Free amino acids and bioactive profile of Pastırma during its processing

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Abstract

This study is focused on the characterization of the proteolysis occurred during the processing of Pastırma, a traditional Turkish dry-cured meat product, which is responsible for its final characteristics. Thus, the evaluation of naturally generated free amino acids and peptides present at 0, 2, 5, 10, and 21 days of processing and the bioactivity of peptide fractions have been approached. Peptides were examined by MALDI-TOF and results showed differences in the amount of generated peptides at different times of processing, and a total of 29 peptides were newly generated at Day 21 in comparison with Day 2 during processing. The water soluble fraction of Pastırma at the end of the curing period (Day 21) was also analyzed by size-exclusion chromatography and some of the collected fractions demonstrated strong ACE-inhibitory and antioxidant activities. In fact, Pastırma showed an ACE inhibitory activity higher than 86% from 220 to 270 mL corresponding to molecular masses between 900 and 1500 Da, and also a DPPH radical-scavenging activity above 60% at 250 to 300 mL corresponding to molecular masses between 700 and 2000 Da. Thus, Pastırma represents a good source of natural ACE-

inhibitory and antioxidant peptides which might be due to the proteolysis occurred by endogenous enzymes and the contribution of the çemen paste used in production

Key words: Pastırma, Proteolysis, Free amino acids, Bioactive peptides, Antioxidant activity, Antihypertensive effect

Highlights

- An intense proteolysis occurred during Pastırma processing.
- Individual and total free amino acids content increased during processing of Pastırma.
- MALDI-TOF spectra confirmed naturally generated peptides of a wide range of molecular masses.
- Pastırma bioactive peptides had strong antihypertensive and antioxidant activity.
- Çemen paste contributed to the high bioactivity of Pastırma.

1. Introduction

Pastırma is a Turkish traditional dry-cured meat product which is produced using whole muscles obtained from certain parts of slaughtered cattle. Dry-curing (salting), drying, pressing, coating with çemen paste, and final drying with çemen (ageing) are the traditional processing steps in the manufacturing of Pastırma (Öztan, 2005). Pastırma production is a fast process that generally takes 3-4 weeks (Gök, Obuz, & Akkaya, 2012) where salt diffusion occurs rapidly. In this sense, the time required to reach homogenous salt concentration in the muscle is similar to other dry-cured products such as dry-cured loin probably due to its similarity in size and muscle composition (Sentandreu & Toldrá, 2001).

Proteolysis is the main process contributing to the unique characteristics of dry-cured meat products (Aristoy & Toldrá, 1995; Lametsch et al., 2003; Toldrá, 1998; Toldrá & Flores, 1998), resulting in the accumulation of large amounts of free amino acids (Sforza et al., 2001; Toldrá, Aristoy, & Flores, 2000) and peptides of different sizes (mainly small peptides) (Mora, Sentandreu, Fraser, Toldrá, & Bramley, 2009; Mora et al., 2009; Sentandreu et al., 2003). Endogenous muscle enzymes (Toldrá, 1998; Toldrá & Flores, 1998; Toldrá, Rico, & Flores, 1993) are mainly responsible for degradation of the proteins (Toldrá, Cerveró, & Part, 1993). These biochemical changes as well as the addition of çemen paste give rise to the characteristic taste and flavor of Pastırma.

Some studies have described proteolysis in Pastırma, mainly with conventional methods such as the determination of non-protein nitrogen content, SDS-PAGE, etc., (Ahhmed, 2013; Ahhmed et al., 2013; Ahhmed et al., 2014; Kızılkaya, 2012; Soyer, Uğuz, & Dalmış, 2011; Uğuz, 2007), while Ceylan and Aksu (2011) analyzed individual free amino acids in Pastırma sold in local markets. Nevertheless, no studies have been published dealing with the

characterization of generated peptides during Pastırma processing and the study of their bioactivity.

Bioactive peptides have various health promoting effects such as antioxidant and prebiotic properties, opiate, antithrombotic or antimicrobial activity, immunomodulatory, mineral-binding ability or cholesterol and blood pressure lowering (ACE-inhibitory) effects (Chernukha, Fedulova, & Kotenkova, 2015; Korhonen & Pihlanto, 2003; Walther & Sieber, 2011). The bioactivity of peptides is based on their amino acid composition (peptides that usually contain between 3 and 20 amino acid residues), sequence, size, configuration and molecular mass, which are also affected by processing conditions (Korhonen & Pihlanto, 2003; Matsui & Matsumoto, 2006; Pihlanto-Leppälä, 2000). Some recent studies have shown Spanish dry-cured ham as a natural source of peptides with antioxidant activity and antihypertensive effect (Escudero, Aristoy, Nishimura, Arihara & Toldrá, 2012; Escudero, Toldrá, Sentandreu, Nishimura & Arihara, 2012; Mora, Escudero, Fraser, Aristoy & Toldrá, 2014). To the best of our knowledge no study has been published on the naturally generated bioactive peptides in Pastırma and the molecular weight distribution of these peptides. In this context, one of the objectives of the present work was to monitor the profiles of free amino acids (FAAs) and peptides generated in the traditional Turkish meat product Pastırma to better understand the possible relation of the specific components with the production of bioactive peptides during its processing. What is more, the present study also involves the bioactivity assessment of generated peptides by analysing for the first time ACE-inhibitory and antioxidant activity.

2. Materials and Methods

2.1. Pastırma production and sample preparation

Pastırma production was conducted using methods reported by Aksu (1999), Soyer et al. (2011) and Yagli and Ertas (1998), with some modifications. Beef *Longissimus dorsi* muscles (n=6), after trimming visible connective tissue and fat, were used in the production.

Processing steps were presented in Figure 1. Çemen paste was prepared by mixing 50% çemen flour, also known as fenugreek flour (*Trigonella foenum graceum*), 35% crushed fresh garlic and 15% paprika powder (w:w), then, by dissolving this dry çemen mixture in water (1:2, w:v). Samples were analyzed at days 2 (curing), 5 (first pressing), 10 (third drying) and 21 (end product) of Pastırma processing.

2.2. Characterization of Pastırma

2.2.1. Moisture content

The moisture content (%) of Pastırma samples was measured at each sampling day using electronic moisture analyser HB-43 halogen from Mettler Toledo (Barcelona, Spain) at 175°C by triplicate.

2.2.2. Salt content estimated from Na determination

Salt content was estimated from sodium (Na^+) concentration. Samples from inside, surface and whole Pastırma at each sampling point were measured in triplicate according to Armenteros, Aristoy, Barat and Toldrá (2012). A Metrohm 838 Advanced Sample Processor (Metrohm® Ltd., Herisau Switzerland) in an Advanced Compact IC 861 ion chromatograph (IC) equipped with a conductivity detector and a Metrosep C3 (250×0.4 mm) column (Metrohm® Ltd.). Software IC Net 2.3 (Metrohm® Ltd.) was used to monitor separation. Samples were taken with a cylindrical borer transversally (3 cm of diameter). After taking this cylindrical sample, çemen layer was manually removed. The central part, consisting of approximately one third of the

whole length of cylindrical sample, was taken out and used as the inside sample. The remaining two thirds were used as surface part.

2.2.3. Analysis of free amino acids and dipeptide contents

Proteins were extracted from the whole Pastırma samples at each sampling step after manually removal of çemen paste and were deproteinized (Aristoy & Toldrá, 1991). The same procedure was also applied to the çemen paste. N-Leucine (1 mM) was used as internal standard. Amino acids were analyzed after derivatization with phenyl isothiocyanate (Bidleymeyer, Cohen, Tarvin, & Frost, 1986). Phenyl thiocarbamyl amino acid derivatives were separated by reverse phase in a 1200 Agilent HPLC (Agilent Technologies, Santa Clara, CA, USA) using a Pico Tag column (300x3.9 cm) (Waters, Ireland). The mobile phases and separation conditions described in Flores, Aristoy, Spanier, and Toldrá, (1997) were used. Detection was at 254 nm, and amino acids were identified by their retention times compared to standards.

2.3. Extraction and deproteinization

Five g sample of each part was homogenized with 20 mL of 0.01 N HCl in a Stomacher (IUL Instrument, Barcelona, Spain) for 8 min at 4°C. The homogenate was further centrifuged at 12000xg for 20 min at 4°C. The supernatant was filtered through glass wool, deproteinized by the addition of 3 volumes of ethanol, kept at 4°C overnight, and centrifuged at 12000 x g (20 min at 4°C). Ethanol in the supernatant was evaporated in a rotary evaporator and samples were further lyophilized for water evaporation.

2.4. Peptide-mass mapping by MALDI-TOF MS

Whole samples at each sampling step, and inside and surface parts of final Pastırma samples after removal of their çemen layer were analyzed by MALDI-TOF-MS to determine the molecular mass of peptides generated at different times of processing. Dried samples were

resuspended in 50 μ L acetonitrile:H₂O (2:98, v/v) with 0.1% (v/v) of trifluoroacetic acid (TFA) and 1 μ L of each sample was directly spotted onto the MALDI target plate and then allowed to air dry. A 5800 MALDI-TOF instrument (AB Sciex, MA, USA), which was used in a range from 800 to 2000 Da as previously indicated in Lassoued et al. (2015). The analysis of data was done using mMass software (<http://www.mmass.org/>).

2.5. Size-exclusion chromatography (Molecular mass fractionation)

Molecular mass fractionation was performed in the final Pastırma product after removal of the çemen layer in the samples from the whole, inside and surface parts. Five millilitres of each deproteinized extract were subjected to size-exclusion chromatography (SEC) using a Sephadex G25 gel filtration column (2.5x65 cm, Amersham Biosciences, Uppsala, Sweden), which was previously equilibrated by passing 3 column volumes of 0.01 N HCl, in order to fractionate the peptides according to their size (Escudero, Mora, & Toldrá, 2014). Fractions were then monitored by ultraviolet (UV) absorption at 214, 254 and 280 nm (Agilent 8453 UV spectrophotometer, Agilent Technologies, Palo Alto, CA, USA) to obtain the chromatograms. Fractions corresponding to elution volumes from 100 mL (20th fraction) to 600 mL (120th fraction) were lyophilized. The remaining residue was dissolved in 1 mL of bi-distilled water, and then consecutive fractions (in pairs) were pooled (e.g. 19th and 20th fractions, 21th and 22th fractions), and stored at -20°C until being used for ACE inhibitory activity and antioxidant activity assays.

2.6. Assay of angiotensin I-converting enzyme inhibitory activity

The angiotensin I-converting enzyme (ACE) inhibitory activity of the peptide fractions obtained after G-25 separation in the extracts from inside, surface and whole Pastırma parts were measured in triplicate according to the method developed by Sentandreu and Toldrá (2006) with minor changes. In brief, 50 μ L of each pooled fraction was mixed with 50 μ L of

150 mM Tris-base buffer (pH 8.3) containing 7.5 mg/mL of ACE. The reaction was started by the addition of 200 μ L of 150 mM Tris-base buffer (pH 8.3) containing 1.125 M NaCl and 10 mM Abz-Gly-Phe-(NO₂)-Pro. At the end, the reaction mixture was incubated at 37°C for 60 min. The release of o-aminobenzoylglycine (Abz-gly) by the action of ACE generated a fluorescence that was measured at 355 nm excitation and 405 nm emission wavelengths, respectively. ACE inhibition of each collected fraction is expressed as a percentage. Çemen paste was also analyzed to test ACE inhibitory activity. Five grams of çemen paste was mixed with 20 mL of 0.01 N HCl, centrifuged (çemen paste concentration in this extract was 0.25 g/mL) and 50 μ L of the supernatant was subjected to analysis as indicated above. This supernatant was also used for antioxidant activity assays. .

2.7. Antioxidant activity

2.6.1. 2,2-Diphenyl-1-picrylhydrazylradical-scavenging assay

2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical-scavenging activity was measured in triplicate as described by Bersuder, Hole, and Smith (1998). The DPPH radical-scavenging activity (%) was calculated as:

$$\text{DPPH activity (\%)} = \frac{(\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}})}{\text{Abs}_{\text{control}}} \times 100$$

where, Abs_{sample} is the absorbance of sample and Abs_{control} is the absorbance of the control..

2.6.2. Ferric-reducing antioxidant power (reducing power)

The assay was based on the method described by Huang, Tsai, and Mau (2006). Distilled water and BHT was used as the negative control and positive control, respectively. .

2.8. Statistical analysis

The analysis of variance (ANOVA) procedure was conducted and significant differences in contents of free amino acid and peptides content between sampling time were evaluated by Tukey HSD Multiple Comparison Test using SPSS software (SPSS 17.0 for Windows, SPSS Inc, Chicago, IL, USA) ($p<0.05$).

3. Results and Discussion

3.1. Characterization of the Pastirma product

3.1.1. Moisture and sodium contents

In the production of dried-cured meat products, due to the fast sodium chloride diffusion during processing, muscle proteins were solubilized and water activity was reduced, resulting in an ultimate product with a pleasant salty taste (Martin, 2001). During process stages at day 2, 5, 10, and 21, moisture contents of whole Pastirma samples were 69.6 ± 1.01 , 68.6 ± 1.45 , 62.6 ± 1.48 and 52.4 ± 2.10 , respectively while samples from the inside parts showed a sodium content (determined on dry basis) of $14.54\pm4.65\%$; 11.06 ± 3.01 ; $11.58\pm2.89\%$; and $11.25\pm2.14\%$, respectively, and samples from the surface $16.60\pm3.48\%$; $12.15\pm2.52\%$; $8.68\pm2.13\%$; and $8.36\pm2.25\%$, respectively.

3.1.2. Free amino acids and peptide contents

Glutamine, alanine, taurine, lysine and leucine were the predominant amino acids identified at day 2, constituting 27.2%, 15.9%, 8.19%, 5.97% and 5.75% of total FAAs, respectively (Table 1). Although curing was initiated at this stage, the samples had similar FAA to raw meat reported in the literature because of the short period of time the meat remained in the curing agents. At day 5, as in the first stage of processing, alanine, glutamine, lysine, leucine and taurine were predominant amino acids with some changes in their ratios. The predominant amino acids determined at day 10 were lysine, alanine, arginine, proline and glutamic acid.

Concentrations of serine, asparagine, arginine, proline and all essential amino acids at day 10 increased in comparison to the amounts at days 2 and 5, with the exception of threonine and isoleucine ($p < 0.05$). The most abundant FAA at day 21 was proline, followed by lysine, alanine, leucine, arginine and glutamic acid. Ceylan and Aksu (2011) reported that glutamine, tyrosine, lysine, glutamic acid, leucine and arginine were other predominant FAA in Pastırma. There were differences in the amounts of individual FAA in the aforementioned study and those in the present study, likely because of the differences in the source of samples.

The concentration of all individual amino acids increased during processing between 2 (glycine, β -alanine and alanine) and 20 (proline) times, except for taurine and glutamine. Taurine, a bioactive compound in meat (Arihara & Ohata, 2008) possessing antioxidant and radio-protective characteristics (Huxtable, 1992), did not exhibit significant changes during processing because this compound is not part of proteins (Purchas, Rutherford, Pearce, Vather & Wilkinson, 2004). Glutamine showed significant decreases during Pastırma processing because of its degradation to glutamic acid and NH_3 (Young, Frost, & Agnew, 2012). Similarly, Flores et al. (1997) reported a decrease in glutamine during short and long processes of Spanish Serrano dry-cured ham.

Total FAA contents increased over time ($p < 0.05$). The most noticeable increases were observed during later stages of processing, i.e. after days 10 and 21. Overall, when changes in FAA contents are considered, it is obvious that there is an intense proteolysis during Pastırma processing. Changes in FAA content at the earlier stages of processing are not that prominent because degradation of peptides to FAA occurs at the later stages of the proteolysis phenomena (Toldrá, 1998).

Increase in the relative carnosine and anserine content was observed during processing until day 10, and then, both decreased at day 21. This decrease in the relative carnosine content could

be attributed to the incorporation of çemen paste in the process steps after day 10. Çemen paste did not contain carnosine and anserine (**Table 1**). Carnosine and anserine as the most abundant antioxidants in meats (Arihara & Ohata, 2008), are considered bioactive peptides because of their important role in the prevention of stress related diseases, wound healing recovery from fatigue, and reducing certain proteolytic reactions associated with cell ageing (Arihara & Ohata, 2008; Hipkiss, Brownson, Bertani, Ruiz, & Ferro, 2002).

In the present study, besides dipeptides, carnosine and anserine, the profile of other peptides generated during processing of Pastırma was studied using MALDI-TOF mass spectrometry.

3.2. Peptide-mass mapping by MALDI-TOF MS

MALDI-TOF MS spectra are shown in **Figure 2** and in **Figure 3**. Proteolysis stages can also be confirmed by the gel view given at the top of figures that were obtained using mMass software, which can be used to effectively compare the ions detected at different sampling times.

The first spectrum obtained at day 2 of processing indicates the initial peptide profile after incorporating curing agents into the meat. This spectrum represents peptides naturally present in meat and those resulting from the first steps of protein degradation. At day 5, an intensive accumulation of peptides was clearly observed, which supports FAA contents at this stage where slight increases were observed in the concentration of individual FAAs (Table 1). The peptide profile at day 10 showed a decrease in the amount of ions detected in comparison to the peaks obtained at day 5, probably due to the breakdown of 800 - 2000 Da peptides to smaller ones and FAAs. Overall, the peptide profiles obtained during various stages of Pastırma processing include a mixture of peptides present in a wide range of molecular masses. A fair distribution of peptides from 800 to 2000 m/z was obtained at all different times of processing

(**Figure 2**). Differences in the spectra between Day 2 and Day 21 were also analyzed in detail by mMass software. In fact, 29 peptides were newly generated at Day 21.

MALDI-TOF MS spectra was also determined in the inside and surface part of the final product (see in **Figure 3**). In comparison to the inside parts, the spectra obtained from the surface showed a higher amount in peptides probably due to the influence of processing parameters such as temperature, humidity, or çemen paste that directly affects the surface of the sample.

3.3. Isolation of the peptides by size exclusion chromatography and bioactive study

Size-exclusion chromatography (SEC) with Sephadex G25 gel filtration column was used for fractionation of the peptide mixture according to their size. Collected fractions were monitored by ultraviolet (UV) absorption at 214 nm, 254 nm and 280 nm. Figure 5a shows the obtained chromatogram from the elution volumes 100 mL (20th fraction) to 600 mL (120th fraction), that were those showing any response (absorbance signal). The absorbance signal in the chromatograms is saturated since a high amount of the sample was injected to ensure the detection of the ACE and antioxidant activity in the fractions and also, to know the approximate size of the peptides having these activities.

ACE-I inhibitory properties of fractions are presented in **Figure 4**. ACE inhibitory values of inside, surface and whole samples of the final product varied from 0% to 99.5% (negative values of inside samples showed no activity). Maximum activity was observed in the fractions between 220 and 270 mL in whole, inside and surface samples, ranging between 72.9-99.5%, 63.9-99.9% and 80.1-99.9 %, respectively. These fraction volumes correspond to molecular masses between 900 and 1500 Da (results not shown). The fractions eluted before and after these volume ranges exhibited lower ACE inhibitory activity. Also, in the surface sample, activity of fractions eluting from 130 to 210 mL was relatively high (between 40 and 65%). On the other hand, DPPH radical-scavenging activity from 60% in the elution zone between 250

and 300 mL was observed for a whole sample (**Figure 5B**). These volumes correspond to molecular masses between 700 and 2000 Da (results not shown). Escudero et al. (2012) noted that Spanish dry-cured ham is rich in antioxidant peptides naturally generated during the dry-curing process, with a strong DPPH radical-scavenging activity in the elution zone between 220 and 345 mL (radical-scavenging activity from 39% to 92%). It was emphasized that possible factors responsible for greater antioxidant activity were adequate length, amino acid composition, and amino acid position in the sequence (Mora et al., 2014). **Figure 5C** shows main results obtained for ferric reducing power in the SEC fractions of Pastırma. The strongest antioxidant power was monitored in the elution area between 230 and 320 mL. In this area, whole samples demonstrated generally the higher antioxidant power with absorbance values greater than 0.94.

Within the sample parts evaluated, surface samples appeared to be the most active in ACE-inhibitory and antioxidant assays, probably due to the effect of çemen paste applied on the surface of the product. ACE inhibitory activity of çemen paste would result from the components, fenugreek, garlic and pepper. Hamden, Kesskes, Belhaj, Mnafgui, and Allouche (2011) reported that fenugreek seeds represent a significant antihypertensive effect which arises from its essential oil composition. ACE inhibitor dipeptides derived from garlic have already been isolated and characterized (Suetsuna, 1998). Both dried and aqueous extracts as well as wild garlic have a significant blood pressure lowering effect (Hosseini, Shafiee, & Baluchnejadmojarad, 2007; Sendl et al., 1992; Sharifi, Darabi, & Akbarloo, 2003). At the same, many paprika species exhibit anti-hypertension activity (Ranilla, Kwon, Apostolidis, & Shetty, 2010). Thus, although çemen paste was removed before ACE-inhibitory analysis, some of these compounds could have migrated to the meat resulting in higher activities in its surface. As in the case of ACE-inhibitory activity, natural antioxidant compounds could have diffused in the meat due to their miscibility in the fatty fraction allowing the enrichment of the surface in

natural antioxidants. Regarding volumes of fractions, small peptides represented the highest antioxidant activity, which is in agreement with the results obtained in the ACE inhibitory activity assay. The ACE inhibitory effect as well as the antioxidant activity observed might have resulted from the synergistic effect of çemen ingredients together with the generated peptides. In fact, çemen paste has been previously described as antimicrobial against *Escherichia coli*, *Staphylococcus aureus* and *Yersinia enterocolitica* showing a higher activity than the individual ingredients, probably due to the synergistic effect between compounds (Yetim, Sagdic, Dogan & Ockerman, 2013).

Meat is a good source of bioactive peptides (Arihara, Nakashima, Mukai, Ishikawa, & Itoh, 2001; Escudero et al., 2012; Katayama et al., 2003; Mora, Aristoy & Toldrá, 2016), and among processed meat products dry-cured ham is the only product reported as a natural source of antihypertensive and antioxidant peptides (Arihara & Ohata, 2008; Escudero et al., 2012). Escudero et al. (2012) succeeded in identifying three novel peptides from Spanish dry-cured ham possessing *in vivo* antihypertensive activity. High stability of these ACE inhibitory ham peptides during heat treatment and *in vitro* gastrointestinal digestion were also proved by Escudero et al., (2014).

In the current study, strong inhibition of ACE probably resulted from high concentrations of amino acids, i.e. proline, alanine and leucine in certain positions of the generated peptides in final Pastırma product. What is more, main differences observed in inside and surface samples in comparison with whole sample could be due to a synergistic effect occurred between the generated bioactive peptides and the çemen paste.

3.4. Characterization of çemen paste

Çemen paste is used to cover the pastırma and influences both flavour and preservation of the final product. Thus, this mixture of ingredients has been reported to contribute to the color,

texture, and flavor of pastırma, as well as has resulted very effective against microbial contamination and preventing excessive drying during its processing. FAA contents (**Table 1**), sodium content, ACE-I inhibitory activity, DPPH radical scavenging and ferric reducing antioxidant power of çemen paste (Table 2) were also analyzed in this study. Çemen paste showed 4.87% sodium and 1007.86 mg/100g (dry matter) of total FAA content. The most abundant amino acids found in çemen paste were arginine, proline, asparagine, glutamic acid and lysine. ACE-I inhibitory activity of çemen paste was 93.75% at 0.25 g/mL (Table 2) which was close to maximum activity determined in particular peptide fractions of Pastırma samples (Figure 4). The higher percentages of ACE inhibitory activity obtained in surface sample could be due to the action of çemen paste. DPPH radical scavenging and ferric reducing antioxidant power of 0.25 g/mL çemen paste were found to be 89% and 0.88 (absorbance value), respectively (Table 2). These values indicate that the high antioxidant capacity of çemen paste could influence the strong antioxidant activity of Pastırma products.

4. Conclusions

Intense proteolysis during processing of Pastırma evidenced by a significant increase in free amino acids and peptides content was monitored by using modern proteomic techniques in this novel study. Formation of peptides in a wide range of molecular masses that were naturally generated during dry-cured processing was confirmed by MALDI-TOF spectra. The water soluble peptide fraction of Pastırma showed an ACE inhibitory activity higher than 86% in SEC fraction corresponding to molecular masses between 900 and 1500 Da, and also a strong DPPH radical-scavenging activity above 60% corresponding to molecular masses between 700 and 2000 Da. The promising results from this work showed that Pastırma represents a good source of natural bioactive peptides capable of reducing free radicals and inhibiting ACE activity. The novel scientific gains from the current study will be a guide for further studies on the

identification, quantification and sequencing of bioactive peptides of Pastırma by mass spectrometry in tandem.

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FIGURE CAPTIONS

Figure 1 Pastirma processing flow.

Figure 2 MALDI-TOF MS spectra of peptides generated at days 2, 5, 10 and 21 of Pastirma processing. Spectrum displays molecular masses between 800 and 2000 m/z . Numbers indicate m/z ($[MH^+]$) of the peptides.

Figure 3 MALDI-TOF spectra of end product at 21 days in the inside and the surface of the Pastirma. Spectrum displays molecular masses between 800 and 2000 m/z . Numbers indicate m/z ($[MH^+]$) of the peptides.

Figure 4 ACE-inhibitory activity of fractions collected on a Sephadex G-25 gel filtration column from Pastırma extracts.

Figure 5 (a) UV absorbance values of peptide elution volumes from 100 mL to 600 mL at 214 nm after fractionation of Pastırma extracts on a Sephadex G-25 gel filtration column; (b) DPPH radical-scavenging activity of the obtained fractions; (c) Ferric-reducing antioxidant power of the obtained fractions.

Figure 1

Figure 1 Pastırma processing flow

Figure 2

Figure 1 MALDI-TOF MS spectra of peptides generated at Days 2, 5, 10 and 21 of pasturama processing. Spectrum displays molecular masses between 800 and 2000 Da. Numbers indicate m/z ([MH⁺]) of the peptides. Gel view was obtained by using mMass software.

Figure 3

Figure 1 MALDI-TOF MS spectra of end product inside and surface. Spectrum displays molecular masses between 800 and 2000 Da. Numbers indicate m/z ($[MH^+]$) of the peptides. Gel view was obtained using mMass software

Figure 4

Figure 1 ACE-inhibitory activity of fractions collected on a Sephadex G-25 gel filtration column from pastirma extracts

Figure 5

(a)

(b)

(c)

Figure 1 (a) UV absorbance values of peptide elution volumes from 100 mL (20th fraction) to 600 mL (120th fraction) at 214 nm after fractionation of pastirma extracts on a Sephadex G-25 gel filtration column; (b) DPPH radical-scavenging activity of these fractionated peptides (c) Ferric-reducing antioxidant power of these fractionated peptides

Table 1 Amino acid and natural dipeptide concentrations of Pastırma samples (mg per 100g of dry matter) at days 2, 5, 10 and 21, and çemen paste (mg/100g dry matter)*

<i>Amino acids</i>	2nd day	5th day	10th day	21st day	Çemen paste
Essential FAA					
Histidine	5.06 ^a ±0.69	10.26 ^a ±2.18	18.48 ^b ±4.88	28.45 ^c ±6.70	13.74±0.93
Threonine	9.47 ^a ±0.44	8.09 ^{ab} ±1.71	17.92 ^b ±4.99	41.66 ^c ±10.33	16.42±1.77
Valine	14.57 ^a ±1.30	26.06 ^a ±3.44	42.37 ^b ±8.81	84.02 ^c ±15.21	38.12±4.61
Methionine	8.17 ^a ±1.10	12.30 ^a ±1.57	19.24 ^b ±3.65	37.29 ^c ±5.25	9.82±1.28
Isoleucine	12.63 ^a ±1.19	17.26 ^{ab} ±2.01	26.24 ^b ±5.25	56.98 ^c ±9.73	16.71±1.48
Leucine	21.78 ^a ±1.86	32.45 ^a ±2.95	49.84 ^b ±9.57	111.55 ^c ±17.91	30.03±3.55
Phenylalanine	11.09 ^a ±1.16	16.81 ^a ±2.20	31.1 ^b ±5.87	70.86 ^c ±11.09	24.23±2.72
Tryptophan	2.52 ^a ±0.19	3.65 ^a ±0.57	7.02 ^b ±1.46	14.33 ^c ±2.41	10.60±1.44
Lysine	22.61 ^a ±3.12	36.40 ^a ±6.55	88.12 ^b ±21.21	140.23 ^c ±37.50	70.00±13.25
Non essential FAA					
Aspartic acid	1.35 ^a ±0.21	0.91 ^a ±0.19	3.14 ^a ±0.93	9.31 ^b ±7.34	24.99±3.06
Glutamic acid	-	-	55.74 ^a ±5.84	92.99 ^b ±28.19	76.31±9.62
Serine	12.99 ^a ±0.68	12.49 ^a ±1.82	21.58 ^b ±4.92	44.28 ^c ±11.95	20.63±2.30
Asparagine	4.99 ^a ±0.19	5.71 ^a ±0.72	24.18 ^b ±4.64	39.22 ^c ±7.78	79.11±9.79
Glycine	16.24 ^a ±1.18	12.68 ^a ±2.33	14.70 ^a ±3.71	29.32 ^b ±9.45	10.69±1.41

Glutamine	102.94 ^a ±5.97	56.57 ^a ±13.35	51.73 ^a ±4.53	49.09 ^b ±11.07	52.04±6.37
Alanine	60.26 ^a ±4.51	61.09 ^a ±10.10	67.57 ^a ±8.73	133.17 ^b ±18.90	38.12±4.61
Arginine	17.71 ^a ±1.29	22.32 ^a ±1.58	66.16 ^b ±14.56	109.22 ^c ±27.55	288.43±41.91
Proline	7.69 ^a ±0.69	11.94 ^a ±2.70	58.57 ^b ±9.95	151.00 ^c ±31.31	171.42±20.11
Tyrosine	12.14 ^a ±2.67	23.97 ^b ±3.12	32.46 ^c ±5.41	59.60 ^d ±4.94	26.41±3.19
Ornithine	2.42 ^a ±0.50	2.53 ^a ±0.54	4.25 ^a ±3.64	14.27 ^a ±16.40	2.11±0.36

Other FAA

β-Alanine	1.34 ^a ±0.20	1.76 ^a ±0.44	1.83 ^{ab} ±0.57	2.54 ^b ±0.49	2.03±0.34
Taurine	31.04 ^a ±7.56	29.89 ^a ±5.38	22.60 ^a ±7.41	25.89 ^a ±8.35	3.26±0.42

Dipeptides

Carnosine	560.41 ^{ab} ±108.06	662.21 ^{bc} ±88.95	712.18 ^c ±66.71	459.41 ^a ±99.23	-
Anserine	75.41 ^a ±19.39	96.40 ^a ±21.19	106.72 ^a ±20.46	76.41 ^a ±18.89	-
Total**	347.98^a±9.48	374.82^a±32.70	702.27^b±48.84	1319.38^c±202.02	1007.86±132.17

*Least square means±standard deviations.

**Taurine, carnosine and anserine are not included.

^{a-d} LS Means with different letters in the same line indicate significant differences (p< 0.05).

Table 2 Bioactivity characterization of çemen paste*

Bioactive characterization	
ACE-I inhibitory activity (%)	93.75±2.422
DPPH radical scavenging (%)	89.00±0.007
Reducing power**	0.88±0.008

*Least square means±standard deviations. Çemen paste concentration in the extract used for bioactivity characterization was 0.25 g/mL.

**Values for ferric-reducing antioxidant power were based on the absorbance values measured at 700 nm.