

# Effect of the use of proteases on the physical-chemical and sensory characteristics of fermented sausages

Petrón M.J., Broncano J.M., Carrapiso A.I., 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). Manufacturing sausages with proteases increased the  $a^*$  values while decreased TBARs and values of hardness, gumminess and chewiness. Samples with proteases were also the best evaluated by a tasting panel.

**Keywords**— texture analysis, lipid oxidation, meat color, proteases

## I. INTRODUCTION

Lipid oxidation is the major quality deteriorative process in meat and meat products resulting in a variety of breakdown products which produce off-odours and flavours [1]. In order to prevent foods from undergoing such deterioration it is very important to inhibit lipid peroxidation occurring in foodstuffs. Antioxidants are used to preserve food products by retarding discolouration and deterioration as a result of oxidation [2].

The meat industry is increasingly searching for natural solutions to minimize oxidative rancidity and extend the shelf-life of meat products rather than synthetic additives [3]. Thus, the search for alternative methods to retard oxidative processes in meat has led to the research of alternative natural antioxidants.

Several proteolytic enzymes from microbial, plant and animal sources have been already used for meat tenderization as well as for fermentation acceleration and sensory enhancement in fermented meat products in meat industry [4, 5]. Moreover, several authors have observed a decrease in TBARs values in fish meat using fish protein hydrolysates [6].

The purpose of the present experimental work was to improve the oxidative stability of Iberian "chorizo" by using a commercial protease and to evaluate the use of the added protease on its sensorial quality.

## 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 sausage was 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 stored at 18°C and continuous light exposure until analysis (days 0, 15, 30 and 45).

### II.2. Thiobarbituric acid-reactive substances (TBARs)

TBA reactive substances (TBARs) were measured following the extraction method extensively described by Sørensen and Jørgensen [7] on days 0, 15, 30 and 45.

### II.3. Instrumental color evaluation

Color changes in sausages samples (batches 1 and 2) during storage were monitored by evaluating  $a^*$  values (redness) at an interval of 15 days (day 0, 15, 30 and 45) [8]. Colorimetric analysis was performed using a Chroma Meter CR-300 (Minolta Corporation, Osaka, Japan).

### II.4. Textural profile analysis (TPA)

Texture profile analysis (TPA) was performed by using a Universal TA-XT plus texture analyser (Stable

Micro Systems, UK). A double compression cycle test was performed up to 25% compression of the original portion height. Force–time deformation curves were obtained with a 25 kg load cell applied at a cross-head speed of 2 mm/s.

## II.5. Sensory analysis

In order to evaluate the influence of the added protease on the sensory characteristics of sausages, samples from batch 1 and 2 were assessed by an untrained panel of 346 members. Panellists were asked to evaluate general acceptability of the samples on a nine-point hedonic scale (4 = like extremely, 3 = like very much, 2 = like moderately, 1 = like slightly, 0 = neither like nor dislike, -1 = dislike slightly, -2 = dislike moderately, -3 = dislike very much, and -4 = dislike extremely).

## II.6. 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. Oxidative stability of dry-cured sausage extracts during storage.

The results indicate that the highest levels of TBARs values were found in batch 1 from day 0 to day 45 (1.2 to 2.8 vs 0.8 to 2.4 mg MDA/kg).

The evolution of the oxidative stability is presented on figure 1. MDA content increased during the first 15 days of storage. After day 15, TBARs values remained constant. The intense lipid oxidation during the first 15 days may be due to the residual oxygen in sausages packages and the oxygen permeability of films [9]. Once the residual oxygen (during the first 15 days of storage) was consumed, the lipid oxidation (TBARs value) remained stable (between 15 and 45), or even decreased (on day 30 of storage).

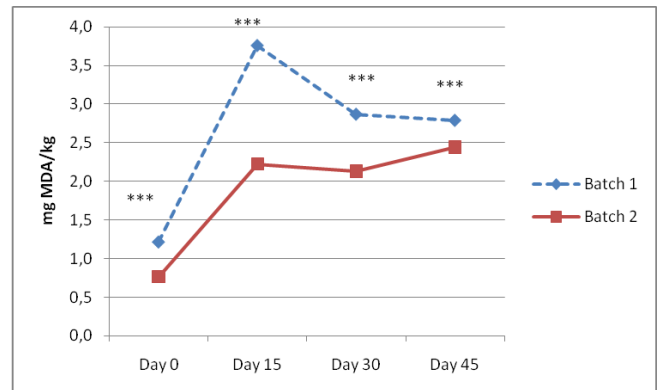


Figure 1. TBARs (mg MDA kg<sup>-1</sup> sample) during storage: Batch 1 (without proteases), Batch 2 (fungal protease). \*\*\* p<0,001.

The same trend was observed for the evolution of redness (a\*) as showed in figure 2.

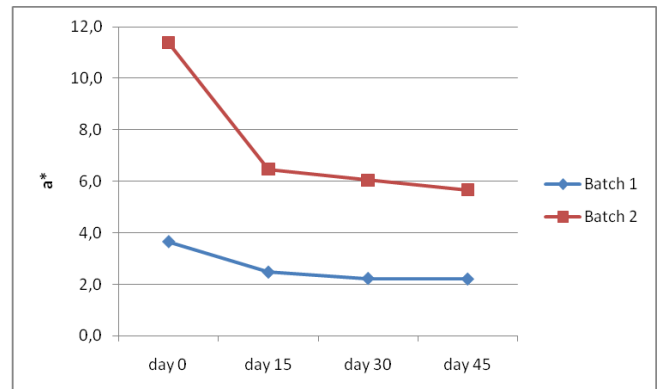


Figure 2. Evolution of a\* (redness) during storage. Batch 1 (without proteases), batch 2 (fungal protease).

The higher lipid and color oxidation for batch 1 (without proteases) may be due to the protection against oxidation of batch 2 as a result of the use of proteases. The use of proteases in sausages could increase low molecular weight compounds with potential antioxidant activity (data not shown). In this sense, other authors have also reduced the TBARs values in meat and fish by using protein hydrolysates rich in peptides and free amino acids [2, 6].

### III.2. Textural profile analysis

Table 1 shows results from TPA analysis when a significant effect of protease was found. Hardness,

gumminess and chewiness were significantly affected by the use of proteases ( $P < 0.001$ ), these parameters decreasing when proteases were added. The decrease in these parameters could be ascribed to a higher proteolysis due to the action of proteases [10]. Not significant differences were observed for the rest of textural parameters.

Table 1. Texture analysis profile.

	Hardness (N)	Gumminess (N)	Chewiness (N×cm)
Batch 1	270.0	175.6	117.3
Batch 2	132.4	64.5	33.7
P Batch	$P < 0.001$	$P < 0.001$	$P < 0.001$

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

### III.3. Sensory analysis

Results of sensory analysis show a clear preference of panelists for sausages of batch 2 (with proteases) (figure 3).

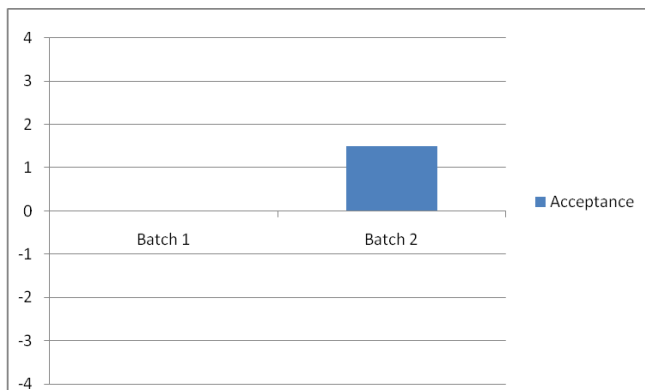


Figure 3. Acceptability of the samples on a nine-point hedonic scale.

This higher preference of panelist for sausages of batch with proteases could be related to the higher hardness in samples of batch 1 [10].

## IV. CONCLUSIONS

The use of proteases seems to be an inhibitor of lipid oxidation in fermented meat product as Iberian dry-cured sausage further enhancing its acceptance by consumers.

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