet İrfan Aksu*

Abstract

BACKGROUND: Pastirma is a dry-cured and semi-fermented meat product, produced from whole beef or water buffalo muscles. Sixteen to twenty different types of pastirma can be produced from a carcass, and each pastirma type has a different name, shape and quality characteristics. The objective of this study was to determine the free amino acid (FAA) composition, pH and moisture values of 'sırt', 'bohca' and 'sekerpare' pastirma.

RESULTS: Significant differences were determined among the pastirma types in glutamic acid ($P < 0.05$), threonine ($P < 0.05$), arginine ($P < 0.05$), alanine ($P < 0.01$), methionine ($P < 0.01$), tryptophan ($P < 0.05$), proline ($P < 0.01$) and valine ($P < 0.01$). Threonine, methionine and valine were higher in sırt pastirma than the others; glutamic acid, arginine and tryptophan were higher in sekerpare pastirma. The predominant FAA in the all pastirma samples was alanine, while the lowest was asparagine. The highest mean pH was in sırt pastirma, and the lowest mean pH in bohca pastirma. There was no significant difference in mean moisture values among pastirma types.

CONCLUSION: Significant differences were determined among pastirma types in some FAA. Analysis results indicated that quality and nutritional properties for different pastirma types are different. The most advantageous pastirma types in terms of FAA were bohca and sırt, which contain the highest quantity of threonine, methionine and valine.
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Keywords: pastirma types; dry-cured meat; free amino acids; pH; moisture

INTRODUCTION

Pastirma is a dry-cured and semi-fermented meat product, produced from whole beef or water buffalo muscles.[1] Low-temperature and short-time ripening/production are typical processes in production of pastirma, which is different from that of the dry-cured ham. Some factors, such as the product's composition, type of muscle, temperature, pH and the available substrate for proteolytic enzymes are highly effective in the formation of free amino acids (FAAs) in pastirma and other dry-cured meat products processed in pieces.[2,3] Proteolysis is the most significant reaction placing in pieced and processed and dry-cured meat products during ripening. FAAs in pastirma and other similar meat products are generated by aminopeptidases originating in the meat's structure at the last stage of proteolysis.[4] Some researchers have determined that the compounds which are a potential source for the formation of volatile and non-volatile aromatic ingredients are generated by degradation of FAAs, which contribute to tastiness.[5]

Pastirma is produced from whole muscle and/or muscles obtained from certain parts of beef and water buffalo carcasses. To produce the pastirma, the main processes are curing, drying, pressing, re-drying, re-pressing and coating with a cement consisting of garlic, red pepper, paprika, flour ground from seed of *Trigonella foenum-graecum*, and water, and then re-drying to a maximum of 40% moisture. This process takes approximately a month, and significant structural changes take place during this period. Sixteen to twenty different types of pastirma can be produced from a carcass, and each pastirma type has different name, shape and quality characteristics.[1,6–8] Also, the quality of pastirma is ranked according to muscles used in pastirma production as first, second or third class according to the Turkish Pastirma Standards. In the Pastirma Standard,[9] 'sırt', 'sekerpare' and 'bohca' pastirma types are defined as first class. Thus the production of sırt, bohca and sekerpare pastirma types is generally more widespread compared to other pastirma types, thanks to their high-quality properties. The fat distribution in these pastirma types, which are more tender than the other types, is more evident and the color displays variation from pink to red.[9]

Sırt pastirma is rectangular, 60–70 cm long, 15–20 cm wide and 3–4 kg in weight. It is more delicious, crispier and more easily digestible than the other pastirma types. Bohca pastirma is triangular, on average 60 cm long and 15 cm wide and 1.5–2 kg in weight. Sekerpare pastirma is rectangular, 55–60 cm long, 10–12 cm wide and 1.2–1.5 kg in weight. While sırt pastirma is produced from the muscles/muscle groups found in the loin part

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of the carcass, bohca and sekerpare pastirma types are produced from muscles/muscle groups obtained from the round section of carcass.[6,10]

Many studies have been conducted on traditional Turkish meat products in the last decade on determining and improving the chemical properties that contribute to the quality and functional properties of pastirma produced by different methods.[3,11–13] Non-protein nitrogen and FAAs have been found to increase during production of pastirma.[3,11–13,14] Similarly, in dry-cured meat products, as a consequence of the decomposition of the proteins into peptides and amino acids due to proteolytic enzymes, the amount of non-protein nitrogen has been reported to increase.[2] However, there is no research concerning the free amino acid composition of pastirma types. Thus the present study was conducted to determine the FAA composition, moisture and pH values of sırt, bohca and sekerpare pastirma types.

MATERIALS AND METHODS

Materials

A total of 39 pastirma samples (14 sırt, 16 bohca and 9 sekerpare) collected from four different provinces in Turkey were analyzed. All samples were placed in a plastic bag and transported to the laboratory and stored at 4 °C until analysis. After slicing and cutting with a steel knife, small pieces (1–2 mm × 1–2 mm) were used for analysis. The free amino acid composition, pH and moisture values were obtained for all samples.

pH and moisture analysis

The pH value was determined by homogenizing (after Nach Prof. P. Willem, Janke and Kunkel KG, IKA Werk, Staufen I. Breisgau) a 10 g sample with 100 mL distilled water, then measuring pH using a star probe (Orion 3-Star, pH benchtop meter, Thermo Scientific, Singapore). Moisture value was determined as weight loss of ∼10 g sliced and cut pieces of pastirma samples after drying at 100 ± 2 °C for 18 h.

Free amino acid analysis

FAAs were analyzed by high-performance liquid chromatography (HPLC) according to Aristoy and Toldra,[15] Antoine *et al*.,[16] and Henderson *et al*.,[17] with some modifications. Sample preparation stages for FAAs are shown in Fig. 1.

Samples were analyzed by an Agilent (Germany) 1200 model liquid chromatography device (with double-detectors: ultraviolet (UV) and florescence detector (FLD)) with an autosampler and UV (338 nm) for cysteine, valine and hydroxyproline, and an FLD (florescence, 262 nm) detector for aspartic acid, glutamic acid, asparagine, serine, glutamine, histidine, glycine, threonine, arginine, alanine, tyrosine, methionine, tryptophan, phenylalanine, isoleucine, leucine, lysine and proline. The column was a Zorbax Eclipse-AAA 4.6 × 150 mm, 3.5 µm (Agilent PN 963 400-902). As the derivatization reagent for amino acids, *ortho*-phthalaldehyde (OPA; Agilent PN 5061-3335) and 9-fluorenylmethyl chloroformate (FMOC; Agilent PN 5061-3337) were used, while 0.4 mol L^{-1} borate (Agilent PN 5061-3339) (pH 10.2) was employed as the buffer solution. The following were used as the mobile phase in the chromatography system: mobile phase A: 40 mmol L^{-1} NaH_2P0_4 (pH 7.8); mobile phase B, acetonitrile (ACN)–methanol (MeOH)–water (45 : 45 : 10, v/v/v) solutions. The flow rate of the mobile phase moved through the system at 2 mL min^{-1} and the column temperature was 40 °C.

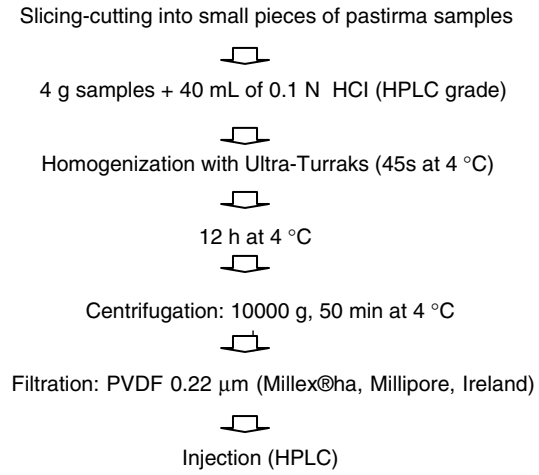

Figure 1. Sample preparation flowchart for FAA analysis.

The chromatographs related to the amino acid standard used in the research are displayed in Figs 2 and 3. The calibration table has been prepared such that the amino acid standards are moved through the system at five different concentrations, which are 10, 25, 100, 250 and 1000 pmol μL^{-1} respectively. While hydroxyproline, asparagine, glutamine and tryptophan (PN 5062-2478) were prepared at five different concentrations, valine, aspartic acid, glutamic acid, serine, histidine, glycine, threonine, arginine, alanine, tyrosine, methionine, phenylalanine, isoleucine, leucine, lysine and proline were obtained from Agilent Technologies as a mixture of a 17-amino acid solution at five different molarities, which were 10 pmol μL^{-1} (PN 5061-3334), 25 pmol μL^{-1} (PN 5061-3333), 100 pmol μL^{-1} (PN 5061-3332), 250 pmol μL^{-1} (PN 5061-3331) and 1000 pmol μL^{-1} (PN 5061-3330).

Statistical analysis

The study was carried out according to a completely randomized design procedure. The type of pastirma (sırt, bohca, sekerpare) was chosen as a single factor. One-way analysis of variance (ANOVA) via the SPSS 10.01 (SPSS 1996) program package was conducted. Comparisons of the averages from the variation sources that were found to be significant were made using Duncan's multiple range test, and the results of statistical analysis are shown as mean values and standard deviations in the tables.

RESULTS AND DISCUSSION

Free amino acids

The amounts of free amino acids determined in different pastirma types, their significance levels and Duncan's test results are shown in Table 1 and Fig. 4. The three pastirma types had the same free amino acids, but in different amounts. Pastirma type significantly affected glutamic acid ($P < 0.05$), threonine ($P < 0.05$), arginine ($P < 0.05$), alanine ($P < 0.01$), methionine ($P < 0.01$), tryptophan ($P < 0.05$), proline ($P < 0.01$) and valine ($P < 0.01$) amounts. The highest glutamic acid ($P < 0.05$) was detected in sekerpare pastirma, whereas the lowest amount was detected in bohca pastirma (Fig. 4). Glutamic acid is the most commonly found amino acid in beef (14.4% protein).[18] However, Banon *et al*.[19] determined that only 3.08% free amino acid was detected in dry-cured hams and this was constituted of glutamic acid, while

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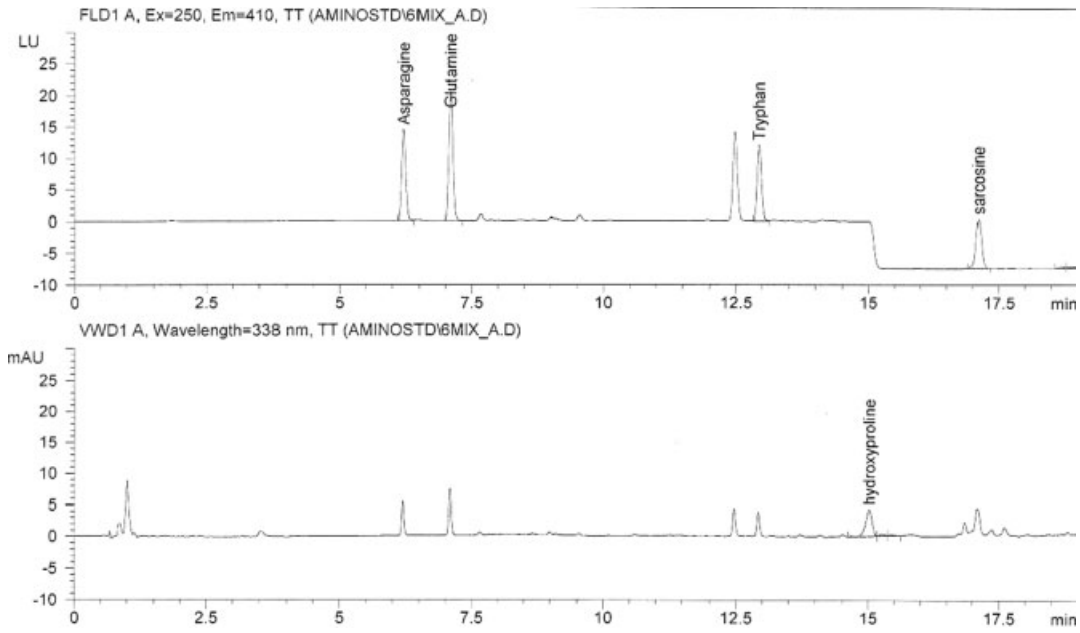

Figure 2. Chromatogram of hydroxyproline, asparagine, glutamine and tryptophan.

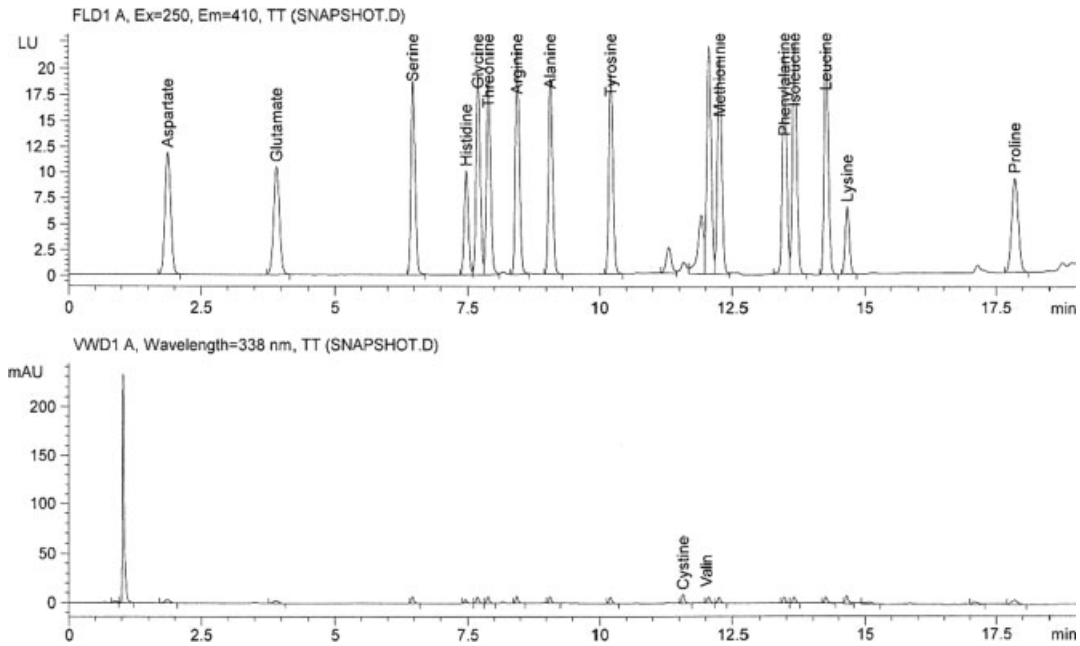

Figure 3. Chromatogram of valine, aspartic acid, glutamic acid, serine, histidine, glycine, threonine, arginine, alanine, tyrosine, methionine, phenylalanine, isoleucine, leucine, lysine and proline.

tyrosine was the most commonly free amino acid at 70.54%. The glutamic acid quantity in Iberian hams was 947.4 mg 100 g^{-1} dry matter (DM).[20] The amount of glutamic acid as a free amino acid in the cured hams produced from the *biceps femoris*, semitendinosus and *semimembranosus* muscles was 455.9 mg 100 g^{-1} DM.[21] El-Mahdy and El-Sebaiy[22] concluded that in the seeds of *Trigonella foenum-graecum*, which is used in the preparation of the cement for pastirma production, the most commonly found amino acids were glutamic and aspartic acids.

The threonine amount was significantly ($P < 0.05$) affected by pastirma types. The highest threonine amount was determined in sırt pastirma (50.75 mg 100 g^{-1} dry matter) and the lowest (41.01 mg 100 g^{-1} dry matter) in bohca pastirma (Table 1). In Iberian hams produced by dry-curing, threonine amount was reported to be 330.6 and 395.0 mg 100 g^{-1} DM.[21] However, the amount of threonine in hams produced from *semimembranosus* muscles is higher than that of the hams produced from *biceps femoris* muscles.[23]

The amount of arginine in sekerpare pastirma was notably higher compared to other pastirma types. In general, a higher amount was observed in pastirma types such as bohca and sekerpare produced from the round section of the carcass. It has been determined that the amount of arginine as an FAA in hams produced from *semimembranosus* muscle used in the production

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Table 1. Amounts of FAAs in sırt, bohca and sekerpare pastirma types (expressed as mg 100 g^{-1} dry mass)

	Type of pastirma			
Free amino acid	Sırt	Bohca	Sekerpare	Significance
Aspartic acid	16.70 ± 10.79 (2.47–39.02)	11.57 ± 6.46 (2.72–24.31)	14.01 ± 8.03 (3.61–30.41)	n.s.
Glutamic acid	117.67 ± 40.93[ab] (39.53–217.94)	105.74 ± 23.43b (60.72–166.29)	135.11 ± 40.74a (74.25–210.51)	*
Asparagine	4.22 ± 1.26 (1.47–6.79)	4.79 ± 1.42 (2.56–8.55)	4.98 ± 1.26 (2.89–6.84)	n.s.
Serine	57.44 ± 20.31 (16.56–102.18)	49.83 ± 15.81 (26.44–84.31)	54.46 ± 16.17 (31.80–84.50)	n.s.
Glutamine	130.82 ± 83.18 (12.22–319.77)	170.22 ± 90.08 (38.36–394.16)	137.56 ± 79.43 (53.14–275.43)	n.s.
Histidine	35.41 ± 12.16 (10.93–62.02)	30.06 ± 9.74 (16.35–50.95)	30.72 ± 6.56 (19.33–41.36)	n.s.
Glycine	49.28 ± 21.96 (11.41–109.52)	42.13 ± 12.36 (23.90–80.59)	42.56 ± 14.83 (20.82–71.48)	n.s.
Threonine	50.75 ± 17.94a (15.01–94.63)	41.01 ± 13.39b (19.82–70.30)	46.41 ± 11.45[ab] (29.40–69.08)	*
Arginine	80.36 ± 21.57b (39.07–111.20)	93.35 ± 27.85a[b] (37.45–153.03)	101.10 ± 30.41[a] (38.15–141.68)	*
Alanine	434.88 ± 90.83b (273.31–650.49)	530.39 ± 101.81[a] (339.89–729.90)	523.45 ± 125.51[a] (305.09–680.85)	**
Tyrosine	128.44 ± 73.86 (50.40–293.03)	141.60 ± 49.10 (38.96–231.31)	120.09 ± 58.70 (46.17–224.40)	n.s.
Methionine	17.73 ± 12.82a (4.07–55.89)	11.54 ± 8.73b (4.28–52.61)	9.50 ± 2.86b (5.64–15.34)	**
Tryptophan	16.07 ± 4.73[ab] (5.68–24.81)	14.96 ± 3.68b (7.25–24.55)	18.62 ± 5.91a (10.16–28.85)	*
Phenylalanine	65.84 ± 22.35 (30.01–115.05)	65.82 ± 23.33 (27.18–117.25)	71.76 ± 16.27 (40.78–101.74)	n.s.
Isoleucine	56.16 ± 18.90 (22.86–98.11)	54.23 ± 21.66 (2.08–104.95)	49.86 ± 21.62 (2.21–81.39)	n.s.
Leucine	102.08 ± 35.79 (43.53–183.05)	100.79 ± 38.01 (44.15–198.37)	110.40 ± 25.04 (57.20–154.26)	n.s.
Lysine	123.00 ± 45.18 (47.42–237.80)	107.60 ± 38.45 (53.03–197.05)	112.24 ± 25.64 (55.01–148.66)	n.s.
Proline	35.24 ± 12.66a (17.28–57.71)	24.68 ± 8.59b (12.58–49.04)	33.45 ± 10.12a (17.81–51.55)	**
Cysteine	8.99 ± 5.68 (3.42–28.02)	8.22 ± 4.68 (2.39–22.84)	8.64 ± 3.02 (4.75–15.46)	n.s.
Valine	48.50 ± 31.44a (19.84–121.69)	32.59 ± 12.33[b] (15.11–64.97)	35.68 ± 7.71b (19.12–46.68)	**
Hydroxyproline	24.71 ± 6.36 (13.12–36.48)	21.87 ± 6.02 (14.95–33.34)	24.00 ± 6.10 (13.70–35.23)	n.s.

Values ± standard deviation.
Values with different letters along rows are significantly different ($P < 0.05$).
* $P < 0.05$;
** $P < 0.01$; n.s., means not significant.

of sekerpare pastirma is also higher than in hams produced from other muscles (*biceps femoris*).[23]

There was a significant difference ($P < 0.01$) in the alanine amount of pastirma types. Among the pastirma types, the lowest value was found for the sırt pastirma (434.88 mg 100 g^{-1} DM) ($P < 0.05$) and there was no difference between bohca (530.39 mg 100 g^{-1} DM) and sekerpare (523.45 mg 100 g^{-1} DM) pastirma (Table 1). As can be seen from Fig. 4, alanine amount in pastirma types produced from the round section of the carcass was higher than it was in sırt pastirma types. Among the pastirma types produced from the round section, the alanine amount of bohca pastirma was found to be higher than that of sekerpare. This amino acid has also been reported as the most abundant in beef meat and dry-cured ham. Regarding the FAA composition of beef, it has been reported that the amount of alanine is higher than that of other amino acids.[24] The most common FAA

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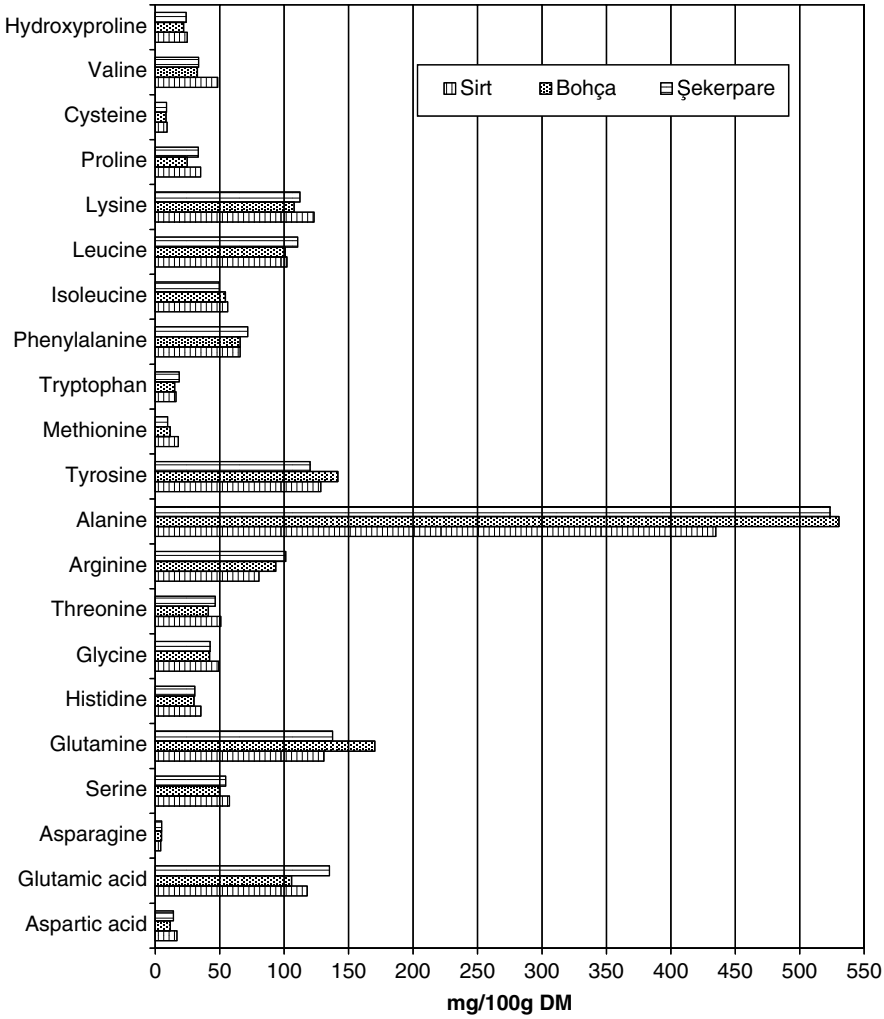

Figure 4. Typical profiles of the free amino acids in sırt, bohca and sekerpare pastirma types.

found in hams produced from the *semimembranosus* and *biceps femoris* muscles was alanine.[23] Flores *et al*.[20] also determined that the most abundant free amino acid in Iberian dry-cured hams is alanine, but Perez-Juan *et al*.[21] found that alanine constitutes the largest amount after lysine and leucine in dry-cured ham.

The different pastirma types showed significant differences in methionine. Among the pastirma types, the highest value was found in the sırt pastirma (17.73±12.82 mg 100 g^{-1} DM) ($P < 0.05$) but there was no difference between bohca (11.54 ± 8.73 mg 100 g^{-1} DM) and sekerpare (9.50 ± 2.86 mg 100 g^{-1} DM) pastirma (Table 1) in methionine. Also, as can be seen from Fig. 4, the amount of methionine in bohca and sekerpare pastirma produced from the muscles of the round part of the carcass was lower than in sırt pastirma. The methionine in the hams produced from the *semimembranosus* and *biceps femoris* muscles was 36 and 32 µmol g^{-1}, respectively.[25] Similarly, it was found that the amount of methionine in dry-cured hams produced from *biceps femoris*, semitendinosus and *semimembranosus* muscles was 232.6 mg 100 g^{-1} DM.[21] Aristoy and Toldrá[26] determined that, in pork carcass, methionine in *longissimus dorsi* muscle is higher than in the *semimembranosus* muscle.

There was a significant difference ($P < 0.05$) in tryptophan in the pastirma types. Among the pastirma types, the amount of tryptophan was highest in sekerpare pastirma, with 18.62 mg 100 g^{-1} DM, and lowest in bohca pastirma, with 14.96 mg 100 g^{-1} DM. Tryptophan is the free amino acid that demonstrates the largest increase during ham production. The researchers have stated that the amount of tryptophan in *semimembranosus* muscles at the beginning of production is 1.2 ± 01 µmol 100 g^{-1} DM, and this amount increased to 963.4 ± 17.2 µmol 100 g^{-1} DM in seasoned hams. Also an increase of 900-fold in hams made from *biceps femoris* muscle has been reported.[23] Perez-Juan *et al*.[21] also reported that the amount of tryptophan in hams was 114.2 mg 100 g^{-1} DM.

There was a significant difference ($P < 0.01$) in proline amount of pastirma types. Among the pastirma types, the lowest value (24.68 mg 100 g^{-1} dry matter) was found for bohca pastirma, while there was no difference between sırt and sekerpare pastirma (Table 1). However, it was detected that no difference exists between *longissimus dorsi* and *semimembranosus* muscles with respect to the amount of proline FAA.[26]

It was observed that there was a very significant ($P < 0.01$) difference in valine among pastirma types, the highest valine being determined in sırt pastirma type at 48.50 mg 100 g^{-1} DM (Table 1). The amounts of valine in bohca and sekerpare pastirma types produced from the round part of the carcass were similar and also less than the value detected in sırt pastirma. The amount

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Table 2. Moisture and pH values of sırt, bohca and sekerpare pastirma types

Property	Types of pastirma			Significance
	Sırt	Bohca	Sekerpare	
Moisture (%)	47.17 ± 5.00 (35.83–55.70)	46.61 ± 5.42 (36.31–57.34)	47.96 ± 3.21 (41.40–53.53)	n.s.
pH	5.86 ± 0.12a (5.60–6.06)	5.79 ± 0.12b (5.58–6.12)	5.82 ± 0.10ab (5.67–5.98)	*

Values ± standard deviation.
Values with different letters along rows are significantly different ($P < 0.05$).
* $P < 0.05$; n.s., means not significant.

of valine as FAA found in the *longissimus dorsi* muscles was higher than in the *semimembranosus* muscle.[26]

Pastirma types had no effect ($P > 0.05$) on the amounts of aspartic acid, serine, glutamine, histidine, glycine, phenylalanine, isoleucine, leucine, lysine, proline, cysteine and hydroxyproline (Table 1). In meat products produced by the dry-curing method and long-duration ripening, the value of aspartic acid as an FAA is reported as 516.5 mg 100 g^{-1} DM,[20] 218.2 mg 100 g^{-1} DM[21] and 1048.3 ± 11.2 µmol 100 g^{-1} DM.[23] The amount of serine was reported to be 399.1–401.3 mg 100 g^{-1} DM.[20,21] The amount of glutamine as an FAA in hams produced from *semimembranosus* muscles was reported to be 1730.3 ± 30.1 µmol 100 g^{-1} DM and in those made from *biceps femoris* muscles to be 1955.0 ± 30.3 µmol 100 g^{-1} DM.[23] Histidine is considered to be an essential amino acid for children and constitutes 2.9% of the raw protein in beef. As for histamine, it is formed as a result of decarboxylation of histidine by the enzyme bacterial histidine decarboxylase.[18] The amount of histidine was considerably lower in all pastirma types than in dry-cured hams.[20,21,23] This is probably because the processing time of pastirma is shorter than that of hams. Glycine is one of the most frequently encountered FAAs in beef.[24] In Iberian hams produced by the dry-curing principle, the amount of phenylalanine was reported as 417.8 mg 100 g^{-1} DM.[20] The phenylalanine amount in dry-cured hams was also reported to be 449.4 mg 100 g^{-1} DM.[21] These values are very different from the data collected in the current research. An important factor behind this difference is that hams have been ripened for a long duration.

The average isoleucine values of the pastirma types can be found in Table 1. Even though it is not statistically significant, the average value for the sırt pastirma type is higher than the other types. Free isoleucine amount in *longissimus dorsi* muscle is higher than in the *semimembranosus* muscle.[26] Leucine is one of the most commonly found FAAs in dry-cured meat products such as ham.[20,21,23]

Hydroxyproline values determined in pastirma types were very similar (Fig. 4). Hydroxyproline is a constituent amino acid in the structure of connective tissue. The nutrient value of meat decreases with increasing proportion of collagen or elastin content.[18] The fact that hydroxyproline is one of the FAAs detected in pastirma types indicates that degradation occurs in connective tissue proteins during the process of pastirma production.

Moisture and pH

The average values of moisture and pH determined in sırt, bohca and sekerpare pastirma types are shown in Table 2. When the evaluation of average moisture values was compared in the three pastirma type studied, it was observed that there is no significant differences amongst groups ($P > 0.05$). However, the moisture values in the five samples of sırt pastirma, in the four samples of bohca pastirma and in the two samples of sekerpare pastirma were determined to be above the established maximum value (50%) for Pastirma Standard. Several researchers have determined a moisture content of pastirma ranging from 34.4% to 56.0%.[27,28]

The average pH values of sırt, bohca and sekerpare pastirma were determined as 5.86 ± 0.12, 5.79 ± 0.12 and 5.82 ± 0.10, respectively. Statistical analysis showed that there was a significant ($P < 0.05$) difference among pastirma types for pH values (Table 2). The highest average pH value was obtained in sırt pastirma, while the lowest value was in bohca pastirma (Table 2). pH values of pastirma types made from round section are lower than those of pastirma types produced from loin. According to the Pastirma Standard,[9] the pH value of pastirma should be between 4.5 and 5.8. However, there are no data about the pH values of pastirma types in the standard. The pH values in 10 of the 14 sırt pastirma samples, in seven of the 16 bohca samples and in seven of the nine sekerpare pastirma samples were determined to be above the established maximum value of 5.8 for Pastirma Standard. Some factors such as the quality of the meat used in production, occurrence of a partial fermentation during production, conditions of production and drying probably affected the pH of pastirma.[11] Doğruer *et al*.[28] determined that pH value of the pastirma ranged from 5.56 to 6.54 (average 5.88). Aksu *et al*.[29] determined that the pH value in pastirma was 5.66.

CONCLUSIONS

Results from present study showed that amounts of the free amino acids of sırt, bohca and sekerpare pastirma types were generally significantly difference. The most advantageous pastirma types in terms of essential amino acids were bohca and sırt, which contain the highest quantity of threonine, methionine and valine compared to sekerpare pastirma. The highest alanine amounts were detected in bohca and sekerpare, while the highest proline amount was determined in sırt and sekerpare.

The highest amount of FAA, in each of the three pastirma types was alanine, followed by glutamine, tyrosine, glutamic acid, lysine, leucine, arginine, phenylalanine, isoleucine, serine, threonine, glycine, valine, histidine, proline, hydroxyproline, tryptophan, aspartic acid, cysteine and serine.

This research is the first study conducted to determine the FAA composition of pastirma or pastirma types. By taking into account the variations among pastirma types that have been observed within the scope of this research, it will be beneficial to determine in the next study the changes that take place in the production

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stages of each pastirma type with respect to FAAs and the effects of different production and curing techniques on these components.

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