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# Identification of peptides with antioxidant and antihypertensive capacities by RP-HPLC-Q-TOF-MS in dry fermented camel sausages inoculated with different starter cultures and ripening times

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**Running title**: Identification of bioactive peptides in dry fermented camel sausages

**ABSTRACT**

Low molecular weight peptides are produced during meat fermentation. They contribute to generate flavor compounds but they can also exert certain bioactivities. The aim of this work was to evaluate, for the first time, the generation of bioactive peptides during the preparation of dry fermented camel sausages and to study the influence of the ripening time and the starter culture on bacteria growing, peptide concentration and size, and antioxidant and antihypertensive capacities of peptides. Camel meat sausages inoculated with different starter bacteria and non inoculated were ripened up to 28 days. Results demonstrated that bacteria population, peptide concentration, and peptide size were affected by the ripening time and the inoculated bacteria. Moreover, the ripening process resulted in an increasing antioxidant and antihypertensive capacity showing the highest bioactivities in fractions with peptides below 3 kDa. Peptides in these fractions were identified by RP-HPLC-Q-TOF-MS analysis. Identified peptides showed common features with peptides with antioxidant or antihypertensive activity.

K**eywords**: fermented sausage, bioactive peptide, starter culture, antioxidant capacity, antihypertensive capacity, mass spectrometry.

## 1. Introduction

The manufacture of dry fermented sausages represents an important part of the meat industry, particularly in Mediterranean countries (Laranjo et al., 2015). Many studies have examined the possibilities for reformulation of fermented sausages using different meat types, such as goat, sheep, and mutton (Zhao et al., 2011; Stajic, Perunovic, Stanisic, Zujovic & Zivkovic, 2013). The potential of camel meat is receiving increasing attention due to its nutritional value and its low fat content. However, not much has been done in relation to its use for the preparation of fermented sausages.

Many biochemical reactions involving proteins, carbohydrates, and lipids occur during the fermentation of sausages and they determine the characteristics of the final fermented product (Candogan, Wardlaw & Acton, 2009). Proteolysis, catalyzed by either endogenous enzymes present in meat tissues or by those of microbial origin from added starter cultures, is one of them.

The use of starter cultures for the production of fermented foods is needed to guarantee safety and desirable technological properties. Most promising bacteria cultures are those isolated from the endogenous microflora of traditional meat products. These microorganisms are well adapted to the meat environment and are capable of dominating product microflora (Essid & Hassouna, 2013; Aro Aro et al., 2010; Drosinos et al., 2005; Tabanelli et al., 2012; Zhao et al., 2011). The two main categories of bacteria that play a significant role and are commonly found in fermented sausages are lactic acid bacteria (LAB) and coagulase negative *Staphylococci* (CNS). They are the most active endogenous microorganisms in the acidification process and in the denitrification, lipolysis, and proteolysis (Casaburi et al., 2007).

LAB, in dry fermented meat products, is responsible for the rapid fermentation of carbohydrates leading to a decrease in pH. The acidification below the isoelectric point of muscle proteins affects the protein coagulation which is responsible for the slice ability, firmness, and cohesiveness of the final product (Drosinos et al., 2007). On the other hand, CNS participates in the development and stability of product color by the nitrate reductase activity, that leads to the formation of nitrosomyoglobin and the reduction of nitrate to nitrite, which also limits lipid oxidation (Talon, Walter, Chartier, Bariere, & Montel, 1999). Moreover, LAB and CNS also play a significant role in the development of the flavor in fermented meats contributing to the formation of low molecular weight compounds such as peptides (Simonová et al., 2006).

In addition to contribute to the development of a characteristic flavor in dry fermented products, peptides can also exert different bioactivities. In fact, a peptide is a functional ingredient if it has been successfully demonstrated its beneficial effect on one or more functions of the body beyond its nutritional effects, so that their effect is significant for health, in general, or enables to reduce the risk to suffer a disease (Diplock et al., 1999). Bioactive peptides can present diverse activities (antioxidant, antihypertensive, hypocholesterolemic, immunostimulating, *etc*) although antioxidant and antihypertensive activities are the most studied (García, Puchalska, Esteve & Marina, 2013). Antioxidant and antihypertensive peptides have been observed in other meat products such as Spanish dry cured ham and Spanish dry fermented sausages (Escudero et al., 2013; Mora, Escudero, Aristoy & Toldrá, 2015) but they have never been studied in camel sausages.

The aim of this work was to evaluate the presence of antioxidant and antihypertensive peptides in dry fermented camel sausages, to study the influence of the ripening time and the starter culture, and to identify those peptides showing the highest bioactivities by peptidomic analysis.

## 2. Materials and methods

### *2.1. Chemical and reagents*

All chemicals were of analytical grade purity. Water was daily obtained from a Milli-Q system from Millipore (Bedford, MA, USA). Acetonitrile (ACN), acetic acid (AA), acetone, ethanol, methanol (MeOH), and hexane were purchased from Scharlau Chemie (Barcelona, Spain). Tris(hydroxymethyl)aminomethane (Tris), hydrochloric acid (HCl), sodium dihydrogen phosphate, ammonium bicarbonate, and sodium dodecyl sulphate (SDS) were from Merck (Darmstadt, Germany). Sodium hydroxyde, trifluoroacetic acid (TFA), bovine serum albumin (BSA), o-phthalaldehyde (OPA), sodium tetraborate, 2-mercaptoethanol, glutathione (GSH), 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), potassium persulphate, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), 2,2-diphenyl-1-picrylhydrazyl (DPPH), 1,10-phenantroline, ferrous sulphate, hydrogen peroxide, potassium ferricyanide, trichloroacetic acid (TCA), ferric chloride, ammonium thiocyanate, ferrous chloride, angiotensin converting enzyme (ACE) from rabbit lung, hippuryl-histidyl-leucine (HHL), 2-[4-(2-hydroxyethyl)-1-piperazinyl]-ethanesulphonic acid (HEPES), and sodium chloride were all purchased from Sigma-Aldrich (Saint Louis, MO, USA).

### *2.2. Bacterial strains and culture conditions*

*Staphylococci xylosus*, *Lactobacillus plantarum* and *Lactobacillus pentosus* strains were isolated from a camel meat sausage obtained by fermentation with endogenous microflora. Strains of *S. xylosus* were isolated on Mannitol Salt Agar (Pronadisa, Madrid, Spain) after incubation at 30°C for 48 h. Morphology, Gram-staining, and coagulase tests were carried out to isolate only Gram-positive and coagulase-negative cocci. Strains of *L. plantarum* and *L. pentosus* were isolated and purified on Man Rogosa and Sharpe agar (MRS)

(Pronadisa) at 30°C for 24 h. Only Gram-positive catalase-negative isolates were employed. A commercial mixture, recommended for the production of all types of fermented sausages and containing a strain of *L. sakei* and *S. carnosus* was kindly donated by Chr. Hansen (F-SC-111 Bactoferm™, Christian Hansen A/S, Horsholm, Denmark).

### *2.3. Sausage manufacture*

Camel sausages were produced with lean meat (70% w/w), camel fat (22% w/w), salt (4%,w/w), glucose (1 %, w/w), $KNO_3$(0.01%, w/w), and spices (black pepper, coriander, and paprika). After homogenization, the mixture was divided into four batches: batch A was inoculated with isolated strains of *S. xylosus* and *L. plantarum* ($10^7$ CFU/g); batch B was inoculated with isolated starters of *S. xylosus* and *L. pentosus* ($10^7$ CFU/g); batch C was inoculated with commercial starters containing a strain of *S. carnosus* and *L. sakei* ($10^7$ CFU/g); and batch D was non inoculated and was used as control. Mixtures were manually stuffed into fibrous casing (20-25 cm of length and about 4 cm of diameter) of approximately 250 g and placed in a drying ripening chamber (BCR, CF 1 B, Antony, France). Sausages were subjected to the following conditions of temperature and relative humidity (RH): fermentation for 5 days at 24°C and 85-90% RH followed by drying at 14°C and 75-80% RH for 23 days. Samples were taken after 0, 7, 14, 21, and 28 days of ripening for their analyses.

### *2.4. Extraction and fractionation of peptides*

Protein and peptides from dry fermented sausages were extracted according to a method previously developed by the research group (Castro-Rubio, García, Rodríguez & Marina, 2005) with some modifications. All samples were defatted before their analysis by the following procedure: 10 g of fermented sausages was ground with an automatic miller, homogenized with 25 mL of acetone in an Ultra-Turrax mixer (IKA, Staufen, Germany) for 3 min, submitted to agitation for 15 min, and centrifuged (4000×g, 30 min, 25°C). The

supernatant was removed and the pellet was extracted again with 25 mL of acetone following the same procedure. Finally, the pellet was dried overnight at 60°C to remove the remaining acetone. Peptides were extracted by mixing defatted fermented sausage (0.5g) with 5 mL of a 30 mM Tris-HCl buffer (pH 8.0) containing 0.5% (v/v) 2-mercapthoethanol with ultrasonic agitation for 10 min at 50°C, and centrifuging for 20 min at 4000×g. The supernatant was mixed with 10 mL of cold acetone and kept in the fridge for 30 min. Finally, the solution was centrifuged for 10 min at 4000×g and the precipitate was redissolved in phosphate buffer (PB) (pH 8.0). Extracts were filtered through regenerated cellulose filters (0.45 µm) and fractionated by ultrafiltration through molecular weight (Mw) cut-off filters of 5 kDa (Vivaspin 500 PES, Sartorius Stedim Biotech, Goettingen, Germany) and 3 kDa (Amicon, Millipore).

### *2.5. Peptide concentration*

Peptide concentration in fractions obtained by ultrafiltration were determined by the OPA (o-phthalaldehyde) assay (Wang et al., 2008). The procedure was as follows: 2.5 µL of sample were mixed with 100 µL of OPA mixture (2.5 mL of sodium tetraborate, 1 mL of 5% (m/v) SDS, 100 µL of 40 mg/mL OPA in MeOH, 10 µL of 2-mercaptoethanol, and 1.39 mL of water). Solutions were left for 8 min at room temperature and signals were measured at 340 nm. Peptide content was calculated by interpolation in a GSH standard calibration curve in the range from 0 to 5 mg/mL. All measurements were carried out a minimum of three times.

### *2.6. ABTS radical scavenging capacity*

ABTS assay was performed according to a previously developed method (Wiriyaphan, Chitsomboon & Yongsawadigul, 2012) with modifications. In this assay, ABTS oxidation is produced by its reaction with potassium persulphate resulting in the formation of deep green $ABTS^{\bullet+}$. $ABTS^{\bullet+}$stock solution containing 7.4 mM ABTS and 2.6 mM potassium persulphate

in 10 mM PB (pH 7.4) was prepared and kept in the dark for, at least, 16 h. The working solution was prepared daily by mixing the $ABTS^{\bullet+}$ stock solution with 10 mM PB (pH 7.4) to obtain an absorbance of 0.70 ± 0.01 at 734 nm. The assay was carried out by mixing 1 µL of sample with 100 µL of $ABTS^{\bullet+}$ working solution for 6 min at room temperature. Afterwards, the absorbance corresponding to $ABTS^{\bullet+}$ was measured at 734 nm. $ABTS^{\bullet+}$scavenging capacity was calculated using the following equation:

$$ABTS\ radical\ scavenging\ capacity\ (\%) = \left(\frac{Abs_{blank} - Abs_{sample}}{Abs_{blank}}\right) x\ 100$$

where $Abs_{sample}$ is the absorbance of 1 µL of sample with 100 µL of $ABTS^{\bullet+}$ working solution and $Abs_{blank}$ is the absorbance of 1 µL of buffer with 100µL of $ABTS^{\bullet+}$ working solution. Trolox (1 mM) was employed as positive control. Three replicates of each sample were measured, at least, three times. Solvent blanks were measured for every assay.

### *2.7. DPPH radical scavenging capacity*

DPPH radical scavenging capacity was evaluated through a method proposed by You, Zhao, Regenstein & Ren (2011) with some modifications. Sample (50 µL) was mixed with 50 µL of 0.1 mM DPPH in 95% ethanol and kept for 30 min (at room temperature) in the dark. The absorbance of the resulting solution was measured at 540 nm. The DPPH radical-scavenging capacity was calculated as follows:

$$DPPH\ radical\ scavenging\ capacity\ (\%) = \left(1 - \frac{Abs_{sample} - Abs_{control}}{Abs_{blank}}\right) x\ 100$$

where $Abs_{sample}$ is the absorbance of the sample with DPPH solution; $Abs_{control}$ is the absorbance of the sample without DPPH solution; $Abs_{blank}$ is the absorbance of the sample solvent (without peptides) with the DPPH solution. GSH (0–5 mg/mL) was used as positive

control. Three replicates of each sample were measured, at least, three times. Solvent blanks were measured for every assay.

### *2.8. Inhibition of the formation of hydroxyl radicals*

The capacity to inhibit the formation of hydroxyl radicals was evaluated by the method of Ajibola, Fashakin, Faqbemi & Aluko (2011) with modifications. Hydroxyl radicals were generated by the reaction of hydrogen peroxide with Fe (II) that was monitored by its reaction with 1,10-phenanthroline. Antioxidant compounds inhibit the oxidation of Fe (II) to Fe (III) and the formation of •OH. The assay consisted of mixing 50 µL of 3 mM 1,10-phenanthroline with 0.1 M PB (pH 7.4), 50 µL of 3 mM ferrous sulphate, 50 µL of sample, and 50 µL of 0.01% (v/v) hydrogen peroxide. The mixture was incubated for 1 h at 37°C and the absorbance corresponding to the complex Fe (II)-1,10 phenantroline was measured at 536 nm. Inhibition of the hydroxyl radical formation was calculated according to the following equation:

$$Hydroxyl\ radical\ scavenging\ capacity\ (\%) = \left(\frac{Abs_{sample} - Abs_{blank}}{Abs_{control} - Abs_{blank}}\right) x\ 100$$

where $Abs_{sample}$ is the absorbance of the sample, $Abs_{blank}$ is the absorbance of the buffer, and $Abs_{control}$ is the absorbance of the solution resulting when adding the buffer instead of the sample and water instead of hydrogen peroxide. GSH (1 mg/mL) was employed as positive control. Three replicates of each sample were measured at least three times. Solvent blanks were measured for every assay.

### *2.9. Ferric reducing antioxidant power (FRAP)*

This assay monitors the increase in absorbance when Fe (III), from the ferricyanide complex, turns into Fe (II) and it was evaluated according to a method previously proposed by

Ajibola et al. (2011) with modifications. For that purpose, 12.5 µL of hydrolysate, 12.5 µL of 0.2 M PB (pH 6.6), and 25 µL of potassium ferricyanide were mixed and incubated for 20 min at 50°C. Thereafter, the reaction was stopped by adding 25 µL of 10% (w/v) TCA. For the assay, 50 µL of that solution, 40 µL of water, and 10 µL of 2.5% (w/v) ferric chloride were mixed and, after 3 min at room temperature, the absorbance was measured at 700 nm. The reducing power was calculated according to the following equation:

$$Reducing\ power = \frac{Abs_{\ sample}}{Peptide\ content}$$

where $Abs_{sample}$ is the absorbance corresponding to the hydrolysate and the peptide content was calculated by the OPA assay. The positive control was GSH (0-1 mg/mL). The percentage of inhibition was calculated taking as maximum reduction power that obtained with 1 mg/mL GSH. Three replicates of each sample were measured, at least, three times. Solvent blanks were measured for every assay.

### *2.10. ACE inhibition capacity*

ACE inhibition capacity was evaluated following the method described by Geng, He, Yang & Wang (2010) with slight modifications. This method is based on the reaction of the tripeptide HHL into hippuric acid (HA) by the action of ACE and it measures the capacity of hydrolysates to inhibit ACE activity. The procedure involved mixing 2.5 µL of sample (or buffer for the control) with 10 µL of ACE (0.05 U/mL in water), 17.5 µL of 50 mM HEPES buffer (pH 8.3) that contained 300 mM NaCl, and 5 µL of HHL (1.3 mg/mL in HEPES with NaCl). Then, the mixture was incubated at 37°C and the reaction was stopped by adding 50 µL ACN at -20°C. The separation of HHL and HA was performed in an HPLC equipment with a Chromolith® Performance RP-18 endcapped column (100 × 4.6 mm) (Merck, Darmstadt, Germany). The chromatographic conditions were: gradient, 5-95% B in 10 min;

flow rate, 1 mL/min; temperature, 25°C; injection volume, 10 µL; and UV detection at 228 nm. Mobile phases were: A, water with 0.025% (v/v) TFA and B, ACN with 0.025% (v/v) TFA. Inhibition of ACE was calculated using the following equation:

$$ACE\ inhibition\ \% = \frac{HA_{blank} - HA_{sample}}{HA_{blank}}\ x\ 100$$

where $HA_{blank}$ is the HA peak area without ACE inhibition and $HA_{sample}$ is the HA peak area in the sample. Three replicates of each sample were measured, at least, three times. Solvent blanks were measured for every assay.

### *2.11. Identification of peptides by RP-HPLC-ESI-Q-TOF-MS/MS*

The identification of peptides was carried out using a high sensitivity mass spectrometry Quadrupole/Time-of-Flight (Q/TOF) (Series 6530) coupled to a liquid chromatograph (model 1100), both from Agilent Technologies (Palo Alto, CA, USA). HPLC separation was obtained using an Ascentis Express Peptide ES-C18 column (100 mm × 2.1 mm I.D., 2.7 µm particle size) with an Ascentis Express Guard column (5 mm × 2.1 mm I.D., 2.7 µm particle size), both from Supelco (Bellefonte, PA, USA). Mobile phases were: A, Milli-Q water with 0.3% of AA (v/v), and B, ACN with 0.3% of AA (v/v). Three different elution gradients were employed depending on the sample. Peptides in control sausage and batch B sausage were eluted using a gradient at 5% B for 3 min, 5-70% B in 35 min, and 70-95% B in 2 min. A gradient at 35% B for 12 min, from 35 to 75% B in 28 min, and from 75 to 95% B in 7 min was employed for the separation of peptides in batch A sausage. Finally, a gradient from 5 to 95% B in 35 min was used for the separation of peptides in batch C sausage. In all cases, the flow rate was 0.3 mL/min, the column temperature was 25°C, and the injection volume was 15 µL.

The mass spectrometry (MS) detection was carried out in the positive ion mode and the mass range was from 100 to 1500 *m*/*z*. ESI Jet Stream conditions were: fragmentator voltage, 200 V; nebulizer pressure, 50 psig; capillary voltage, 3500 V; gas temperature, 350°C; gas flow, 12 L/min; and skimmer voltage, 60 V. Source sheath gas temperature and flow were 400°C and 12 L/min, respectively. MS/MS was performed employing the auto mode and the following optimized conditions: 1 precursor per cycle, dynamic exclusion after two spectra (released after 1 min), and collision energy of 5 V for every 100 Da. Precursor mass tolerance threshold was 10 ppm and the maximum fragment mass error was set at 0.5 Da. The identification of peptides was carried out with PEAKS STUDIO 7 software (Bioinformatics Solutions Inc., Waterloo, Canada) using the database search tool with an improved algorithm that validates and assists the database search with *de novo* sequencing results. Proteome of meat proteins (*Cetartiodactyla* proteome containing 162,867 entries) from UniProt databases (SwissProt and TrEMBL) was used for the database search. The carbamidomethylation of cysteines, methionine oxidation, and serine phosphorilation were selected as variable modifications during the search. Results were refined using a false discovery rate (FDR) of 1%. Peptides identified above appropriate -10logP threshold have been treated as a true positive. *Do novo* peptide sequencing using an average local confidence (ALC% indicates the expected percentage of correct amino acids in the peptide sequence) above 80% was also used. Identifications were carried out using two independent samples injected by duplicate.

### *2.12. Statistical analysis*

Data were statistically analyzed using one-way ANOVA using SPSS Statistic Software Version 17. Tukey's multiple range test was employed to determine any significant difference between mean values using $P < 0.05$.

## 3. Results and discussion

Three dry fermented camel meat sausages were prepared using different mixtures of starter cultures. Starter cultures strains were selected taking into account results from a previous work in which 130 LAB and 120 CNS strains (all Gram-positive) were isolated from traditional dry sausages and characterized by their proteolytic activity (Mejri & Hassouna, 2016). *L. plantarum*, *L. pentosus*, and *S. xylosus* strains showed the highest halo diameter around the proteolytic bacteria in the hydrolysis of caseins and the most intense values for the hydrolysis of azocasein. Therefore, these strains were selected for the preparation of fermented camel sausages. Batch A sausages were prepared by adding *S. xylosus* and *L. plantarum* cultures, batch B sausages were inoculated with *S. xylosus* and *L. pentosus*, and batch C sausages contained commercial starters of *S. carnosus* and *L. sakei*. Additionally, a fourth batch (batch D) was prepared without adding any starter and was used as control. All batches were ripped during 28 days and samples at 0, 7, 14, 21, and 28 days were taken for their analyses.

### 3.1. Comparison of bacteria growing during ripening of inoculated and non inoculated dry fermented camel meat sausages

Bacteria growing in the non inoculated sausage (control) and in sausages (batch A, B, and C) inoculated with $10^7$ CFU/g of different LAB and CNS during ripening is shown in Figure 1. As expected, the bacteria population in inoculated samples was higher that the observed for the control. The LAB count in the first day of fermentation was 4.57 log CFU/g for control sausages against 7.78, 7.09 and 7.44 log CFU/g in batch A, batch B and batch C, respectively. Similarly, the initial *Staphylococci* counts were around 6.15 log CFU/g in the inoculated sausages and 4.42 log CFU/g in the control ones. For all batches, it was observed the same bacteria growing pattern with a maximum bacteria population on the $14^{th}$ day of ripening.

Comparing bacteria strains, LAB was the dominant microflora for the inoculated and non inoculated sausages. The faster growth rates of LAB during fermentation and ripening of sausages demonstrated a better adaptation of LAB to the meat environment than CNS (Casaburi et al., 2007; Drosinos et al., 2005; Zhao et al., 2011).

### 3.2. Evaluation of the release of peptides during the ripening of inoculated and non inoculated dry fermented camel meat sausages

Protein and peptides in sausages for every batch (A, B, C, D) and at different ripening times were extracted using a method previously developed by our research team (Castro-Rubio et al., 2005). Moreover, every extract was fractionated by ultrafiltration to obtain fractions with protein/peptides with Mw higher than 5 kDa, from 3 to 5 kDa, and smaller than 3 kDa. Peptide concentration in fractions was determined and results are summarized in Table 1. As expected, peptide concentration in fractions above 5 kDa and from 3 to 5 kDa decreased during ripening in the control and in inoculated sausages while peptide concentration increased in fractions containing peptides below 3 kDa. In general, there were not significant differences when comparing peptide concentrations in fractions from different inoculated samples and control.

### 3.3. Evaluation of the antioxidant capacity of peptides released during the ripening of inoculated and non inoculated dry fermented camel meat sausages

The antioxidant capacity of whole extracts and fractions obtained by ultrafiltration (with peptides > 5 kDa, 3-5 kDa, and < 3 kDa) was evaluated. Since there is not a standardized method for that purpose and there are different antioxidant mechanisms, a correct evaluation of antioxidant capacity requires the use of different assays. Therefore, the capacity to scavenge two different radicals (DPPH and ABTS), the capacity to inhibit the formation of hydroxyl radicals, and the ability to reduce Fe (III) were evaluated in every

sample. As example, Figure 2 shows the variation of the antioxidant capacity evaluated by the hydroxyl radical assay and by the ABTS assay for the whole extracts and fractions obtained from the control and batch A sausages. The capacity to scavenge DPPH radicals, to inhibit the formation of hydroxyl radicals, and the reducing capacity of whole extracts decreased with the ripening time while they increased in all fractions. Nevertheless, this increase was faster for the fractions with peptides below 3 kDa than for the fractions with peptides from 3-5 kDa and above 5 kDa. Therefore, the highest capacity to inhibit the formation of hydroxyl radicals, to scavenge DPPH radicals, and to reduce Fe (III) was observed for the fractions below 3 kDa at the highest ripening time. Similar behavior was observed for batches B and C. Other authors have also demonstrated an increase in DPPH radical scavenging activity (from 58 to 68%) of peptides below 3 kDa from Iberian dry-cured sausage after 30 days of ripening (Broncano et al., 2011). Furthermore, Sun et al. (2009) observed a great DPPH radical scavenging activity (92%) in the fraction of peptides below 5 kDa from fermented pork sausages after 72 h of drying.

Regarding the capacity to scavenge ABTS radicals, in general, it was higher than the capacity to scavenge DPPH radicals, the capacity to inhibit the formation of hydroxyl radicals, and the reducing capacity. Moreover, like in the other assays, ABTS radical scavenging capacity decreased with the ripening time for the whole extracts and increased for the fractions with peptides < 3 kDa. Nevertheless, unlike in the other assays, fractions with peptides above 5 kDa and from 3 to 5 kDa yielded a decreasing ABTS radical scavenging capacity with the ripening time. Table 2 shows the antioxidant capacity determined using the different assays for the fractions below 3 kDa at all ripening times. Comparing batches, the increase in antioxidant capacity was faster for the fraction < 3 kDa in batch A while this increase was slower for the control and batch C (commercial starter). An intermediate situation was observed for batch B that differed in the kind of added LAB. The increase in

antioxidant capacity could be due to the increase in peptide concentration (see Table 1) and to an increase in peptide hydrophobicity (Ajibola et al., 2011; Moslehishad et al., 2013). This fact was in agreement with Vaštag et al. (2010) that revealed the relationship between antioxidant activity and degree of proteolysis. Indeed, after 30 days ripening of a pork fermented sausage, the degree of hydrolysis increased from 6 to 12% and this fact came along with an increase in DPPH scavenging activity and reducing power.

### 3.4. Evaluation of the ACE inhibition capacity of peptides released during the ripening of inoculated and non inoculated dry fermented camel meat sausages

Figure 3 shows the ACE inhibition capacity of whole extracts and fractions obtained by ultrafiltration (> 5 kDa, 3-5 kDa, < 3 kDa). The percentage of ACE inhibition increased significantly ($p < 0.05$) in the whole extract and in all fractions during the ripening. Nevertheless, the highest ACE inhibition capacity was always observed for the fraction with peptides below 3 kDa. This result confirmed suggested formulated by Escudero et al. (2013) and Mora et al. (2015) who stated that low molecular weight peptides (< 3 kDa), contributing to the development of flavor in dry fermented meat products, could also exert antihypertensive activity. Moreover, ACE inhibition through ripening was significantly affected by the addition of starter ($p < 0.05$). In fact, the highest ACE inhibition capacity was observed in batch A sausages (Figure 3A) and the lowest in the control (Figure 3D) while batch B sausages (Figure 3B) yielded a similar behavior to the control and the sausages inoculated with commercial starter (Figure 3C) resulted in an intermediate situation. Since batch A and B sausages contained a different LAB culture, this could be the explanation to this difference in ACE inhibition capacity. These results were in accordance with those of Flores & Toldrá (2011) and Mora et al. (2015) that studied the contribution of microbial starters to uniformity and safety during the manufacture of fermented sausages. In fact, they found that the action of microorganisms such as LAB could influence the last period of

fermentation, contributing to the generation of small peptides and free amino acids. We have confirmed these results and we have also demonstrated that these peptides can yield ACE inhibiting ability.

### *3.5. Identification of peptides*

Fractions with peptides < 3 kDa, obtained at the highest ripening time for all batches and control sausages, exerted the highest antioxidant and antihypertensive capacities. Nevertheless, significant differences were observed among them. In order to have a deep insight, peptides present in fractions below 3 kDa from the control and the different batches were identified using RP-HPLC-ESI-Q-TOF-MS/MS and PEAKS software. Identification of peptides was firstly carried out by the database search tool. In case no match with database proteins was observed, *de novo* identification was used for sequencing peptides. Table 3 shows those peptides matching to proteins by database search and Table 4 groups those peptides identified by *do novo*. Matching scores (ALC (%) when doing *de novo* sequencing or *-10 log P* when doing database search identification), the experimental molecular mass, mass accuracy, and protein accession in the Uniprot database (when using database search) were also included in Tables 3 and 4.

Peptides were firstly searched against *Camelus* database but, due to the low number of entries, most peptides were searched against *Cetartiodactyla* database with 162,867 entries. Number of peptides identified by database search ranged from 9 to 15 and, due to the low number of entries from Camelus, most of them were attributed to related species proteomes. Peptides contained between 5 and 23 amino acids which is in accordance with results from bibliography for other meats (López, Brue, Vignola & Fadda, 2015). Most peptides identified by database search came from main meat proteins actin, myosin and troponin T. Despite there were common peptides among some samples, no common peptide among all samples was

identified when doing database search. An exception was sequence FAGDDAPR that appeared as an independent peptide or within the sequence of a bigger peptide in all samples. Unlike this, *de novo* identification enabled to find peptide EDEEVEH in all samples. The number of identified peptides by *do novo* ranged from 4 to 11 with a number of amino acids from 4 to 12. Figure 4 shows the total ion chromatogram (TIC) obtained by RP-HPLC-ESI-Q-TOF-MS/MS for the fraction of peptides below 3 kDa from the control sausage and the MS/MS spectrum corresponding to EDEEVEH peptide. The analysis of the amino acid composition of identified peptides showed high amounts of acid (glutamic acid (E) and aspartic acid (D)) and hydrophobic amino acids (valine (V), proline (P), alanine (A) and leucine/isoleucine (L/I)). Moreover, basic amino acid histidine (H) was also present. The presence of these amino acids has been reported to be usual among antioxidant peptides (Sarmadi & Ismail, 2010). In fact, acid and basic peptides show capacity to chelate metal ions and donate hydrogen while hydrophobic amino acids can enhance solubility of peptides in lipids making easier the accessibility to hydrophobic radical species. Moreover, amino acids such as proline (P), arginine (R) or lysine (K) at C-terminal position are usual in antihypertensive peptides (Puchalska, 2015) and were also found in many identified sequences.

## 4. Conclusions

Despite bacteria growing in inoculated and non inoculated meat sausages showed identical patterns with a maximum in the 14$^{th}$ ripening day, bacteria population in inoculated samples was higher than in the control showing a higher growing in lactic acid bacteria than in *Staphylococci*. This bacterial growing resulted in a decreasing concentration in peptides with molecular weights above 3 kDa and an increasing amount of peptides with molecular

weights below 3 kDa. This could explain why this fraction always showed the highest antioxidant and antihypertensive capabilities in comparison with fractions above 3 kDa. The highest antioxidant and antihypertensive capacities were observed at the end of ripening. In general, antioxidant and antihypertensive capacities in non inoculated sausages were lower than the observed for inoculated sausages, especially for the sausage inoculated with *S. xylosus* and *L. plantarum* (Batch A). Analysis of fractions below 3 kDa by RP-HPLC-ESI-Q-TOF-MS/MS enabled the identification of 13-22 peptides in every sample with a number of amino acids that ranged from 4 to 23. Peptides showed many common features with other antioxidant and antihypertensive peptides. Peptide EDDEVEH and sequence AGDDAPR were observed in the control and all batches.

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**References**

Ajibola, C.F., Fashakin, J.B., Faqbemi, T.N., & Aluko, R.E. (2011). Effect of peptide size on antioxidant proprieties of African yam bean sead (*Sphenostylis Stenocarpa*) protein hydrolysate fractions. *International Journal of Molecular Sciences, 12*, 6685-6702.

Aro Aro, J. M., Nyam-Osor, P., Tsuji, K., Shimada, K., Fukushima, M., & Sekikawa, M. (2010). The effect of starter cultures on proteolytic changes and amino acid content in fermented sausages. *Food Chemistry*, *119*, 279-285.

Broncano, J. M., Timón, M. L., Parra, V., Andrés, A. I., & Petrón, M. J. (2011). Use of proteases to improve oxidative stability of fermented sausages by increasing low molecular weight compounds with antioxidant activity, *Food Research International*, *44*, 2655-2659.

Candogan, K., Wardlaw, F. B., & Acton, J. C. (2009). Effect of starter culture on proteolytic changes during processing of fermented beef sausages. *Food Chemistry*, *116*, 731-737.

Casaburi, A., Aristoy, M. C., Cavella, S., Di Monaco, R., Ercolini, D., Toldrá, F., & Villani, F. (2007). Biochemical and sensory characteristics of traditional fermented sausages of Vallo di Diano (Southern Italy) as affected by the use of starter cultures. *Meat Science*, *76*, 295-307.

Castro-Rubio, F., García, M. C., Rodríguez, R., & Marina, M. L. (2005). Simple and inexpensive method for the reliable determination of additions of soybean proteins in heat-processed meat products: an alternative to the AOAC official method. *Journal of Agricultural and Food Chemistry*, *53*, 220-226.

Diplock, A. T., Aggett, P. J., Ashwell, M., Bornet, F., Fern, E. B., & Roberfroid, M. B. (1999). Scientific concepts of functional foods in Europe: consensus document. *British Journal of Nutrition*, *81*, 1-27.

Drosinos, E. H., Mataragas, M., Xiraphi, N., Moschonas, G., Gaitis, F., & Metaxopoulous, J. (2005). Characterization of the microbial flora from a traditional Greek fermented sausage. *Meat Science, 69,* 307-317.

Drosinos, E. H., Paramithiotis, S., Kolovos, G., Tsikouras, I., & Metaxopoulos, I. (2007). Phenotypic and technological diversity of lactic acid bacteria and staphylococci isolated from traditionally fermented sausages in Southern Greece. *Food Microbiology, 24,* 260-270.

Escudero, E., Mora, L., Fraser, P. D., Aristoy, M. C., Arihara, K., & Toldrá, F. (2013). Purification and identification of antihypertensive peptides in Spanish dry-cured ham. *Journal of Proteomics, 78,* 499-507.

Essid, I., & Hassouna, M. (2013). Effect of inoculation of selected *Staphylococcus xylosus* and *Lactobacillus plantarum* strains on biochemical, microbiological and textural characteristics of a Tunisian dry fermented sausage. *Food Control, 32,* 707-714.

Flores, M. & Toldrá, F. (2011). Microbial enzymatic activities for improved fermented meats. *Trends in Food Science and Technology, 22,* 81-90.

García, M. C., Puchalska, P., Esteve, C., & Marina, M. L. (2013). Vegetable foods: A cheap source of proteins and peptides with antihypertensive, antioxidant, and other less occurrence bioactivities. *Talanta, 106,* 328-349.

Geng, F., He, Y., Yang, L., & Wang, Z. (2010). A rapid assay for angiotensin converting enzyme activity using ultra-performance liquid chromatography-mass spectrometry. *Biomedical Chromatography, 24,* 312–317.

Laranjo, M., Agulheiro-Santos, A. C., Potes, M. E., Cabrita, M. J., García, R., Fraqueza, M. J., & Elias, M. (2015). Effects of genotype, salt content and calibre on quality of traditional dry-fermented sausages. *Food Control*, *56*, 119-127.

López, C. M., Brue, E., Vignola, G. M., Fadda, S. G. (2015). Identification of small peptides arising from hydrolysis of meat proteins in dry fermented sausages. *Meat Science*, *104*, 20–29.

Mejri, L. & Hassouna, M. (2016). Characterization and selection of Lactobacillus plantarum species isolated from dry fermented sausage reformulated with camel meat and hump fat. *Applied Biological Chemistry*, *59*, 533-42.

Moslehishad, M., Ehsani, M. R., Salami, M., Mirdamadi, S. R., Ezzatpanah, H., Naslaji, A. N., & Moosavi- Movahedi, A. A. (2013). The comparative assessment of ACE-inhibitory and antioxidant activities of peptide fractions obtained from fermented camel and bovine milk by *Lactobacillus rhamnosus* PTCC 1637. *International Dairy Journal*, *29*, 82-87.

Mora, L., Escudero, E., Aristoy, M.C., & Toldrá, F. (2015). A peptidomic approach to study the contribution of added casein proteins to the peptide profile in Spanish dry-fermented sausages. *International Journal of Food Microbiology*, *212*, 41-48.

Puchalska, P., Marina, M. L., & García, M. C. (2015). Isolation and characterization of peptides with antihypertensive activity in foodstuffs, *Critical Reviews in Food Science and Nutrition*,*55*, 521-551.

Sarmadi, B. H., & Ismail, A. (2010). Antioxidative peptides from food proteins: A review. *Peptides*, *31*, 1949-1956.

Stajic, S., Perunovic, M., Stanisic, N., Zujovic, M., & Zivkovic, D. (2013). Sucuk (Turkish-style dry-fermented sausage) quality as an influence of recipe formulation and inoculation of starter cultures. *Journal of Food Processing and Preservation*, *37*, 870-880.

Sun, W., Zhao, H., Zhao, Q., Zhao, M., Yang, B., Wu, N., Y. Quian (2009). Structural characteristics of peptides extracted from Cantonese sausage during drying and their antioxidant activities. *Innovative Food Science & Emerging Technologies*, *10*, 558-563.

Tabanelli, G., Coloretti, F., Chiavari, C., Grazia, L., Lanciotti, R. & Gardini, F. (2012). Effects of starter cultures and fermentation climate on the properties of two types of typical Italian dry fermented sausages produced under industrial conditions. *Food Control*, *2*6, 416-426.

Talon, R., Walter, D., Chartier, S., Barière, C., & Montel, M. C. (1999). Effect of nitrate and incubation conditions on the production of catalase and nitrate reductase by staphylococci. *International Journal of Food Microbiology*, *52*, 47-56.

Vaštag, Z., Popović, L., Popović, S., Petrović, L., & Peričin, D. (2010). Antioxidant and angiotensin-I converting enzyme inhibitory activity in the water soluble protein extract from Petrovac sausage. *Food Control*, *21*, 1298-1302.

Wang, D., Wang, L., Zhu, F., Zhu, J., Chen, X. D., Zou, L., Saito, M., & Li, L. (2008). In vitro and in vivo studies on the antioxidant activities of the aqueous extracts of Douchi (a traditional Chinese salt-fermented soybean food). *Food Chemistry*, *107*, 1421-1428.

Wiriyaphan, C., Chitsomboon, B., & Yongsawadigul, J. (2012). Antioxidant activity of protein hydrolysates derived from threadfin bream surimi byproducts. *Food Chemistry*, *132*, 104-111.

You, L., Zhao, M., Regenstein, J. M., & Ren, J. (2011). In vitro antioxidant activity and in vivo anti-fatigue effect of loach (*Misgurnus anguillicaudatus*) peptides prepared by papain digestion. *Food Chemistry*, *124*, 188-194.

Zhao, L., Jin, Y., Ma, C., Song, H., Li, H., Wang, Z., & Xiao, S. (2011). Physico-chemical characteristics and free fatty acid composition of dry fermented mutton sausages as affected by the use of various combinations of starter cultures and spices. *Meat Science*, *88*, 761-766.

**Figure captions**

**Figure 1.** Evolution of lactic acid bacteria LAB (A) and *Staphylococci* (B) in control (batch D) (□) and starter-inoculated sausages (batch A) (■), (batch B) (■), and (batch C) (■).

**Figure 2.** Variation of the antioxidant capacity evaluated by the capacity to inhibit the formation of hydroxyl radicals and the capacity to scavenge ABTS radicals of whole extracts (♦) and fractions (> 5 kDa (■), 3-5 kDa (▲), and < 3 kDa (×)) obtained for the control and batch A sausages.

**Figure 3.** ACE inhibition capacity (%) of whole extracts (♦) and fractions (> 5 kDa (■), 3-5 kDa (▲), and < 3 kDa (×)) obtained from non inoculated (control (D)) and inoculated camel meat sausages (batch A (A), batch B (B), and batch C (C)).

**Figure 4.** Total ion chromatogram (TIC) obtained by RP-HPLC-ESI-Q-TOF-MS/MS for the fraction with peptides below 3 kDa from the control sausage and spectrum corresponding to the EDEEVEH peptide.

**Table 1**. Peptide concentration (mg/mL) in fractions obtained from starter inoculated (batch A, B, and C) and non inoculated (control) camel meat sausages during 28 days ripening.

| | | Peptide concentration (mg/mL) | | | |
|---|---|---|---|---|---|
| Ripening time (days) | Sample | Control | Batch A | Batch B | Batch C |
| **0** | >5 kDa | 5.32 ±0.09 | 4.02± 0.03 | 5.57 ±0.01 | 5.34 ±0.14 |
| | 3-5 kDa | 5.24 ±0.01 | 4.81±0.17 | 4.39±0.14 | 5.06 ±0.04 |
| | <3kDa | 3.33± 0.01 | 2.99±0.15 | 3.46 ±0.15 | 3.63±0.04 |
| **7** | >5 kDa | 4.58 ± 0.17 | 3.65±0.21 | 4.33±0.27 | 4.48±0.23 |
| | 3-5 kDa | 5.13 ± 0.02 | 4.06±0.02 | 3.50 ±0.35 | 5.15 ±0.03 |
| | <3 kDa | 3.63 ±0.06 | 3.07 ±0.07 | 3.95±0.02 | 3.91±0.04 |
| **14** | >5 kDa | 3.88± 0.25 | 3.27±0.02 | 3.53 ±0.10 | 2.46±0.06 |
| | 3-5 kDa | 4.00 ± 0.06 | 3.84 ±0.01 | 2.43 ±0.03 | 2.95±0.01 |
| | <3 kDa | 3.74± 0.04 | 3.53±0.07 | 4.35±0.16 | 4.47±0.01 |
| **21** | >5 kDa | 2.56 ±0.02 | 3.05 ±0.03 | 2.27±0.30 | 2.05 ±0.09 |
| | 3-5 kDa | 2.09 ±0.16 | 3.46 ±0.04 | 2.22±0.07 | 2.63±0.24 |
| | <3 kDa | 4.21 ± 0.16 | 4.37±0.33 | 4.94 ±0.03 | 4.59 ±0.06 |
| **28** | >5 kDa | 2.56 ±0.06 | 2.82 ±0.20 | 2.04±0.14 | 2.62 ±0.05 |
| | 3-5 kDa | 1.85 ±0.03 | 3.32±0.01 | 3.00 ±0.08 | 2.21±0.16 |
| | <3 kDa | 4.73 ±0.03 | 5.23±0.19 | 5.26 ±0.23 | 4.99 ±0.09 |

**Table 2.** Antioxidant capacity determined using different assays in fractions containing peptides below 3 kDa obtained from the control sausage and from the starter-inoculated sausages at different ripening times.

| | Antioxidant capacity (%) | | | |
|---|---|---|---|---|
| Ripening time (days) | ABTS assay | DPPH assay | FRAP assay | HYDROXYL assay |
| **0** | | | | |
| Control | 95.08 ± 0.67 | 27.33 ± 1.45 | 15.26 ± 1.35 | 12.60 ± 0.62 |
| Batch A | 99.82 ± 0.15 | 34.87 ± 0.69 | 18.19 ± 0.21 | 14.25 ± 0.65 |
| Batch B | 101.2 ± 1.1 | 29.57 ± 1.01 | 15.87 ± 0.37 | 13.20 ± 1.93 |
| Batch C | 66.29 ± 0.10 | 29.03 ± 0.89 | 13.38 ± 0.45 | 10.61 ± 0.68 |
| **7** | | | | |
| Control | 96.24 ± 0.03 | 41.73 ± 0.92 | 30.50 ± 0.39 | 26.91 ± 1.59 |
| Batch A | 104.30 ± 0.33 | 47.77 ± 1.83 | 32.28 ± 0.16 | 27.33 ± 0.67 |
| Batch B | 98.17 ± 0.38 | 40.37 ± 0.55 | 29.37 ± 0.86 | 26.42 ± 0.23 |
| Batch C | 74.08 ± 0.74 | 43.01 ± 0.92 | 27.15 ± 1.10 | 24.66 ± 0.54 |
| **14** | | | | |
| Control | 99.58 ± 0.33 | 68.54 ± 1.14 | 42.63 ± 0.68 | 37.79 ± 3.16 |
| Batch A | 103.27 ± 0.46 | 72.48 ± 0.70 | 54.56 ± 2.01 | 49.27 ± 0.34 |
| Batch B | 100.2 ± 1.2 | 69.80 ± 1.30 | 45.59 ± 0.30 | 35.30 ± 2.10 |
| Batch C | 98.93 ± 0.95 | 71.39 ± 0.71 | 50.10 ± 0.27 | 34.13 ± 0.04 |
| **21** | | | | |
| Control | 100.4 ± 0.05 | 70.86 ± 1.16 | 57.92 ± 1.14 | 44.35 ± 0.53 |
| Batch A | 101.6 ± 0.18 | 75.48 ± 0.31 | 76.70 ± 0.23 | 54.12 ± 1.25 |
| Batch B | 97.31 ± 0.16 | 71.69 ± 0.97 | 65.90 ± 0.59 | 49.75 ± 0.89 |
| Batch C | 100.10 ± 0.82 | 71.25 ± 1.19 | 66.42 ± 0.31 | 45.39 ± 0.49 |
| **28** | | | | |
| Control | 101.7 ± 1.3 | 76.56 ± 1.75 | 61.04 ± 0.71 | 59.43 ± 0.11 |
| Batch A | 100.11 ± 0.63 | 82.73 ± 0.56 | 84.56 ± 0.33 | 75.21 ± 0.98 |
| Batch B | 95.7 ± 2.2 | 82.09 ± 0.51 | 81.73 ± 1.05 | 61.66 ± 1.27 |
| Batch C | 106.3 ± 2.4 | 80.35 ± 1.31 | 74.74 ± 0.10 | 60.57 ± 0.26 |

**Table 3**. Identification of peptides in fractions below 3 kDa obtained from the control and the starter-inoculated sausages at the highest ripening time using Database search.

| Sample | Peptide | -10lgP | Mass (Da) | ppm | Accession Number |
|---|---|---|---|---|---|
| **Control** | FAGDDAPR | 50.28 | 847.3824 | 4.3 | tr\|Q0PEU1\|Q0PEU1_BUBBU |
| | EVHEPEEKPRPR | 43.26 | 1501.7637 | 2.6 | tr\|Q8MKH9\|Q8MKH9_BOVIN |
| | EVHEPEEKPRP | 42.13 | 1345.6626 | 1.3 | tr\|Q75NH1\|Q75NH1_PIG |
| | LPAPEPKPKPEPE | 34.08 | 1427.766 | 4.7 | tr\|W5Q754\|W5Q754_SHEEP |
| | IPEGEKVDFDDIQKK | 33.78 | 1759.8992 | 1.3 | tr\|Q75NH1\|Q75NH1_PIG |
| | VAPEEHPT | 29.75 | 878.4134 | 2.4 | tr\|Q0PEU1\|Q0PEU1_BUBBU |
| | APPPPAEVHE | 27.68 | 1042.5083 | 0.1 | tr\|Q75NH1\|Q75NH1_PIG |
| | KPPDIPD | 26.67 | 780.4017 | 6.5 | tr\|W5Q754\|W5Q754_SHEEP |
| | APKIPEGEKVDFDDIQK | 26.66 | 1927.989 | 6.7 | tr\|Q8MKH9\|Q8MKH9_BOVIN |
| | AGDDAPR | 26.62 | 700.314 | 0.9 | tr\|Q0PEU1\|Q0PEU1_BUBBU |
| | APPPPAE | 22.99 | 677.3384 | 1 | tr\|Q75NH1\|Q75NH1_PIG |
| | APKIPEGEK | 21.27 | 967.5338 | 1.2 | tr\|Q75NH1\|Q75NH1_PIG |
| | GDDAPR | 20.67 | 629.2769 | 1.3 | tr\|Q0PEU1\|Q0PEU1_BUBBU |
| | DVGDWRKN | 20.19 | 988.4726 | 3.7 | tr\|Q0Q4Z4\|Q0Q4Z4_VICPA |
| | TPKLPP | 20.05 | 651.3955 | 8 | tr\|S9WFG8\|S9WFG8_CAMFR |
| **Batch A (*S. xylosus* + *L. plantarum*)** | VHIITHGEEKD | 52.06 | 1276.6411 | 2 | tr\|Q29RP6\|Q29RP6_BOVIN |
| | FAGDDAPR | 47.1 | 847.3824 | 1.9 | tr\|Q27HS3\|Q27HS3_PIG |
| | EVHEPEEKPRPR | 44.31 | 1501.7637 | 2 | tr\|Q8MKH9\|Q8MKH9_BOVIN |
| | EVHEVHEPEEKPRP | 44.31 | 1501.7637 | 2 | tr\|Q8MKH9\|Q8MKH9_BOVIN |
| | EVHEPEEKPRP | 38.52 | 1710.8325 | 2.2 | tr\|Q8MKH9\|Q8MKH9_BOVIN |
| | DEEEVEH | 34.8 | 885.3352 | 4.4 | tr\|F1N5N3\|F1N5N3_BOVIN |
| | IAFSQY | 29.8 | 727.3541 | 5.7 | tr\|B3VHM9\|B3VHM9_CAPHI |
| | DEGEAGAGGQ | 24.71 | 889.3413 | 3.4 | tr\|W5Q7Z0\|W5Q7Z0_SHEEP |
| | EDGGFAP | 22.79 | 691.2812 | 2 | tr\|D1KKB3\|D1KKB3_PIG |
| | SNGNPGPPGPPGPS | 21.95 | 1230.5629 | 9.2 | tr\|I3LSV6\|I3LSV6_PIG |
| | EEGDQGGLGEVGAQ | 21.64 | 1344.5793 | 7.5 | tr\|W5PPQ0\|W5PPQ0_SHEEP |
| | AIPDEV | 20.77 | 642.3224 | 4.1 | tr\|L8IVP3\|L8IVP3_9CETA |
| **Batch B (*S. xylosus* + *L. pentosus*)** | VHIITHGEEKD | 56.09 | 1276.6411 | 4.5 | tr\|A1XQV9\|A1XQV9_PIG |
| | DEEEVEH | 37.2 | 885.3352 | 4.6 | tr\|F1N5N3\|F1N5N3_BOVIN |
| | VAPEEHPT | 36.66 | 878.4134 | 3.3 | tr\|Q0PEU1\|Q0PEU1_BUBBU |
| | VITHGDAKDQE | 35.42 | 1211.5782 | 1.6 | tr\|Q866A7\|Q866A7_PIG |
| | IAFSQY | 30.35 | 727.3541 | 3.9 | tr\|B3VHM9\|B3VHM9_CAPHI |
| | DNGSGLVKAGFAGDDAPRAVFPS | 28.8 | 2247.0918 | 3.3 | tr\|S9X353\|S9X353_CAMFR |
| | FAGDDAPRAVFPS | 28.66 | 1348.6411 | 5.3 | tr\|Q0PEU1\|Q0PEU1_BUBBU |
| | PDAELAAF | 28.53 | 832.3966 | 4.3 | tr\|S9XPF8\|S9XPF8_CAMFR |
| | VITHGDA | 25.1 | 711.3552 | 1.7 | tr\|Q866A7\|Q866A7_PIG |
| | AGDDAPR | 23.45 | 700.314 | 0.2 | tr\|S9X353\|S9X353_CAMFR |
| | YVITHGDAKDQE | 20.42 | 1374.6415 | 2.9 | tr\|Q866A7\|Q866A7_PIG |
| **Batch C (*commercial strains of S. carnosus + L. sakei*)** | DEEEVEH | 57.41 | 885.3352 | 0.7 | tr\|F1N5N3\|F1N5N3_BOVIN |
| | EVHEVHEPEEKPRP | 47.19 | 1710.8325 | 1.2 | tr\|Q75NH1\|Q75NH1_PIG |
| | RSGEGQDDAGELD | 43.67 | 1347.5538 | 5 | tr\|W5Q0I1\|W5Q0I1_SHEEP |
| | EVHEPEEKPRP | 41.73 | 1345.6626 | 1.5 | tr\|Q8MKH9\|Q8MKH9_BOVIN |
| | FAGDDAPRAVFPS | 35.9 | 1348.6411 | 2.9 | tr\|Q27HS3\|Q27HS3_PIG |
| | TLM(+15.99)DD | 21.99 | 609.2316 | 6.2 | tr\|S9XGP7\|S9XGP7_CAMFR |
| | TIM(+15.99)DD | 21.99 | 609.2316 | 6.2 | tr\|A0A0N9DSG4\|A0A0N9DSG4_BALOM |
| | DVGDWRKN | 20.95 | 988.4726 | 0.8 | tr\|Q0Q4Z4\|Q0Q4Z4_VICPA |
| | APAKAK | 20.6 | 584.3646 | 2 | tr\|W5P900\|W5P900_SHEEP |

**Table 4**. Identification of peptides in fractions below 3 kDa obtained from the control and the starter-inoculated sausages at the highest ripening time by *de novo*.

| Sample | Peptide | ALC (%) | Mass (Da) | ppm |
|---|---|---|---|---|
| **Control** | EDEEVEH | 98 | 885.3351 | 0.2 |
| | PVVP | 91 | 410.2529 | 4.9 |
| | TEAGM(+15.99)AP | 87 | 691.2847 | 8.5 |
| | VDFDD | 85 | 609.2282 | 1.5 |
| | DAM(+15.99)APAT | 83 | 691.2847 | 4.7 |
| | EPVPP | 80 | 537.2798 | 6.4 |
| **Batch A (*S. xylosus* + *L. plantarum*)** | LLVE[a] | 96 | 472.2897 | 3.6 |
| | VDFDD | 95 | 609.2282 | 1.1 |
| | TVFL[a] | 90 | 478.2791 | 0.8 |
| | VPVP | 90 | 410.2529 | 3.5 |
| | DAM(+15.99)APAT | 83 | 691.2847 | 4.6 |
| | LPHEPEEQRPPR[a] | 80 | 1483.7532 | 1.6 |
| | EDEEVEH | 79 | 885.3351 | 5.8 |
| **Batch B (*S. xylosus* + *L. pentosus*** | EDEEVEH | 98 | 885.3351 | 0.8 |
| | APPPP | 96 | 477.2587 | 2.3 |
| | LTM(+15.99)F[a] | 95 | 526.2461 | 4.7 |
| | VPVP | 91 | 410.2529 | 3.5 |
| | ALFSQY[a] | 90 | 727.3541 | 0.5 |
| | TVFL[a] | 90 | 478.2791 | 1.5 |
| | GDDAPR | 86 | 629.2769 | 3.1 |
| | PRPAGP | 85 | 593.3285 | 2.2 |
| | DVFDD | 84 | 609.2282 | 0.8 |
| | RPVP | 81 | 467.2856 | 2.1 |
| | EDHTKAHVV | 80 | 1034.5144 | 1.3 |
| **Batch C (*commercial strains of S. carnosus* + *L. sakei*)** | EDEEVEH | 98 | 885.3351 | 3.3 |
| | DAM(+15.99)APAT | 85 | 691.2847 | 8.7 |
| | DADGAFP | 82 | 691.2813 | 0.4 |
| | DADGAPF | 82 | 691.2813 | 3.8 |

[a] Since it is not possible to differentiate I from L by MS due to their equal molecular masses, only isoforms with L were presented in our results, although peptide sequences containing I amino acid instead of L are also possible.

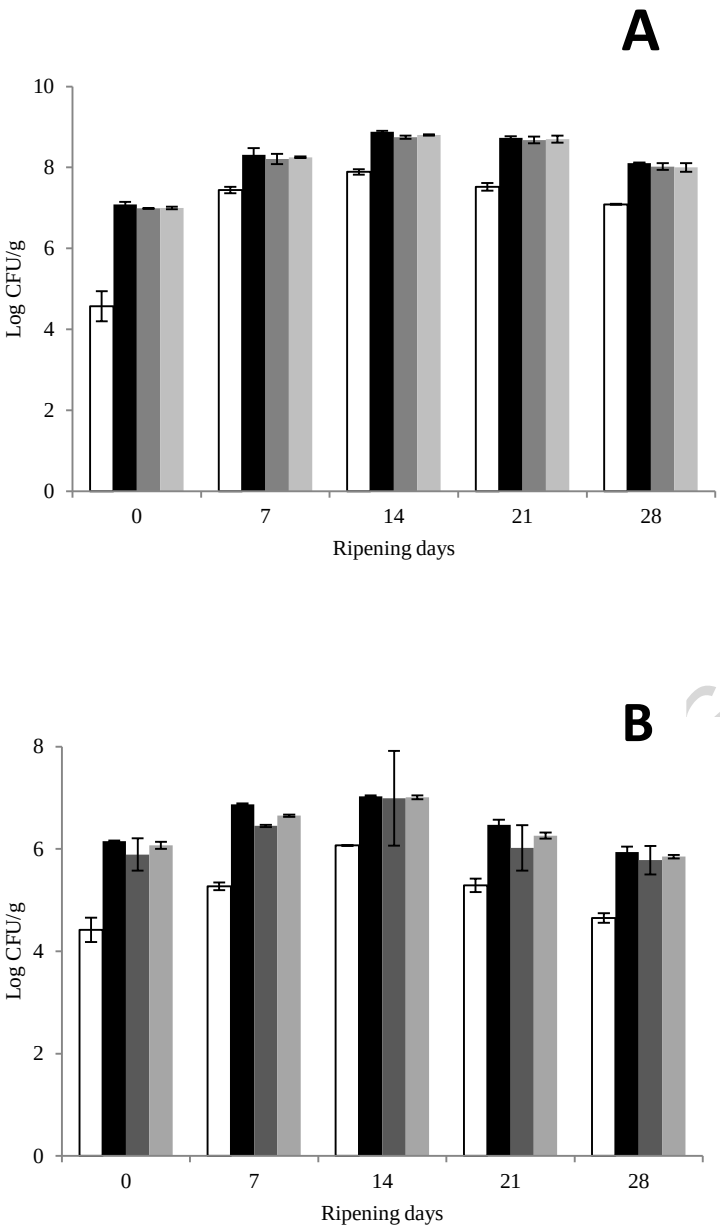



**Figure 1.**

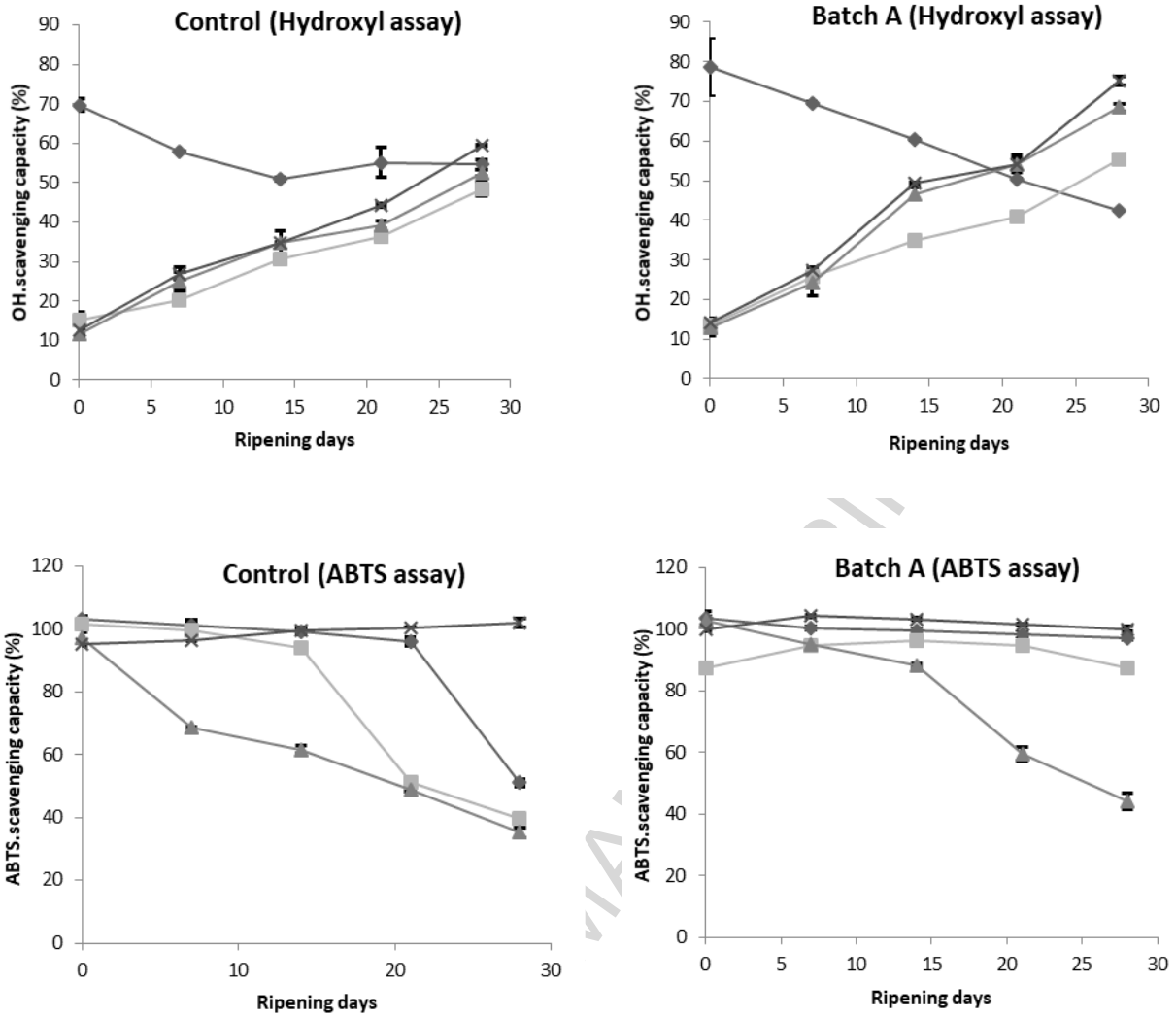



**Figure 2.**

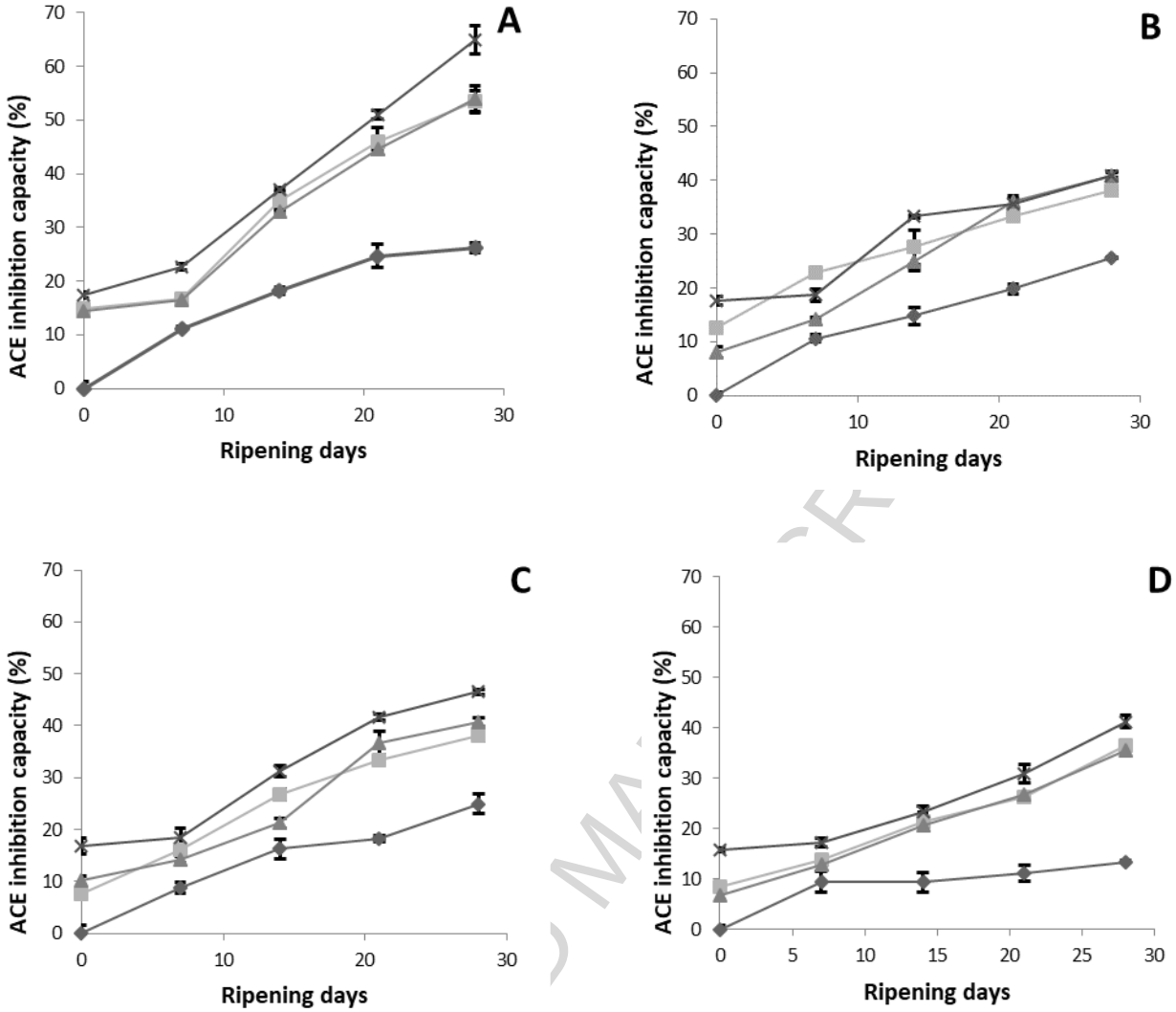



**Figure 3.**

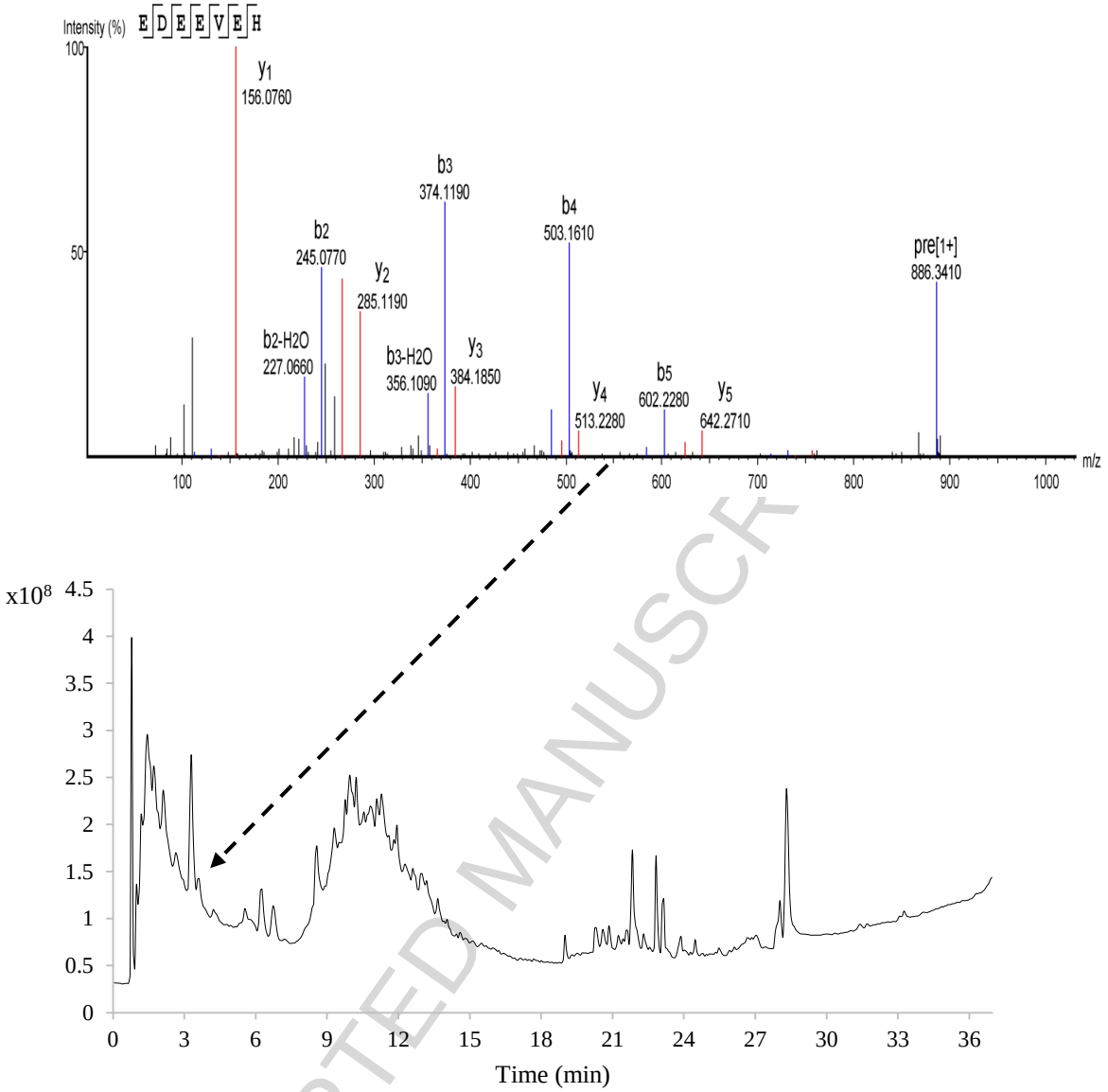
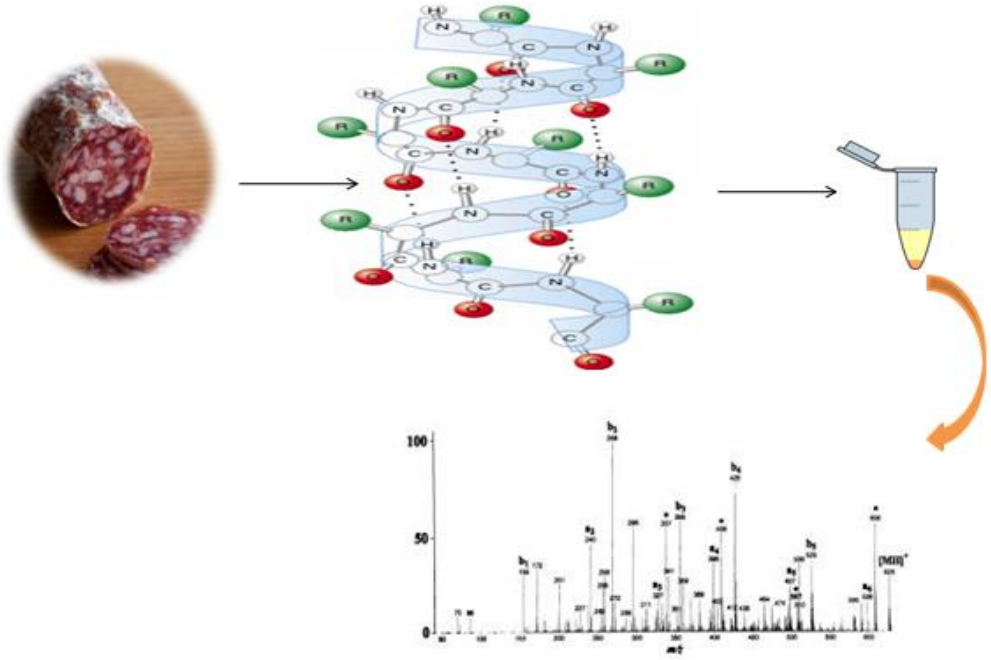



**Figure 4**

**Graphical Abstract**



**Highlights**

- First study of the production of bioactive peptides in dry fermented camel sausages.
- Ripening time and starter culture affected peptide concentration, size, and activity.
- Peptides < 3 kDa showed the highest antioxidant and antihypertensive capabilities.
- Highest bioactivities were observed at the end of ripening.
- Peptides in fraction < 3 kDa were identified by RP-HPLC-Q-TOF-MS.