EFFECT OF STARTER CULTURES ON FREE AMINO ACID CONTENT OF DRY-CURED FOAL SAUSAGE

P.E. Munekata[1], R. Domínguez[2], R. Agregán[2], P. Borrajo[2], D. Franco[2] and J.M. Lorenzo[2*]

[1]Department of Food Engineering, Faculty of Animal Science and Food Engineering, University of São Paulo, 225 Duque de Caxias Norte Ave , Jardim Elite, postal code 13.635-900, Pirassununga, São Paulo, Brazil. CNPq scholarship holder

[2]Centro Tecnológico de la Carne de Galicia, Rúa Galicia Nº 4, Parque Tecnológico de Galicia, San Cibrao das Viñas, 32900 Ourense, Spain

*jmlorenzo@ceteca.net

Abstract – The present study deals with the effect of commercial starter cultures CX (*Staphylococcus carnosus* + *Staphylococcus xylosus* + *Pediococcus pentosaceus*), FL (*Debaryomyces hansenii* + *Staphylococcus xylosus*), and TH (*Pediococcus pentosaceus* + *Staphylococcus xylosus*) on free amino acid (FAA) on the content of dry-cured foal sausage. Samples were evaluated after 53 days of ripening. The presence of starter cultures significantly (P<0.001) increased the total FAA content (1248.25, 1207.22 and 1266.57 mg/100 g of dry matter for CX, FL and TH treatments, respectively) compared to control (CO) (951.93 mg/100 g of dry matter) samples. The main FAA in dry-cured foal sausage was leucine followed by cysteine, phenylalanine and valine, while the aspartic acid was the less abundant. The three different starter cultures showed different FAA profile. Finally, TH batch showed the highest amount of glutamic acid, glycine and alanine, while CX batch presented the highest content of tyrosine, isoleucine and phenylalanine.

Key Words – Proteolysis, horsemeat, starter culture, dry-cured sausage

I. INTRODUCTION

Consumption of horsemeat holds a minority percentage of meat consumption compared to chicken, beef and pork. The low fat and cholesterol content, high level of unsaturated fatty acids, vitamin B, essential amino acids, Fe-heme, characterize the horsemeat as an important source of nutrients and healthy alternative to beef and pork meat. The development of products from horsemeat presents nutritional benefits that are in accordance with consumer expectations for healthier food, especially about the risk of cardiac diseases and obesity [1-4].

The traditional production of fermented sausages by endogenous microorganisms does not ensure the safety or quality of final product. In this scenario, the use of starter cultures as means to increase the productivity and technological properties allows a better control of fermentation and ripening process, restraining other undesirable microorganisms. Production of exogenous proteases by starter cultures can enhance the hydrolysis of proteins and accelerate the ripening and flavour development, leading to a better control on the final product [5-7].

The release of FAA is related to aminopeptidase activity, being considered the main agent during drying and ripening. Presence of FAA impacts on basic taste of dry fermented sausages and also contributes to typical aroma. Such effect is due the formation of volatile compounds from FAA degradation. Previous studies relate sensory attributes, such as fresh taste, sweet, bitter, sour and salty to specific amino acids [7,8]. Thus, the aim of the present study was to evaluate the effect of starter cultures on FAA content of dry-cured foal sausage.

II. MATERIALS AND METHODS

II. 1 Sausage production

Four different batches of foal sausage were manufactured according to traditional techniques, one of them without starter culture and the other three batches with addition of different commercial starter cultures in a proportion defined by the manufacturer in each case. The batches were named as follows: (i) CO batch, control without starter culture, (ii) CX: with CXP (Cargill) (*Staphylococcus carnosus* + *Staphylococcus*

xylosus + *Pediococcus pentosaceus*), (iii) FL: with Flavor Start P406 (Cargill) (*Debaryomyces hansenii* + *Staphylococcus xylosus*), (iv) TH: with Lyocarni THM-17 (Sacco) (*Pediococcus pentosaceus* + *Staphylococcus xylosus*). The four batches mentioned before were manufactured with the same ingredients, formulation and technology in May and June 2014.

Foal sausage formulation includes lean foal meat (85%), pork back fat (15%), NaCl (25 g/kg), lactose (20 g/kg), dextrin (20 g/kg), sodium caseinate (20 g/kg), glucose (7 g/kg), black pepper (1.5 g/kg), white pepper (1 g/kg), sodium ascorbate (0.5 g/kg), sodium nitrite (0.15 g/kg) and potassium nitrate (0.15 g/kg). All the foals used in this study were reared in an extensive production system in freedom regimen. The lean foal meat and the pork back fat were ground through a 10 mm diameter mincing plate and vacuum mixed together with the other ingredients for 3 min. The mix was maintained at 4 °C for 24 h and then stuffed into natural casings with a diameter of 60 mm and a length of 40 cm. The sausages were fermented for 2 days at 20 °C and 80% of relative humidity and then transferred into a drying-ripening chamber where they were kept for 51 more days at 11 °C and 75% relative humidity. Samples after 53 days of processing were taken for subsequent analysis.

II. 2 Free amino acid analysis

Amino acids were extracted following the procedure described by Pérez-Palacios *et al.* [9] with some modifications. Samples were prepared by homogenizing 5 g of sample with 25 mL of hydrochloric acid 0.1 N, in an Ika Ultra-Turrax for 8 min while cooled by submerging the extract in ice. The homogenized samples were centrifuged for 20 min at 5240 g and the supernatant material was filtered through glass wool prior to further analyses. 200 µL of this extract was deproteinized by adding 800 µL of acetonitrile and centrifuged for 3 min at 5240 g. The derivatization of standards and samples and chromatographic analysis conditions were as follows: 10 µL of sample was buffered to pH 8.8 (AccQ-Fluor borate buffer) to yield a total volume of 100 µL. Derivatization was initiated by the addition of 20 µL of AccQ-Fluor reagent (3 mg/mL in acetonitrile). Reaction of the AccQ-Fluor reagent kit with all primary and secondary amines was rapid, and excess reagent was hydrolyzed within 1 min. Completion of hydrolysis of any tyrosine phenol modification was accelerated by heating for 10 min at 55°C. The HPLC systems used were a high-performance liquid chromatograph Waters 2695 Separations Module and a Waters 2475 fluorescence detector (Waters, Milford, MA). Empower 2 advanced software (Waters) was used to control the system operation and management of results. Separations were carried out using a Waters AccQ-Tag column (3.9 × 150 mm, with a particle size of 4 µm) with a flow rate of 1.0 mL/min and were performed at 37°C. Detection was accomplished by fluorescence with excitation at 250 nm and emission at 395 nm. Amino acids were identified by retention times and quantified by external standard technique using an amino acid standard (Amino Acid Standard H, Thermo, Rockford, USA). The free amino acids results were expressed in mg/100 g of dry matter.

II. 3 Statistical analysis

The effect of different commercial starter cultures on FFA content was examined using a mixed-model ANOVA, where the FAA content was set as dependent variables, commercial starter cultures as fixed effect, and replicate as random effect. The pairwise differences between least-square means were evaluated by Duncan's method. Differences were considered significant if $P<0.05$. The values were given in terms of mean values and standard error (SEM). All analyses were conducted using the IBM SPSS Statistics 19.0 program (IBM Corporation, Somers, NY, USA) software package.

III. RESULTS AND DISCUSSION

The effect of commercial starter cultures on free amino acid content (expressed as mg/100 g of dry matter) of dry-cured foal sausage is shown in Table 1. In the present study, the content of 18 FAA at the end of ripening process was used as a measure of proteolytic activity. Statistical analysis showed that total FAA content was significantly ($P<0.001$) affected by starter culture incorporation at the end of dry-ripening process (1248.25, 1207.22 and 1266.57 mg/100 g of dry matter for CX, FL and TH treatments, respectively) compared to CO samples (951.93 mg/100 g of dry

matter). However, the three starter cultures did not show significantly (P>0.05) differences on the total FAA content. A similar total FAA content was previously reported by Lorenzo & Franco [10] in dry-cured foal sausage. These results were in agreement with those reported by Aro *et al.* [7] who observed a significantly increase on the total FAA content by incorporation of *Staphylococcus xylosus* as starter culture in pork fermented sausages.

Table 1 Effect of commercial starter cultures on free amino acids (expressed as mg/100 g of dry matter) of dry-cured foal sausage (mean ± standard errors of twenty replicates)

	Batch					
FAA	CO	CX	FL	TH	SEM	Sig
Asp	5.85^{ab}	5.76^{ab}	5.50^{a}	6.32^{b}	0.11	ns
Ser	12.95^{a}	17.23^{b}	19.33^{c}	18.37^{bc}	0.40	***
Glu	31.73^{a}	40.14^{b}	44.97^{b}	57.78^{c}	1.53	***
Gly	15.76^{a}	19.68^{b}	20.66^{bc}	22.32^{c}	0.44	***
His	15.83^{a}	17.09^{a}	19.08^{b}	19.90^{b}	0.35	***
Tau	66.93^{a}	89.39^{b}	86.75^{b}	73.40^{a}	1.71	***
Arg	31.35^{a}	31.01^{a}	29.30^{a}	45.83^{b}	1.18	***
Thr	22.07^{a}	28.81^{bc}	30.89^{c}	27.88^{b}	0.62	***
Ala	58.04^{a}	88.68^{bc}	85.46^{b}	95.44^{c}	2.22	***
Pro	53.15^{a}	71.71^{b}	67.05^{b}	72.15^{b}	1.50	***
Cys	121.09^{a}	108.83^{a}	116.52^{a}	153.95^{b}	3.32	***
Tyr	59.11^{b}	74.69^{c}	71.36^{c}	50.71^{a}	1.76	***
Val	73.67^{a}	107.66^{b}	108.21^{b}	108.51^{b}	2.54	***
Met	39.32^{a}	51.75^{b}	52.36^{b}	52.76^{b}	0.91	***
Lys	24.32^{b}	26.01^{bc}	21.36^{a}	28.17^{c}	0.58	***
Ile	67.18^{a}	100.29^{c}	90.31^{b}	92.40^{bc}	2.01	***
Leu	157.16^{a}	245.46^{b}	228.46^{b}	227.66^{b}	5.26	***
Phe	90.34^{a}	125.29^{c}	112.00^{b}	112.68^{b}	2.32	***
Total FAA	951.93^{a}	1248.2^{b}	1207.2^{b}	1266.5^{b}	21.50	***

CO: control without starter culture; CX: (*S. carnosus* + *S. xylosus* + *P. pentosaceus*); FL: (*D. hansenii* + *S. xylosus*) and TH: (*P. pentosaceus* + *S. xylosus*)
[a-c] Mean values in the same row (corresponding to the same parameter) not followed by a common letter differ significantly (P<0.05)
Sig: significance: *** (P<0.001), n.s. (not significant)

The main FAA in dry-cured foal sausage was leucine (157.16, 245.46, 228.46 and 227.66 mg/100 g of dry matter for CO, CX, FL and TH batches, respectively), followed, in decreasing order, by cysteine, phenylalanine and valine (which were generally up to 70 mg/100 g of dry matter), while the aspartic acid was the less abundant with mean values of 5.85 mg/100 g of dry matter. This finding is in disagreement with those found by Lorenzo & Franco [10] who observed that the arginine was the most abundant FAA at the end of dry-cured foal sausage and by Lorenzo *et al.* [11] who found that the lysine was the main FAA at the end of dry-cured foal "cecina".

The three different starter cultures showed different FAA profile (Table 1). In our study, TH batch showed the highest amount of glutamic acid, glycine and alanine, while CX batch presented the highest content of tyrosine, isoleucine and phenylalanine. The proteolysis of meat proteins by endogenous enzymes and aminopeptidases activity are considered the main process on FAA release [10,11,12], however microbial enzymes from starter cultures can exert a significant effect on FAA release [5,7]. The sensory properties of FAA have been well established: glutamic acid and aspartic acid showing pleasantly fresh taste, glycine, alanine and serine being sweet, arginine, leucine, isoleucine, valine, phenylalanine, methionine and histidine being bitter, lysine and proline contributing sweet and bitter tastes and others showing sour or salty taste [13]. Because the concentrations of all FAA detected in the final products were much higher than their sensory thresholds according to Zhu and Hu [13], they might have an important contribution to foal dry-cured sausage.

IV. CONCLUSION

According to the results obtained in this study, the use of the selected commercial starter cultures for the manufacturing of dry-cured foal sausages exhibited a significantly different effect on FAA content. The presence of starter cultures increased the total FAA content compared to control. In our study, TH batch showed the highest amount of glutamic acid, glycine and alanine, while CX batch presented the highest content of tyrosine, isoleucine and phenylalanine. These differences might affect organoleptic characteristics.

ACKNOWLEDGEMENTS

Authors are grateful to Xunta de Galicia (The Regional Government) (Project FEADER 2013/42) for the financial support and the National Council for Scientific and Technological Development (CNPq). Special thanks to Monte Calabar (A Estrada, Pontevedra) for foal samples supplied for this research.

REFERENCES

1. Lorenzo, J. M., Patieiro, M., & Franco, D. (2013). Influence of muscle type on physicochemical and sensory properties of foal meat. Meat Science 94: 77–83.
2. Lorenzo, J. M., & Patiero, M. (2013). Influence of type of muscles on nutritional value of foal meat. Meat Science 93: 630–638.
3. Lorenzo, J.M., Sarriés, M.V., Tateo, A., Polidori, P., Franco, D. & Lanza, M. (2014). Carcass characteristics, meat quality and nutritional value of horsemeat: A review. Meat Science 96: 1478-1488.
4. Verbeke, W., Pérez-Cueto, F. J. A., Barcellos, M. D., Krystallis, A., & Grunert, K. G. (2010). European citizen and consumer attitudes and preferences regarding beef and pork. Meat Science 84: 284–292.
5. Scannell, A. G. M., Kenneally, P. M., & Arendt, E. K. (2004). Contribution of starter cultures to the proteolytic process of a fermented non-dried whole muscle ham product. International Journal of Food Microbiology 93: 219–230.
6. Lorenzo, J. M., Gómez, M., & Fonseca, S. (2014). Effect of commercial starter cultures on physicochemical characteristics, microbial counts and free fatty acid composition of dry-cured foal sausage. Food Control 46: 382–389.
7. Aro, J. M. A., 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.
8. Zhu, S. W., & Hu, J. X. (1993). Studies on Jinhua ham tastes and taste substances. Food Science 159: 8–11.
9. Pérez-Palacios, T., Ruiz, J., Barat, J. M., Aristoy, M. C., & Antequera, T. (2010). Influence of pre-cure freezing of Iberian ham on proteolytic changes throughout the ripening process. Meat Science 85: 121–126.
10. Lorenzo, J. M., & Franco, D. (2012). Fat effect on physic-chemical, microbial and textural changes through the manufactured of dry-cured foal sausage Lipolysis, proteolysis and sensory properties. Meat Science 92: 704–714.
11. Lorenzo, J. M., Fonseca, S., Gómez, M., & Domínguez, R. (2015a). Influence of the salting time on physico-chemical parameters, lipolysis and proteolysis of dry-cured foal "cecina". LWT - Food Science and Technology 60: 332–338.
12. Lorenzo, J. M., Cittadini, A., Bermúdez, R., Munekata, P. E., & Dominguéz, R. (2015b). Influence of partial replacement of NaCl with KCl, $CaCl_2$, and $MgCl_2$ on proteolysis, lipolysis and sensory properties during the manufacture of dry-cured lacón. Food Control 55: 90–96.
13. Zhu, S. W., & Hu, J. X. (1993). Studies on Jinhua ham tastes and taste substances. Food Science 159: 8-11.