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CHANGES IN THE COMPOSITION OF FREE AMINO ACID DURING PRODUCTION OF PASTIRMA CURED WITH DIFFERENT LEVELS OF SODIUM NITRITE

EBRU ERDEMİR and MUHAMMET İRFAN AKSU[1]

Food Engineering Department, Faculty of Agriculture, Ataturk University, Erzurum, Turkey

[1]Corresponding author.
TEL: +90-442-231-2724;
FAX: 90-442-231-5878
EMAIL: miaksu@atauni.edu.tr or miaksu@hotmail.com

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ABSTRACT

Pastırma is a dry-cured meat product produced in many parts of the world. Pastırma has high-value animal protein. So, it is an important food of animal origin for the human diet. An intense proteolysis also occurs during pastırma production, and the amounts of free amino acids (FAA) increase. In this study, the effects of different nitrite levels on composition of FAA were determined during pastırma processing. At the curing stage of pastırma production, meat was treated with 50, 100 and 150 ppm sodium nitrite. Analysis were performed at the raw material, end of curing, end of second drying and pastırma. The nitrite levels ($P < 0.01$) and production stages ($P < 0.01$) had significant effects on the composition of FAA. Compared with control, the amounts of serine, threonine, tyrosine, histidine and lysine increased, while the amounts of glycine, glutamine and arginine decreased in the pastırma containing nitrite. The highest FAA amounts were found in the pastırma consisting nitrite. However, the lowest FAA amounts among the pastırma groups with nitrite were in the 150 ppm.

PRACTICAL APPLICATIONS

This is the first study on determining the effect of usage nitrite on the composition of free amino acid (FAA) during pastırma processing and in the ready-to-eat pastırma. Proteolysis had an important effect on sensory properties of pastırma. The amounts of FAA increasing in the degradation affect on the desirable sensory properties in the final product, directly or transform different components. The results of this study can serve to increase of pastırma quality properties by the effect of nitrite on the composition of FAAs. In addition, results of this study will contribute significantly to efforts to reduce the usage of nitrite in the pastırma production.

INTRODUCTION

The important ones of factors affecting the quality of pastırma, a traditional meat product, are salting/curing and substances used in curing. Curing in meat technology is a process applied to improve characteristics as appearance, colour, texture, taste, aroma and flavor in product and increase resistance by using salt, nitrate or nitrite or together both of them in meat products and adding other some chemical substances and various spices depending on the product type. For this purpose, nitrate or nitrite together with salt in pastırma production can be used (Tekinsen and Dogruer 2000; Aksu *et al.* 2005a; Gokalp *et al.* 2010).

The proteins, which have a very important place on the quality of meat and meat products, are one of the most important macromolecules of meat. Changes occuring in the structure of proteins can affect the physical properties, chemical and microbiological stability, diffusion and production characteristics, sensory and nutritional qualities of meat and meat products (Dickinson 1997; Lantto 2007). Proteolysis which involves fragmentation reaction of proteins is one of the most important biochemical changes occurring during the maturation of fermented and partly fermented meat products such as pastırma. While proteins are fragmented due to the muscle enzymes and the

development of microorganisms which have proteolytic effect during production and ripening of meat products, amounts of water-soluble nitrogenous compounds such as peptides and FAAs increase (Waade and Stahnke 1997; Aksu *et al.* 2005a). Muscle proteins are broken into polypeptides by cathepsins / calpains during ripening, and polypeptides are subsequently fragmented into peptides by the peptidases. FAAs, which contributes to the characteristics flavor of such products, are formed by endogenous aminopeptidases or by bacterial enzymes in the final stages of proteolysis (Toldra 1998). FAAs consisting of activities of proteolytic enzymes in dry-cured meat products processed in pieces are a potential source in the formation of volatile and nonvolatile flavor compounds, and can contribute to flavor directly (Virgili *et al.* 1998; Cornet and Bousset 1999; Toldra *et al.* 2000). An intense proteolysis also occurs during pastırma production, since the amounts of FAAs, nonprotein nitrogenous (NPN) compounds increase while the band densities of myofibrillar and sarcoplasmic proteins decrease (Aksu *et al.* 2002; Aksu and Kaya 2002a,b; Aktas *et al.* 2005; Soyer *et al.* 2011).

However, it was determined in the various studies that proteolysis was influenced by nitrite/nitrate and the different process parameters such as temperature, salt content, the addition of glucose, the reduced pH, the addition of starter cultures and the storage period (Toldra 1998). Nitrite added in the dry-fermented sausages accelerated FAA producing mechanism and reduced the amino acid fragmentation products (Waade and Stahnke 1997). Similarly, Stahnke (1995) remarked that formations of 3-methyl butanol and 2-methyl propionic acid being degradation products of leucine and valin were reduced by the addition of nitrite, and the nitrite had a positive effect on the FAA content because of the prevented amino acid degradation.

The effect of nitrite addition in pastırma production on the FAA composition has not been studied, so far. Therefore, this study was carried out to determine the effects on the FAA composition of the nitrite used at different levels. The changes in amounts of essential amino acids (lysine, leucine, isoleucine, methionine, phenylalanine, valine, threonine and tryptophan) and nonessential amino acids (aspartic acid, glutamic acid, asparagine, serine, glutamine, histidine, glycine, arginine, alanine, tyrosine, proline and sarcosine) were evaluated during pastırma processing.

MATERIAL AND METHODS

Processing of the Pastırma

Pastırma was manufactured according to production process of pastırma reported by Aksu *et al.* (2005b). For pastırma production, beef *M. longissimus dorsi* muscles were obtained from a local slaughterhouse in Erzurum, Turkey. *Longissimus dorsi* muscles were removed from both sides of each carcass, trimmed of visible connective and fat tissue, and then, each muscle was cut vertically into two pieces, and four pastırmas were produced from a carcass. As treatment groups, pastırma samples with 50, 100 and 150 ppm sodium nitrite were used. As a control group, pastırma samples without nitrite were also used. Each treatment was carried out in two replicates (meat from an individual animal equates to one replicate). The production process of pastırma is shown in Table 1.

TABLE 1. THE STAGES OF PASTIRMA PRODUCTION

Production stage	Time (day)	Temperature (°C)	Relative humiditiy (%)
1. Curing*	2 d	6–7	
2. First drying	4 d	15	80–85
3. First pressing†	17 h	7–10	
4. Second drying	3–4 d	20	70
5. Second pressing	7 h	25	
6. The covering by cemen‡	4.5 d	7 ± 0.5	
7. Last drying	2 d	15 ± 0.5	70
	2 d	18 ± 0.5	65
	4-5 d	20 ± 0.5	60

* For 1 kg meat and treatment groups, 50 g curing mixture containing NaCl and 0, 50, 100 and 150 ppm sodium nitrite was used.
† 25 kg weight for each 1kg of meat.
‡ For 1 kg meat, 500 g flour, *Trigonella foenum graecum seed*, 350 g smashed fresh garlic, 75 g paprika, 75 g red pepper and 1200 mL water.

Determination of FAA Composition

FAA compositions of all groups were determined in the raw material, the final dry curing, at the end of second drying and in pastırma stages. The methods used by Aristoy and Toldra (1991), Antoine *et al.* (1999), Henderson *et al.* (1999) and Ceylan and Aksu (2011) were applied to determine the FAA composition. The sample preparation was also made according to the procedure given by Ceylan and Aksu (2011). Agilent 1200 model HPLC with UV detector and Zorbax Eclipse-AAA 4.6 × 150 mm, 3.5 μm (Agilent PN 963400-902) column were used in the determination of the FAA composition of pastırma samples. OPA (ortho-phthalaldehyde) and FMOC (9-fluorenylmethyl chloroformate) as derivatization reagent for amino acids, 0.4 N Borate (Agilent PN 5061-3339; pH 10.2) as the buffer solution were used. Mobile phase A: 40 mM NaH2P04 (pH 7.8) and mobile phase B: Acetonitrile : Methanol : Water/ 45 : 45 : 10, v/v/v solution as the mobile phase in chromatography system was conducted. Flow rate of mobile phase conducted in the system was 2 mL/min, and column temperature was 40C.

Analysis were done by using UV (ultraviolet) detector (338 nm) for amino acids derivatized by OPA (ortho-

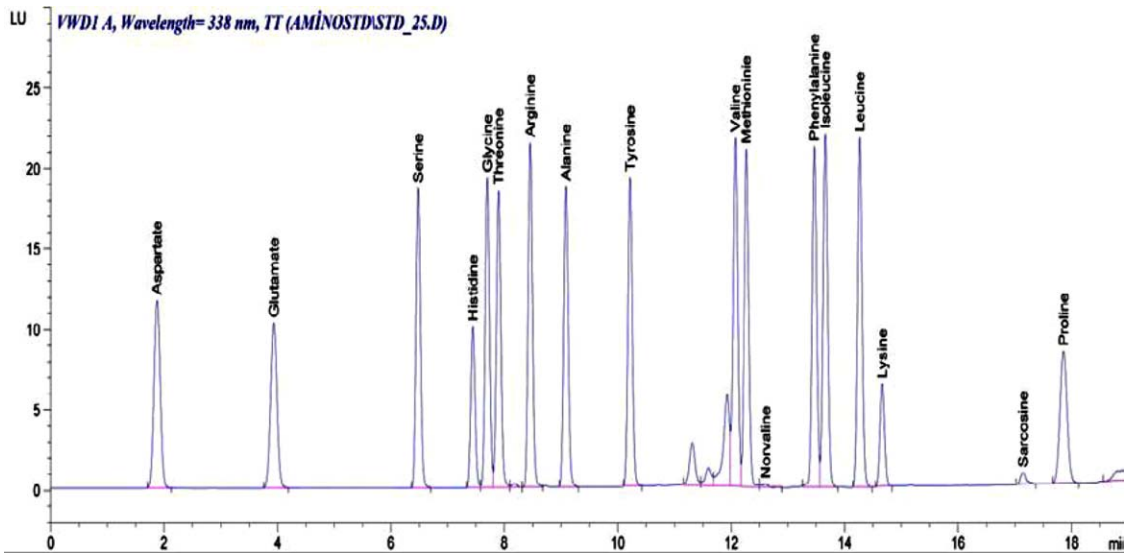

FIG. 1. CHROMATOGRAM OF THE STANDARD AMINO ACID USED IN THE RESEARCH

phthalaldehyde; Agilent PN 5061-3335) reagens and using HPLC for amino acids derivatized by FMOC (9-fluorenylmethyl chloroformate; Agilent PN 5061-3337) reagents. Valine, aspartic acid, glutamic acid, asparagine, serine, glutamine, histidine, glycine, threonine, arginine, alanine, tyrosine, methionine, tryptophan, phenylalanine, isoleucine, leucine, lysine, proline and sarcosine amino acids were determined in this research.

The chromatogram related to amino acid standard used in the research is given in Fig. 1. The calibration table was prepared by amino acid standards including four different concentrations as 25, 100, 250, and 1000 pmol/µl conducted in the system. In a 20s mixture, asparagine, glutamine, tryptophan, valine, aspartic acid, glutamic acid, serine, histidine, glycine, threonine, arginine, alanine, tyrosine, methionine, phenylalanine, isoleucine, leucine, lysine, proline and sarcosine amino acids were obtained from four different concentrations as 25 pmol/µL (PN 5061-3333), 100 pmol/µL (PN 5061-3332), 250 pmol/µL (PN 5061-3331) and 1000 pmol/µL (PN 5061-3330) from Agilent company.

Statistical Analysis

This experiment was conducted according to a completely randomized block design, using two replicates. Analysis of variance by the general linear model of the SPSS Package Program (SPSS. 10.01 1996) and Duncan's multiple range tests were used to find significant differences ($P < 0.05$) between nitrite level and pastırma production stage. The model included the nitrite level (control, 50, 100 and 150 ppm) and the production stage (the raw material, the final dry curing, the end of second drying and pastırma) as main effects, and all their interactions. The results of statistical analysis are shown as mean values ± standard deviations in tables.

RESULTS AND DISCUSSION

Essential Amino Acids

The amounts of FAA found during the production of pastırma including nitrite at different levels are shown in Table 2. The nitrite level ($P < 0.01$), the production stage ($P < 0.01$) and the nitrite level × the production stage interaction ($P < 0.01$) had a significant effect on the amount of lysine (Table 2). The amounts of lysine of samples with nitrite, at all concentrations used in this study, were higher than those of samples without nitrite. These results showed that the addition of nitrite increased the amount of lysine, and the effect of on the degradation of FAA of nitrite was seen clearly. Villaverde *et al.* (2014a) reported that the nitrite had the pro-oxidant effect on carbonylation of meat protein. Waade and Stahnke (1997) reported that the amino-acid-producing mechanisms were expedited by nitrite and/or the degradation of amino acids reduced. The highest increase in the amount of lysine was found between the second drying end and pastırma stages during production (Fig. 2a). This increase was effected by the composition of cemen used as well as changes occuring in cured-dried meat. The researchers found that the flour of the fenugreek seed was rich in lysine (El-Mahdy and El-Sebaiy 1985; Billaud and Adrian

TABLE 2. MEANS AND STANDARD DEVIATIONS OF FREE AMINO ACIDS CONTENTS OF PASTIRMA AT DIFFERENT STAGES OF PROCESSING AND DIFFERENT NITRITE LEVELS, IN MG/100 G DRY MATTER

	Pastırma Production Stage (PPS)					Nitrite Levels (NL)					
Free amino acid	Raw material	End of curing	End of second drying	Pastırma	Sig.	0 ppm (Control)	50 ppm	100 ppm	150 ppm	Sig.	PPS × NL interaction
Essential Amino Acids (EAAS)											
Lys	41.62 ± 9.29^{c}	48.47 ± 8.60^{c}	111.70 ± 40.14^{b}	223.96 ± 44.05^{a}	**	91.68 ± 64.75^{c}	118.93 ± 97.53^{a}	104.30 ± 85.40^{b}	110.84 ± 71.54^{b}	**	**
Ile	12.72 ± 9.56^{b}	8.80 ± 5.64^{c}	13.00 ± 8.37^{b}	28.46 ± 5.70^{a}	**	14.76 ± 10.00^{b}	18.67 ± 10.52^{a}	14.81 ± 12.47^{b}	14.75 ± 9.43^{b}	**	ns
Leu	52.82 ± 37.89^{c}	33.90 ± 6.99^{d}	72.42 ± 16.17^{b}	94.30 ± 21.61^{a}	**	64.61 ± 31.96^{b}	76.28 ± 37.30^{a}	56.40 ± 31.69^{c}	56.14 ± 25.74^{c}	**	**
Met	102.34 ± 34.78^{c}	145.84 ± 21.19^{a}	112.62 ± 11.23^{b}	84.75 ± 9.52^{d}	**	103.78 ± 27.98^{b}	110.63 ± 31.5^{b}	105.39 ± 21.21^{b}	125.72 ± 37.98^{a}	**	**
Phe	51.12 ± 10.43^{c}	36.09 ± 8.47^{d}	56.85 ± 13.12^{b}	76.32 ± 13.28^{a}	**	51.32 ± 15.23^{b}	59.39 ± 21.50^{a}	52.12 ± 20.32^{b}	57.56 ± 16.04^{a}	**	**
Val	20.46 ± 7.35^{b}	21.15 ± 8,38^{b}	20.96 ± 5.19^{b}	33.53 ± 6.34^{a}	**	23.16 ± 6.18^{b}	27.94 ± 9.98^{a}	21.82 ± 9.90^{b}	23.19 ± 7.83^{b}	**	**
Thr	28.34 ± 11.35^{b}	19.74 ± 3.52^{c}	31.74 ± 10.33^{b}	55.67 ± 9.82^{a}	**	29.47 ± 12.86^{c}	38.33 ± 19.08^{a}	32.55 ± 18.27^{bc}	35.14 ± 13.91^{ab}	**	*
Trp	50.41 ± 15.47^{c}	40.02 ± 15.98^{d}	55.14 ± 10.65^{b}	82.04 ± 13.32^{a}	**	55.20 ± 14.73^{b}	62.42 ± 21.84^{a}	53.33 ± 25.17^{b}	56.67 ± 20.86^{b}	**	**
Nonessential Amino Acids (NEAAS)											
Asp	29.90 ± 21.54^{a}	20.23 ± 5.32^{b}	10.70 ± 1.86^{c}	20.39 ± 8.39^{b}	**	21.74 ± 12.43^{a}	21.28 ± 13.90^{a}	16.72 ± 13.94^{b}	21.46 ± 14.23^{a}	**	**
Glu	100.76 ± 28.36^{b}	99.18 ± 38.03^{b}	85.57 ± 35.61^{c}	192.57 ± 31.46^{a}	**	122.58 ± 41.80^{a}	127.42 ± 64.82^{a}	112.48 ± 61.38^{b}	115.60 ± 48.82^{b}	**	**
Asn	34.81 ± 10.40^{b}	28.22 ± 6.48^{c}	26.19 ± 5.71^{c}	48.65 ± 7.22^{a}	**	32.14 ± 7.15	35.69 ± 12.30	34.43 ± 15.81	35.61 ± 10.15	ns	ns
Ser	33.47 ± 4.97^{c}	32.28 ± 2.99^{c}	49.97 ± 14.99^{b}	70.96 ± 12.18^{a}	**	41.84 ± 14.50^{c}	51.30 ± 22.89^{a}	43.86 ± 19.39^{b}	49.69 ± 16.69^{a}	**	**
Gln	297.96 ± 210.03^{b}	361.97 ± 170.41^{a}	230.92 ± 93.11^{c}	129.47 ± 45.50^{d}	**	293.06 ± 182.59^{a}	256.65 ± 181.09^{b}	217.90 ± 113.49^{c}	252.70 ± 182.90^{b}	**	**
His	45.37 ± 8.07^{a}	37.09 ± 7.86^{c}	38.59 ± 5.18^{bc}	40.23 ± 10.06^{b}	**	36.10 ± 7.78^{b}	40.57 ± 7.54^{a}	42.81 ± 9.67^{a}	41.80 ± 7.59^{a}	**	**
Gly	77.75 ± 48.53^{b}	85.60 ± 43.53^{a}	63.54 ± 31.14^{c}	58.37 ± 22.60^{d}	**	79.64 ± 45.20^{a}	74.78 ± 42.87^{b}	61.68 ± 27.85^{d}	69.16 ± 37.26^{c}	**	**
Arg	38.60 ± 13.49^{c}	42.07 ± 23.96^{c}	52.95 ± 19.32^{b}	222.02 ± 41.27^{a}	**	68.50 ± 63.95^{b}	97.10 ± 76.56^{a}	91.44 ± 99.66^{a}	98.60 ± 87.46^{a}	**	**
Ala	866.60 ± 264.43^{b}	1037.83 ± 187.06^{a}	848.70 ± 135.39^{b}	642.35 ± 73.73^{c}	**	804.81 ± 232.04^{c}	856.05 ± 206.09^{b}	796.47 ± 115.38^{c}	938.15 ± 299.01^{a}	**	**
Tyr	229.51 ± 66.88^{b}	276.15 ± 29.25^{a}	229.19 ± 37.03^{b}	23653 ± 31.10^{b}	**	220.39 ± 42.71^{c}	241.50 ± 48.55^{b}	235.37 ± 27.46^{bc}	274.11 ± 51.99^{a}	**	**
Pro	25.13 ± 11.21^{a}	21.17 ± 7.11^{b}	18.37 ± 6.42b^{c}	18.02 ± 11.68^{c}	**	22.07 ± 9.17^{ab}	16.80 ± 7.78^{c}	19.35 ± 10.74^{bc}	24.47 ± 9.66^{a}	**	ns
Sar	130.44 ± 19.47^{a}	107.22 ± 9.37^{b}	80.77 ± 11.32^{c}	82.30 ± 16.96^{c}	*	98.00 ± 27.87^{b}	103.38 ± 28.89^{a}	99.90 ± 22.92^{b}	99.46 ± 22.40^{b}	**	**

^{a,b,c,d}Means followed by different superscript alphabets in each line for pastırma production stage and nitrite levels are significantly different ($P < 0.05$).

*$P < 0.05$.

**$P < 0.01$.

ns: Means not significant, ±: Standard deviation.

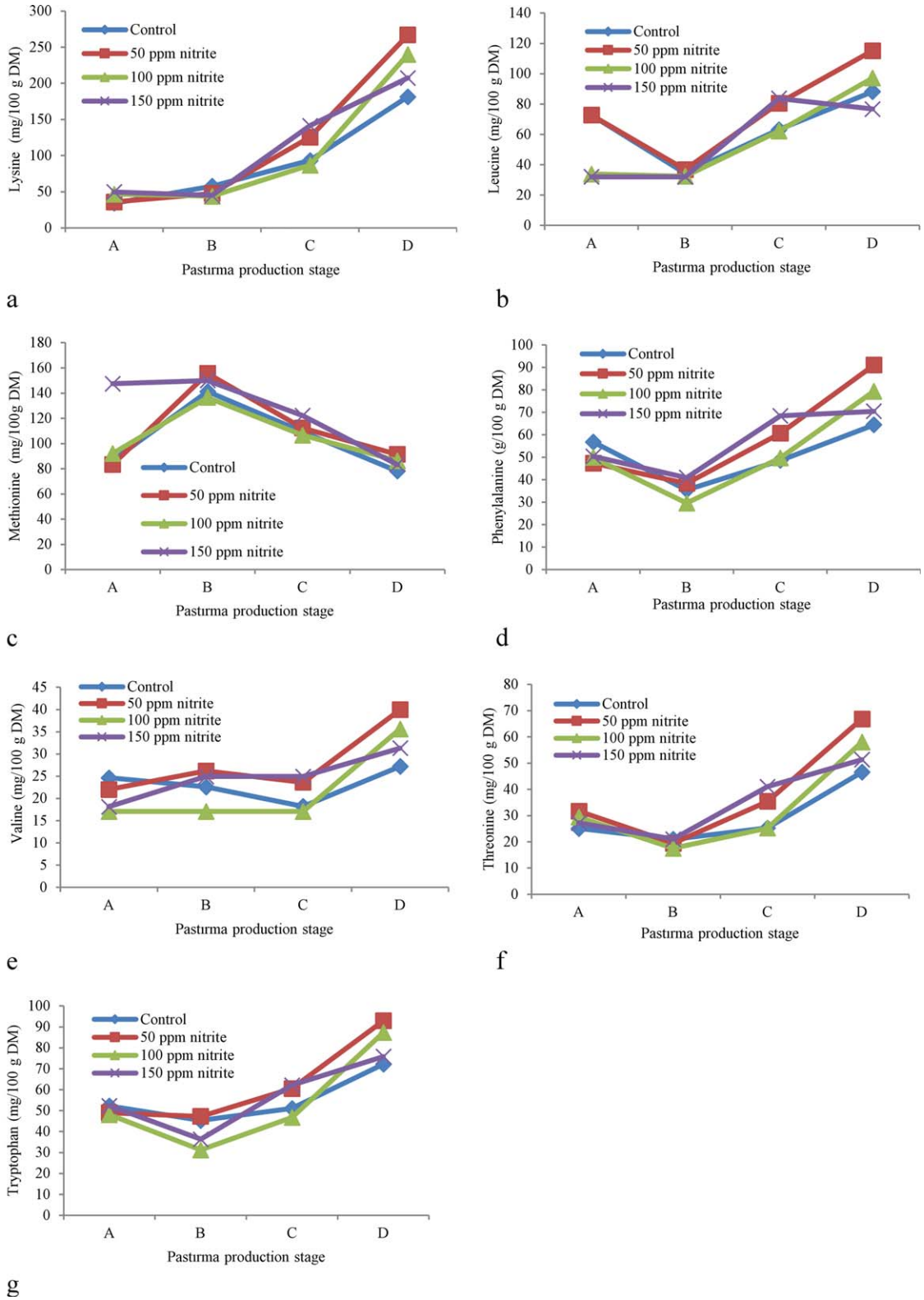

FIG. 2. THE EFFECT OF THE NITRITE LEVEL × THE PRODUCTION STAGE INTERACTION ON THE AMOUNTS OF LYSINE (A), LEUCINE (B), METHIONINE (C), PHENYLALANINE (D), VALINE (E), THREONINE (F) AND TRYPTOPHAN (G) DURING PASTIRMA PROCESSING: A: RAW MATERIAL, B: END OF CURING, C: END OF SECOND DRYING AND D: PASTIRMA

2001). The average values detected in the control and treatment groups including 50, 100 and 150 ppm nitrite were relatively higher than the values determined by Ceylan and Aksu (2011). However, it was indicated that the lysine amino acid increased during production and storage of other meat products, except for pastırma, which proteolysis is generally intense (Cordoba *et al.* 1994; Toldra *et al.* 2000; Hughes *et al.* 2002; Perez-Juan *et al.* 2006; Lorenzo *et al.* 2008).

Nitrite levels had a significant effect ($P < 0.01$) on the amount of leucine (Table 2). While the highest leucine amino acid was determined in the group with 50 ppm nitrite, the lowest values were found in the groups including 100 and 150 ppm nitrite. On the amount of leucine, the production stage had also a very significant effect ($P < 0.01$). The amount of leucine determined in the raw materials decreased in the curing stage, and then increased again. The highest value was also found in pastırma. Villaverde et al. (2014b) found the highest amount of FAA at day 54 of processing in fermented meat. Ceylan and Aksu (2011) determined that the amount of leucine was average 102.08 ± 35.79 mg/100 g DM between 43.53 and 183.05 in sırt pastırma. The average value determined in this study was similar to findings of Ceylan and Aksu (2011). The interaction of the nitrite level × the production stage had a very significant effect ($P < 0.01$) on the amount of leucine (Fig. 2b). It increased until the final product in the group including 100 ppm, until the end of second drying in the group with 150 ppm nitrite. Also, a stable increase was determined after the end of curing stage in the other groups. However, it was reported that the amount of leucine amino acid found in the flour of the fenugreek seed was higher than other amino acids (El-Mahdy and El-Sebaiy 1985). Therefore, it may cause an increase.

The nitrite levels and the production stage had a significant effect ($P < 0.01$) on the isoleucine amino acid (Table 2). The amount of isoleucine in the pastırma group with 50 ppm nitrite was higher than the other groups ($P < 0.05$). The amount of this amino acid decreased by curing and increased gradually in the later stages. The highest value was determined in pastırma while the maximum increase in the amount of isoleucine was found in the end of second drying and pastırma stages. Some researchers reported that the amount of isoleucine increased during the drying and ripening stages of different meat products (Cordoba *et al.* 1994; Waade and Stahnke 1997; Lorenzo *et al.* 2008; Villaverde *et al.* 2014b).

The use of different levels of the nitrite in the pastırma production had highly significant effect ($P < 0.01$) on the amount of methionine. The amounts of methionine in pastırma with 150 ppm nitrite were higher ($P < 0.05$) than other groups. Villaverde *et al.* (2014b) determined that the samples with 150 ppm nitrite had the highest levels of FAA at day 54 of fermented meat processing. However, no difference in the amounts of methionine among the other groups was observed ($P > 0.05$). In this research it was found that the production stages had very significant effects ($P < 0.01$) on the amount of methionine. The highest methionine value was determined at the end of curing in the stages of pastırma production. A steable reduction also occured after curing. The interaction of the nitrite level × the production stage having a very significant effect ($P < 0.01$) on the methionine amount of pastırma is shown in Fig. 2c. In general, the amount of methionine increased by curing in pastırma groups, and then it decreased. It can be expressed that the addition of nitrite had an effect in the final product in terms of methionine. Because the lowest value in pastırma was determined in the control group (Fig. 2c). It was reported that the amounts of methionine in sırt pastırma including *L. dorsi* muscle were found as 4.07–55.89 mg/100 g DM (Ceylan and Aksu 2011).

The nitrite levels, the production stage and the nitrite level × the production stage interaction affected the amount of phenylalanine ($P < 0.01$). The amounts of phenylalanine in the control and pastırma groups with 100 ppm nitrite were lower than the other groups ($P < 0.05$) (Table 2). The amount of phenylalanine was determined as the average 51.12 ± 10.43 mg/100 g DM in the raw materials decreased at the end of curing, and then, it increased continually. The highest amount of phenylalanine was found in pastırma ($P < 0.05$). As is seen from Fig. 2d, the amount of phenylalanine decreased by curing in the all treatment groups. After this stage, the amount of phenylalanine increased in the groups with nitrite. Flores *et al.* (2009) were reported that the amount of phenylalanine in dry cured Iberian ham produced from the fresh meats was found as 42.2 mg/100 g DM at end of the salting and 417.8 mg/100 g DM at the end of ripening during two years.

In this study, it was determined that the level of nitrite had a very significant effect ($P < 0.01$) on the amount of valine. The mean values of the groups with nitrite showed that the highest valine amount in pastırma groups was in the group with 50 ppm nitrite. No significant effect ($P > 0.05$) was observed among the other groups. The production stage had a very significant effect ($P < 0.01$) on the amount of valine, the highest values were determined in pastırma. The valine value found in pastırma was similar with the values determined by Ceylan and Aksu (2011). The interaction of the nitrite level × production stage which had a very significant effect ($P < 0.01$) on the amount of valine in pastırma is shown in Fig. 2e. The amount of valine in control group decreased partially from the raw material to the end of second drying, and increased after this stage. Although, the increase in the valine amount of groups with 50 and 150 ppm nitrite was lower until the end of second drying, the increase continued during production. There was no change in the amount of valine in the groups with

100 ppm nitrite until the end of second drying, but it increased after this stage. Unlike pastırma, the amount of valine was reported that it had consistently increase throughout curing in studies carried out in different meat products cured for a long time (Cordoba *et al.* 1994; Waade and Stahnke 1997; Lorenzo *et al.* 2008). Stahnke (1995) remarked that the product of valine degradation decreased by nitrite addition in dried sausages. Thus, the amount of valine increased during production. Soyer *et al.* (2011) reported that the FAA amount increased during production in pastırma produced by addition of 150 ppm nitrite.

The nitrite level had a very significant effect ($P < 0.01$) on the amount of threonine. The highest value was determined in the group with 50 ppm nitrite while the lowest value was found in the control group ($P < 0.05$; Table 2). On the production stage, the amount of threonine ($P < 0.01$) had also a very significant effect. The amount of threonine found as the average 28.34 ± 11.35 mg/100 g DM in the raw material decreased at the end of curing and increased in the later stages. The amount of threonine was determined to be 55.67 ± 9.82 mg/100 g DM in pastırma ($P < 0.05$). As is seen from Fig. 2f, the amount of threonine decreased in the curing stage, and increased in later stages. Such as the other FAAs, the threonine was affected by the addition of nitrite, and the amount of threonine in the groups with nitrite was higher than those of control groups after the curing stage. The similar changes occured in the some meat products, except pastırma (Waade and Stahnke 1997; Hughes *et al.* 2002). However, Waade and Stahnke (1997) expressed that the degradation of amino acids reduced by nitrite, so the amounts of FAAs increased.

It was determined that the nitrite levels, the production stage and the nitrite level × production stage interaction had a very significant effect ($P < 0.01$) on the amount of tryptophan. The maximum amount of tryptophan was determined in the group with 50 ppm nitrite. However, there was no significant difference in the amount of tryptophan among the control and groups with 100 and 150 ppm nitrite ($P > 0.05$; Table 2). The amount of tryptophan increased consistently after curing was found from 35.12 to 75.18 mg/100 g DM at the end of second drying and from 58.02 to 100.26 mg/100 g DM in pastırma. The least change among pastırma groups occured in the control group during the production (Fig. 2g). The amounts of tryptophan in the groups with 100 and 150 ppm nitrite decreased during curing, and then increased. Villaverde *et al.* (2014a) found in fermented meat that the amount of tryptophan of samples with 150 ppm nitrite was lower than other groups at day 1 of processing and it had the highest concentration followed by the samples with 75 ppm nitrite and those without nitrite after day 54 of processing. The effect of the nitrite in terms of tryptophan was clearly observed in the final product (Fig. 2g). The amount of tryptophan in sırt pastırma was found as 5.68–24.81 mg/100 g DM in a study researched the FAA composition of ready to eat pastırma (Ceylan and Aksu 2011).

Nonessential Amino Acids

The nitrite level had a very significant effect ($P < 0.01$) on the amount of aspartic acid. While the highest aspartic acid contents were detected in groups with 0, 50 and 150 ppm nitrite among pastırma groups, the lowest value was determined in 100 ppm group. The production stages had very significant effects ($P < 0.01$) on the amount of aspartic acid (Table 2). The nitrite level × the production stage interaction had a very significant effect ($P < 0.01$) on the amounts of aspartic acid is shown in Fig. 3a. While significant reduction in the amounts of aspartic acid were observed in the all treatment groups until the end of second drying, the amounts of aspartic acid increased again after the second drying. It is known that important physicochemical and microbiological changes in the second drying stage of pastırma production were occured in present study. At this stage, the important differences were observed among the control group and groups with nitrite in terms of the formation of aspartic acid, the aspartic acid in the control was determined higher than other groups. The lowest aspartic acid was found in the group with 100 ppm nitrite during production. These results showed that nitrite had a significant effect on the formation of aspartic acid. On the other hand, the aspartic acid in the composition of the flour of the fenugreek seed added to cemen of pastırma could cause an increase after the second drying stage. El-Mahdy and El-Sebaiy (1985) determined that the amount of aspartic acid in *Trigonella goeum graecum* L. was relatively high. The values obtained from this study were similar to these values. It was indicated that the amount of aspartic acid in meat products salted for a long time (about 75 days) and ripened in piece such as Iberian ham, except pastırma, increased during production (Cordoba *et al.* 1994). Hughes *et al.* (2002) reported that the amounts of aspartic acid of control samples of semi-dry fermented sausages produced by added 130 ppm nitrite increased during the ripening period.

The nitrite levels, the production stage and nitrite level × production stage interaction had a significant effect ($P < 0.01$) on the amount of glutamic acid. At the end of processing, pastırma samples with 0 and 50 ppm nitrite had a higher glutamic acid content than the samples with 100 and 150 ppm nitrite ($P < 0.05$), while there was no significant difference ($P > 0.05$) between 100 and 150 ppm nitrite samples (Table 2). The amount of glutamic acid increased in pastırma according to raw material ($P < 0.05$), but there was no significant difference between the raw material and the end of curing stages ($P > 0.05$; Table 2). The amounts of glutamic acid in the groups with nitrite were determined lower

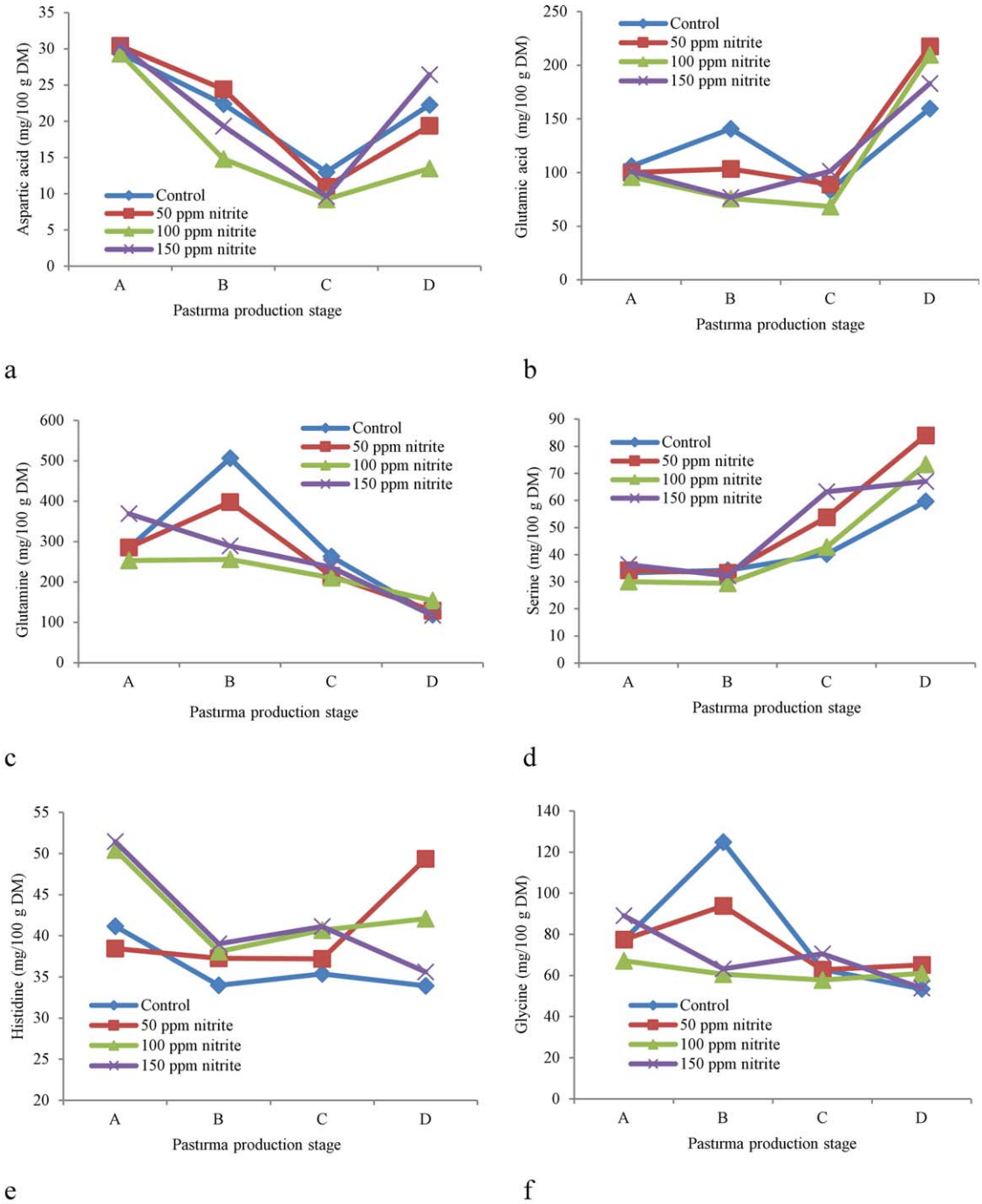

FIG. 3. THE EFFECT OF THE NITRITE LEVEL × THE PRODUCTION STAGE INTERACTION ON THE AMOUNTS OF ASPARTIC ACID (A), GLUTAMIC ACID (B), GLUTAMINE (C), SERINE (D), HISTIDINE (E) AND GLYCINE (F) DURING PASTıRMA PROCESSING A: RAW MATERIAL, B: END OF CURING, C: END OF SECOND DRYING AND D: PASTIRMA

than the control group at the end of curing. There was no significant difference the amounts of glutamic acid in pastırma, but significant increases were observed in all the treatment groups after drying. As can be seen from these results and Fig. 3b, the levels of free glutamic acid in pastırma groups with nitrite were higher than the control sample. This increase was affected by both the nitrite and the fenugreek seed flours used in cemen including glutamic acid at high amount (El-Mahdy and El-Sebaiy 1985). Ceylan and Aksu (2011) reported that the amount of glutamic acid

in sırt pastırma was average 117.67 ± 40.93 mg/100 g DM. These average values were lower than the average values determined in the present study. Dogruer (1992) found that the meat used for pastırma production and pastırma produced from these meats had the highest glutamic acid. The nitrite added in dry fermented sausages accelerated the FAA-producing mechanism, and decreased the amino acid degradation products in a study carried out by Waade and Stahnke (1997).

The lowest glutamine was found in the group with 100 ppm nitrite while no significant difference ($P > 0.05$) was observed between groups with 50 and 150 ppm nitrite. The production stage had a very significant effect ($P < 0.01$) on the amount of glutamine. The amount of glutamine being the average 297.96 ± 210.03 mg/100 g DM at the raw materials increased by the curing process, and decreased steadily in the later stages. The lowest glutamine was determined in pastırma (Table 2). Ceylan and Aksu (2011) reported in their research that the amount of glutamine was the average 130.82 ± 83.18 mg/100 g DM in sırt pastırma. It was found that the nitrite level × the production stage interaction had a very significant effect ($P < 0.01$) on the amounts of glutamine in pastırma (Fig. 3c). As shown in the Fig. 3c, a significant difference was observed among all groups in the raw material and second drying stages. However, the control and the group with 50 ppm nitrite had a higher values than the samples with 100 and 150 ppm at this stage. In addition, the amount of glutamine significantly decreased in all groups after curing. Results were similar to the values determined at sırt pastırma by Ceylan and Aksu (2011).

The production stage had a very significant effect ($P < 0.01$) on the amount of asparagine, but no significant effects ($P > 0.05$) of the nitrite levels and the nitrite level × the production stage interaction were detected on the amount of asparagine (Table 2). The amount of asparagine decreased during curing and drying, and then rised to 48.65 ± 7.22 mg/100 g DM in pastırma ($P < 0.05$). However, no differences in the asparagine values in the curing and drying stages were observed ($P > 0.05$). The amount of asparagine determined in this study was relatively higher than the values found by Ceylan and Aksu (2011).

The use of nitrite in pastırma production increased the amount of serine ($P < 0.01$). The amounts of serine in the groups with 50 and 150 ppm nitrite were higher than the group with 100 ppm nitrite ($P < 0.05$; Table 2). The production stages had also a significant effect ($P < 0.01$) on the amount of serine. The serine values showed an increase (except curing) during the production. However, Askar *et al.* (1993) found that the amount of serine being 68.46 mg in the raw materials was 43.45 mg in pastırma. While the amount of serine increased in all groups during production, the highest change was determined in the control group during production (Fig. 3d). Waade and Stahnke (1997) determined that the amount of the serine FAA in dry fermented sausages increased during 4-week ripening. However, Cordoba *et al.* (1994) found in their study that the amount of serine in long time salting and ripening Iberian Hams was not affected in the salted stage, but increased during ripening.

The nitrite levels, the production stage and nitrite level × production stage interaction ($P < 0.01$) had a significant effect on the amount of histidine (Table 2). The degradation of amino acids was prevented by nitrite added in meat products, and showed a positive effect on the amount of FAA (Stahnke 1995). Thus, the use of nitrite in pastırma production increased the level of histidine ($P < 0.01$; Table 2). The highest histidine was found in the raw materials used in pastırma production, while the lowest was determined at the end of the curing process. After curing, the amount of histidine gradually increased. Although, the amounts of histidine in groups with 100 and 150 ppm nitrite were higher than others groups, decreased in all groups during curing. After this stage, the amount of histidine was observed a significant change in the control and group with 100 ppm nitrite, and the lowest value was determined in the control products in the final products (Fig. 3e).

As shown in Table 2, the nitrite level, the production stage and the nitrite level × the production stage interaction ($P < 0.01$) had a very significant effect on the amount of glycine. The highest glycine amount was found in the control pastırma, and decreased by the nitrite addition ($P < 0.05$; Table 2). During production, the lowest value was determined as 58.37 ± 22.60 mg/100 g DM in pastırma. Hughes *et al.* (2002) and Lorenzo *et al.* (2008) found that an increase at the curing stage in their researches was related to other meat products. However, in contrast to the pastırma production stages, the amount of glycine increased at these meat products (dry cured lacón, fermented sausages) during production. According to Fig. 3f, while the amount of glycine in the control group showed a sharp increase at the curing stage, it declined sharply after this stage. The most important difference in the amount of glycine among the groups was observed at the end of curing during the production stages. But, no differences in the amounts of glycine were observed between the end of second drying and pastırma stages ($P > 0.05$). Ceylan and Aksu (2011) reported that the amount of the glycine amino acid in sırt pastırma was between 11.41 and 109.52 mg/100 g DM.

The nitrite level, the production stage and the nitrite level × the production stage interaction had significant ($P < 0.01$) effects on the amount of alanine. The highest value among pastırma groups was found in group with 150 ppm nitrite, when the lowest value was determined in the group with 100 ppm nitrite ($P < 0.05$). Askar *et al.* (1993) also reported similar change, the amount of alanine in the raw material was higher than pastırma, but the values determined

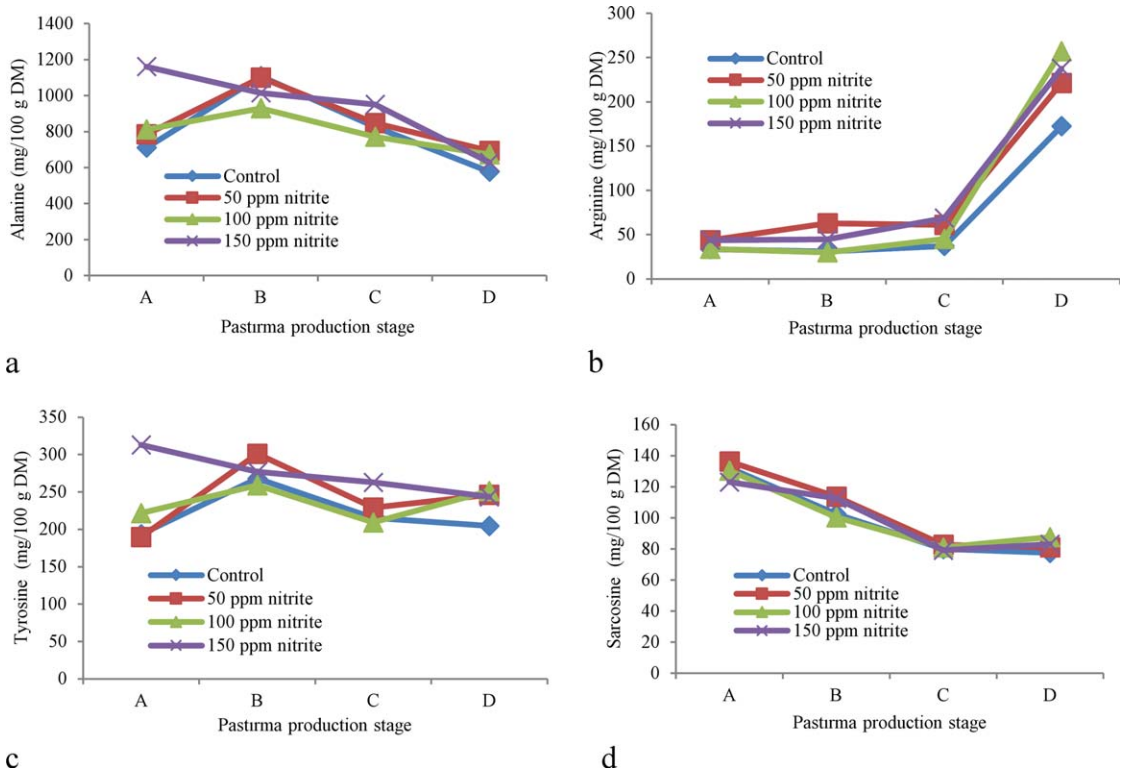

FIG. 4. THE EFFECT OF THE NITRITE LEVEL × THE PRODUCTION STAGE INTERACTION ON THE AMOUNTS OF ALANINE (A), ARGININE (B), TYROSINE (C) AND SARCOSINE (D): A: RAW MATERIAL, B: END OF CURING, C: END OF SECOND DRYING AND D: PASTIRMA

by these researchers were very lower than the present findings. The alanine was the FAA determined as the highest value in beef. Similar results were found in the researches related to meat products, expect pastırma, carried out on this subject (Paleari *et al.* 2003; Cho *et al.* 2008). Proteins disintegrate in 2–3 days salting/curing stage used in the pastırma production, and the amount of water-soluble nitrogen, FAAs and NPN compounds increase increase (Aksu and Kaya 2002a, 2005; Aksu *et al.* 2005a; Soyer *et al.* 2011; Uguz *et al.* 2011). Soyer *et al.* (2011) determined that the NPN amount of meats cured during 2 days by different salt concentrations increased from 5.01% ± 0.23 to 6.95% ± 0.31, the FAA also increased from 12.32% ± 0.97 to15.66% ± 1.65. The amount of alanine and other amino acids increased significantly at the curing stage in this study. According to Fig. 4a, the amount of alanine decreased in all groups at the end of production. This amino acid decreased in the group with 150 ppm nitrite during pastırma production. In addition, the amount of alanine increased in the control and groups with 50 and 100 ppm nitrite during the curing while it decreased continuously at the later stages. As in a lot of FAA, this amino acid in the pastırma with nitrite was higher level than the contol samples. It was thought that this increase occured because the nitrite levels added in the present study accelerated the FAA-producing mechanism in meat products, and decreased the amino acid degradation. The proteolytic activity can decrease due to the use of more than 200 ppm nitrite in the meat products (Toldra *et al.* 1993). Askar *et al.* (1993) and Ceylan and Aksu (2011) determined that the FAA being the most abundant in pastırma was alanine.

The nitrite levels, the production stage and the nitrite level × the production stage interaction had a significant effect ($P < 0.01$) on the amount of arginine. With in increase in nitrite levels in pastırma, there was a slight increase the amount of arginine in pastırma samples. However, there was no significant difference in the amount of arginine among pastırma with nitrite ($P > 0.05$; Table 2). During production stage, the most increase was determined between the second drying end and pastırma in all treatment groups. Waade and Stahnke (1997) determined that the addition of nitrite in the fermented dried sausages had a positive effect on the amount of free arginine. However, between the end of second drying and pastırma, there were significant changes in the amount of arginine among control and pastırma with nitrite (Fig. 4b). These changes in the amount of arginine probably depend on fenugreek seed flours used in making pastırma cemen. In a number of previous studies reported that fenugreek seed flours contains arginine at the high level (El-Mahdy and El-Sebaiy 1985).

The nitrite level, the production stage and the nitrite level × the production stage interaction had significant effects ($P < 0.01$) on the amount of tyrosine during processing. While the lowest tyrosine among the treatment groups was in the control group, the highest value was in the group with 150 ppm nitrite ($P < 0.05$). The used of nitrite in pastırma production increased the amount of tyrosine (Table 2). The amount of tyrosine increased by the curing process (except the group with 150 ppm nitrite), and decreased continuously until drying, the covered by cemen and pastırma stages (Fig. 4c). Ceylan and Aksu (2011) determined that the amount of tyrosine in sırt pastırma was between 50.40 and 293.03 mg/100 g DM.

The nitrite level ($P < 0.01$) has a significant effect on the amount of sarcosine (Table 2). The highest value was found in the group with 50 ppm nitrite among pastırma groups. However, there was no significant difference in the sarcosine levels among the other groups ($P > 0.05$; Table 2). It was determined that the production stage had a significant effect ($P < 0.01$) on the sarcosine value. The amount of sarcosine decreased until the end of second drying, and unchanged after this stage. The nitrite level × the production stage interaction had a significant effect ($P < 0.01$) on the amount of sarcosine in pastırma is shown in Fig. 4d. The amount of sarcosine decreased until the end of second drying in all groups, but the group with 100 ppm nitrite had a slight increase (Fig. 4d). According to these results, it is possible to express that the nitrite addition affected the amount of sarcosine. Villaverde *et al.* (2014b) determined that the amounts of FAA in the fermented meat containing nitrite were higher than the control groups.

The nitrite level and the production stage had significant effect ($P < 0.01$) on the amount of proline. The highest mean value was found in the group with 150 ppm nitrite while the lowest mean value was determined in the group with 50 ppm nitrite ($P < 0.05$; Table 2). The amount of proline was reported as 17.28–57.71 mg/100 g DM in sırt pastırma (Ceylan and Aksu 2011). Villaverde *et al.* (2014b) determined that the samples with 150 ppm nitrite had the highest levels of FAA at day 54 of fermented meat processing.

CONCLUSION

In this research, the findings showed that the use of different nitrite levels in the pastırma production had an effect on the composition and amount of FAA, the amounts of FAA in the samples including 150 ppm nitrite were lower than in the samples with 50 and 100 ppm nitrite between the second drying end and pastırma stages. These findings also showed that proteolytic degradation during pastırma processing was slowed by added 150 ppm nitrite.

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