Use of a commercial protease to increase oxidative stability of Iberian ¨chorizo¨

Petrón M.J., Broncano J.M., Martín L., Parra V., Timón M.L.
Extremadura University, Department of Food Technology, Badajoz, Spain

Abstract— **Fourteen fermented sausages from Iberian pigs were manufactured using a protease with a potential antioxidant activity (batch 1: without proteases, batch 2: fungal protease). The antioxidant properties of extracts from Iberian dry-cured sausages were assessed using reducing power method. Manufacturing sausages with the used protease increased the antioxidant activity of the sausage extracts. Moreover, extracts from dry-cured sausages which were manufactured without proteases showed the highest levels of hexanal content.**

Keywords— **Antioxidant activity, lipid oxidation**

I. INTRODUCTION

The use of commercial proteases is an effective strategy to release food protein-derived active peptides with antioxidant effect. Several studies have shown the generation of this kind of compounds with antioxidant activity, on hydrosilated proteins from many animal and plant sources [1-4]. Moreover, many studies deal with the use of these bioactive antioxidant peptides in meat products showing an intense antioxidant activity against lipid oxidation [5-7].

On the other hand, compounds with physiological activity may be formed during the processing of meat products, such us cured ham or fermented sausages. Studies on the final products of proteolysis have described several low and medium weight peptides, oligopeptides and free amino acids with antioxidant effect in protein extracts from fermented sausages [8, 9].

The use of these compounds with potential antioxidant activity becomes very interesting in Iberian products. These traditional meat products from Spain are highly susceptible to lipid oxidation due to its high polyunsaturated fatty acids content [10].

The purpose of the present experimental work was to improve the oxidative stability of a fermented meat product ”Iberian chorizo” by using different commercial proteases.

II. MATERIAL AND METHOD

II.1. Samples

This study was carried out using fourteen samples of Iberian dry-cured sausages which were manufactured in the pilot plant of the School of Agricultural Engineering of Badajoz. Two batches of dry-cured sausage were made: a control batch (batch 1, n=7), where no protease was added, batch 2 (n=7), with a fungal protease. Commercial enzyme were purchased from Valley Research and used following commercial specifications.

Dry-cured sausages were made from a mixture of pork meat from Iberian pigs, pork fat, paprika and salt. No starter culture was added. The dry-cured sausage mixture was stored at 4°C for 24 h and stuffed in artificial casings. Sausages were stored in a ripening chamber for 2 months. After processing, sausages were vacuum packed, and stored at -80 ˚C until analysis.

II.2. Extraction of low molecular compounds from dry-cured sausage

Low molecular weight compounds were extracted following the method developed by Bauchart et al. [11] with some modifications. Frozen dry-cured sausage samples (2.5 g) were homogenized in 12.5 mL of Perchloric acid (50 ml L-1) in centrifuge tubes for 2 min on ice. The homogenate was centrifuged at 10.000 g for 12 min at 4 ˚C and the supernatant was collected and filtered using a cellulose acetate filter of 0.2 µm pore size. The extracts were then neutralized (pH 7.0) using KOH. The resulting salt was eliminated using a cellulose acetate filter of 0.1 µm size pore filter. The supernatant was submitted to ultrafiltration with 3 kDa cut-off at 4000 g for 30 min. The sausage extracts were finally lyophilized and stored at – 20 ˚C until analysis.

II.3. Reducing power

The RP was determined according to the method described by Oyaizu [12]. A 500 µL test sample (20 mg mL-1 dry-cured sausage extract) was mixed with phosphate buffer (2.5 mL, 0.2 mol L-1, pH 6.6) and potassium ferricyanide (2.5 mL, 10 g L-1). The mixture was incubated at 50 ºC for 20 min. An alliquote (2.5 mL) of trichloroacetic acid (100 g L-1) was added to the mixture, which was then centrifuged at 3000 rpm for 10 min. Supernatant (2.5 mL) was mixed with distilled water (2.5 mL) and FeCl3 (0.5 mL, 1g L-1), and absorbance was measured at 700 nm. The same concentration of ascorbic acid was used as positive control.

II.4. Hexanal content

Hexanal was quantified by headspace-solid phase microextraction and GC/MS AGILENT 6890, coupled to a mass selective detector AGILENT 5973 Network) [13].

II.5. Statistical analysis

Means and standard error of the mean were obtained from the analytical experiments. Results were analysed using an ANOVA test using the GLM procedure of SPSS 15.0 (SPSS Institute Inc., Cary, NC).

III. RESULTS AND DISCUSSION

III.1. Antioxidant activities of dry-cured sausage extracts

Table 1 shows the antioxidant activity of the sausage extracts (<3 kDa) measured by RP (antioxidant activity expressed by absorbance). The RP of sausage extracts ranged from 0.24 to 0.33 . In a previous study on protein extracts from pork fermented sausages RP at 700 nm was close to 0.5 which is in good agreement with the results obtained in the present work [9].

Table 1 Evaluation of antioxidant capacity of Chorizo extract (RP) and oxidative stability of Chorizo samples measured by hexanal content (µg hexanal g-1 muscle).

Batch (n=7)	RP (700nm)	Hexanal
Batch 1	0.237	1.046
Batch 2	0.333	0.149
P Batch	$P < 0.05$	$P < 0.05$

Batch 1 (without proteases), batch 2 (fungal protease concentrate).

The RP of sausage extracts treated with added protease (batches 2) was significantly higher ($P < 0.05$) than those of sausage extracts without added proteases (batch 1) (table 1). Wu et al. [14]. also demonstrated the same trend in fish meat, where a treatment with proteases increased the antioxidant effect of extracts due to increased levels of small peptides and FAAs. Similarly, the use of proteases could have increased the amount of peptides and FAAs with antioxidant activities in our extracts.

It could be suggested that this antioxidant activity in sausage extracts in the present study is due tothe action of proteolysis products that are formed during manufacturing [2].

Other studies have also demostrated the relationship between antioxidant effect and the levels of FAAs and peptides of protein hydrolysates [14, 15]. In this sense, these compounds with potential antioxidant activity, both small peptides and FAAs, have been detected by different authors in fermented sausages [16-18].

Many of these compounds with antioxidant effect have also been identified in fermented sausages from Iberian pig in a parallel study carried out in our laboratory (unpublished results).

III.2. Evaluation the oxidative stability of dry-cured sausage samples

The range of hexanal values in dry-cured sausage was variable (from 1.05 to 0.15 µg hexanal gr^{-1}) (table 1). The reported data agree with previous measurements of hexanal in dry fermented meat product [19, 20] while Rubio et al. [21] described higher values than this study. The results in table 1 indicate that the highest levels of hexanal values were found in batch 1. As mentioned above, extracts from

batch 1 also showed the lowest values for RP assay. Moreover, there was a significant correlation between levels of hexanal compared to the values of RP, yielding a negative Pearson coefficient ($r= -0.660$; $p<0.001$).

IV. CONCLUSIONS

The use of proteases seems to be an inhibitor of lipid oxidation in fermented meat product as Iberian dry-cured sausage.

ACKNOWLEDGEMENTS

This study has been possible thanks to the project (PDT07A005) supported by the Junta de Extremadura and FEDER.

REFERENCES

1. Hogan, S., Zhang, L., Li, J., Wang, H., & Zhou, K. (2009). Development of antioxidant rich peptides from milk protein by microbial proteases and analysis of their effects on lipid peroxidation in cooked beef. Food Chem 117: 438–443.
2. Zhang, L., Li, J., & Zhou, K. (2010). Chelating and radical scavenging activities of soy protein hydrolysates prepared from microbial proteases and their effect on meat lipid peroxidation. Bioresource Technol 101: 2084–2089.
3. Bougatef, A., Hajji, M., Balti, R., Lassoued, I., Triki-Ellouz, Y., & Nasri, M. (2009). Antioxidant and free radical-scavenging activities of smooth hound (Mustelus mustelus) muscle protein hydrolysates obtained by gastrointestinal proteases. Food Chem 114: 1198–1205.
4. Bougatef, A., Nedjar-Arroume, N., Manni, L., Ravallec, R., Barkia, A., Guillochon, D., & Nasri, M. (2010). Purification and identification of novel antioxidant peptides from enzymatic hydrolysates of sardinelle (Sardinella aurita) by-products proteins. Food Chem 118: 559-565.
5. Sakanaka, S., Tachibana, Y., Ishihara, N., & Juneja, L.R. (2005). Antioxidant properties of casein calcium peptides and their effects on lipid oxidation in beef homogenates. J Agr Food Chem 53: 464−648.
6. Rossini, K., Noren, C.P.Z., Cladera-Olivera, F., & Brandelli, A. (2009). Casein peptides with inhibitory activity on lipid oxidation in beef homogenates and mechanically deboned poultry meat. LWT - Food Sci Technol 42: 862–867.
7. Zhang, L., & Zhou, J.L.K. (2010). Chelating and radical scavenging activities of soy protein hydrolysates prepared from microbial proteases and their effect on meat lipid peroxidation. Bioresource Technol 101: 2084−2089.
8. Sun, W., Zhao, H., Zhao, Q., Zhao, M., Yang, B., Wu, N., & Qian, Y. (2009). Structural characteristics of peptides extracted from Cantonese sausage during drying and their antioxidant activities. Innov Food Sci Emer 10: 558-563.
9. 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.
10. Muriel, E., Ruiz, J., Ventanas, J., Petrón, M.J., & Antequera, T. (2004). Meat quality characteristics in different lines of Iberian pigs. Meat Sci 67: 299–307.
11. Bauchart, C., Rémond, D., Chambon, C., Patureau Mirand, P., Savary-Auzeloux, I., Reynès, C., & Morzel, M. (2006). Small peptides (<5 kDa) found in ready-to-eat beef meat. Meat Sci 74: 658–666.
12. Oyaizu, M. (1986). Studies on product of browning reaction prepared from glucose amine. Jpn J Nutr 44: 307–315.
13. Muriel, E., Andrés, A.I., Petrón, M.J., Antequera, T., & Ruiz, J. (2007). Lipolytic and oxidative changes in Iberian dry-cured loin. Meat Sci 75: 315–323.
14. Wu, H-C., Chen, H-M., & Shiau, C-Y. (2003). Free amino acids and peptides as related to antioxidant properties in protein hydrolysates of mackerel (Scomber austriasicus). Food Res Int 36: 949–957.
15. Li, B., Chen, F., Wang, X., Ji, B., & Wu, Y. (2007). Isolation and identification of antioxidative peptides from porcine collagen hydrolysate by consecutive chromatography and electrospray ionization–mass spectrometry. Food Chem 102: 1135–1143.
16. Ansorena, D., Zapelena, M. J., Astiasarán, I., & Bello, J. (1998). Simultaneous addition of Palatase M and Protease P to a dry fermented sausage (dry-cured sausage De Pamplona) elaboration: effect over peptidic and lipid fractions. Meat Sci 50: 37–44.
17. Arihara, K. (2006). Strategies for designing novel functional meat products. Meat Sci 74: 219-229.
18. Toldrá, F. (2002). Characterization of proteolysis. In F. Toldrá (Ed.), dry cured meat products (pp. 113–130). Connecticut: Food Nutrition Press Inc.

19. Zanardi, E., Novelli, E., Ghiretti, G. P., & Chizzolini, R. (2000). Oxidative stability of lipids and cholesterol in salame Milano, coppa and Parma Ham: dietary supplementation with vitamin E and oleic acid. Meat Sci 55: 169–175.
20. Zanardi, E., Ghidini, S., Battaglia, A., & Chizzolini, R. (2004). Lipolysis and lipid oxidation in fermented sausages depending on different processing conditions and different antioxidants. Meat Sci 66: 415–423.
21. Rubio, B., Martínez, B., García-Cachán, M.D., Rovira, J., & Jaime, I. (2008). Effect of the packaging method and the storage time on lipid oxidation and colour stability on dry fermented sausage salchichón manufactured with raw material with a high level of mono and polyunsaturated fatty acids. Meat Sci 80: 1182–1187.