Innovative strategies to enhance the sensory quality of dry fermented sausages containing lactic ingredients by the addition of exogenous enzymes

Short title: Enzyme addition to enhance dry fermented sausages sensory quality

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ABSTRACT

This study investigated the impact of the addition of exogenous enzymes (Accelerzyme CPG, Debitrase DBP20) or cellular preparations (FlavoGard), traditionally used in the cheese industry, to accelerate flavour development of dry fermented sausages with 6% of lactic derivatives content. Sausages were fermented to pH 5.0, dried for 32 days and vacuum packed stored under refrigeration for 60 days. Sausages were analysed for physicochemical parameters, technological microbiota and proteolysis after fermentation, drying/ripening and storage. Similar compositional results were obtained in all products (38-39% humidity in the final product; 38.2 % fat and 40.7 % protein as dry matter throughout the study). Debitrase application positively affected proteolysis by changing the free amino acid profile and increasing Non-protein nitrogen and total free amino acids by 2.2 and 11.8-fold, respectively. Accelerzyme increased ripened cheese flavour and overall sensory quality from 5.1 to 5.8; Debitrase increased ripened cheese odour and flavour, bitterness, umami, adhesiveness, pastiness, and overall sensory quality from 5.0 to 5.9, and decreased acid and hardness. This study highlights the benefits/effects of adding some exogenous enzyme/bacterial preparations traditionally used in the cheese industry to enhance the flavour of dry fermented sausages containing high content of lactic ingredients and increase its sensory quality.

KEYWORDS

Cellular preparations; Flavour development; Proteolysis; Ripening; *Salchichón*

INTRODUCTION

Dry fermented sausages play an important part in many diets, providing valuable protein and fat nutrients with appealing flavours. The flavour of dry fermented sausages is the result of a complex combination of taste compounds. Most of these substances are formed by enzymatic reactions or chemical processes that take place during ripening (Zhao, Schieber & Gänzle, 2016). Proteolysis is one of the main mechanisms, together with lipolysis, for the formation of flavour compounds in dry-fermented sausages. During their production process and due to the activity of endogenous and microbial enzymes, meat proteins are hydrolysed to polypeptides that can be further degraded to large amounts of smaller peptides and free amino acids (Gallego, Mora, Escudero & Toldrà 2018). Studies investigating the biochemical properties of dry fermented sausages revealed that the ingredients, manufacturing conditions, starter cultures, spices and diameter are the primary determinants of the proteolysis pattern and the characteristic flavour properties associated with each sausage type and/or brand (Puolane & Petäjä-Kanninen, 2015; Montanari et al., 2018; Gallego et al. 2018).

However, actual trends for meat products, *e.g.* the reduction of fat and salt contents (European Commission, 2009) and manufacturer's objectives to reduce production costs (Arnau, Serra, Comaposada, Gou, & Garriga, 2007), have an impact on product sensory characteristics. Therefore, innovative strategies able to diversify and improve the odour and flavour of dry fermented sausages are necessary. Among those strategies, the addition of different bacterial starter cultures or yeast strains have been evaluated (Flores, Corral, Cano-García, Salvador, & Belloch, 2015; Aro Aro et al. 2010; Lorenzo, Fonseca, Gómez & Domínguez, 2016).

Additionally, as fermentation is an integral feature of both dry sausage and cheese production, this had led to the evaluation of commercial enzymes or bacterial preparations to diversify the range of flavour in fermented meat and milk products (Arnau, Serra, Comaposada, Gou & Garriga, 2007). Exogenous proteases and lipases have been used to accelerate the ripening of dry fermented sausages, with the primary aim of reducing production costs. However, up to date a

significant improvement in flavour development has been detected only in a minority of studies (Fernández, Ordóñez, Bruna, Herranz, & de la Hoz, 2000; Leroy, Geyzen, Janssens, De Vuyst & Scholliers, 2013).

In previous studies on cheese, the commercial preparations Accelerzyme CPG®, Debitrase DBP 20 and FlavoGard® were shown to be appropriate for cheese flavour development, while acting in different ways on protein breakdown (Kilcawley, Nongonierma, Hannon, Doolan, & Wilkinson, 2012; Kilcawley, Wilkinson, & Fox, 2006). To the best of our knowledge, none of these products had been tested yet in dry fermented sausages with a high lactic derivatives content, which is a common composition in some Spanish fermented sausages (Gou et al., 1998) that increases sweetness, protein content and dry matter and provides a slight lactic nuances. Therefore, and given the consumer demands for safe products of quality and the technological and economical interest of the fermented sausages manufacturers in accelerating the ripening process (Fernández et al., 2000, Leroy et al., 2013), the objective of this study was to evaluate the suitability of the application of Accelerzyme CPG®, Debitrase DBP 20 and FlavoGard® commercial preparations in *salchichón* type dry fermented sausages containing a 6% of powder lactic derivative ingredients (i.e. milk powder, lactose and casein) by evaluating its impact on the microbiology, physicochemical characteristics, proteolysis and sensory properties.

MATERIALS AND METHODS

Sausage manufacturing and enzyme application

Two independent trials were performed on different days, in which 7 batches were produced. For each batch (15 kg), the following ingredients were mixed: 824 g/kg of pork shoulder meat (65%), lean pork meat (19%) and pork fat (17%), 60 g/kg soy mash (Solae LLC, St. Louis, Missouri), 25 g/kg milk powder, 25 g/kg lactose (Firesland Foods Domo USA Inc., Chicago) and 5 g/kg dextrose (Cargill, Martorell, Barcelona, Spain), 25 g/kg NaCl, 15 g/kg decalcified water, 10 g/kg casein (DMV International, The Netherlands), 2.5 g/kg black pepper, 0.5 g/kg erythrobate, 0.15

g/kg potassium nitrate, 0.15 g/kg sodium nitrite and 0.05 g/kg carmine. Meat and fat were previously ground through a 5 mm plate (model PM 114, Castellvall S.A. Riudellots de la Selva, Spain) and the soy mash was prepared by mixing 800 g/kg water, 200 g/kg soy protein, 20 g/kg salt and 0.05 g/kg of carmine in a bowl chopper until it showed a homogenous bright appearance. To every batch, lyophilised starter cultures *Pediococcus pentosaceus* and *Staphylococcus xylosus* (0.25 g/kg, Lyocarni RHM-33, Sacco, Italy) were added. The enzyme/cellular preparations Accelerzyme CPG®, a carboxypeptidase purified from *Aspergillus niger* (DSM Food Specialities, Delft, The Netherlands), Debitrase™ DBP20, a mixture of purified enzymes from *Lactococcus lactis* and *Aspergillus oryzae* (DuPont, Dange St Romain, France) and FlavoGard®, a flavour adjunct culture from *L. lactis* subspecies *cremoris* (DuPont, France) were initially dissolved in 500 ml of sterile water and then applied separately at a low and a high dose rate. The dose rates were chosen based on supplier recommendations and on published data (Kilcawley, Wilkinson, & Fox, 2002a; Zapelena, Ansorena, Zalacain, Astiasarán, & Bello, 1998). Each trial contained a Control without addition of enzyme preparation. The following batches were produced: Control, CPG-Lo, CPG-Hi, DBP-Lo, DBP-Hi, FL-Lo and FL-Hi, details of the trials and doses are provided in Figure 1. The meat batter was mixed for 3 min before and 1 min after the addition of the enzyme/cellular preparations at 0ºC in an AVT-150 mixer under vacuum conditions (Castellvall S.A., Castellar del Vallès, Spain) to achieve a homogenous distribution and subsequently stuffed in 50-60 mm diameter collagen casings (Fibran, Fibran S.A., Sant Joan de les Abadesses, Spain) in pieces of 1 kg (time 0 (t0) samples). Sausages were then fermented for 24-48 h at 22-23 °C at a relative humidity (RH) of 90-95% until the pH decreased to 5.0 (t1). The sausages were then dried for ca. 32 days at 14-16 ºC (RH 75-80%) until a weight loss of ~30% was achieved (t2). At the end of the drying process, sausages were vacuum-packed to avoid further reduction of weight loss and stored under refrigeration (5 ºC) for 60 days (t3). Different randomly selected sausages were used at each sampling time and all the analyses were performed in duplicate.

[insert Figure 1]

Microbiological analysis

For each sampling time (t0,t1,t2 and t3), 25 g of sausage samples were aseptically minced, 1:10 diluted in 0.1% Bacto Peptone (Difco Laboratories, Detroit, MI, USA) with 0.85% NaCl and homogenized for 1 min in a Masticator Classic (IUL S.A., Barcelona, Spain). After doing the appropriate dilutions (in 0.1% Bacto Peptone with 0.85% NaCl), lactic acid bacteria (LAB), gram positive catalase positive cocci (GCC+) and *Lactococcus lactis* (FlavoGard batches) were determined by plating on De Man Rogosa and Sharpe agar (MRS, Merck, Darmstadt, Germany), Mannitol Salt Phenol-red agar (MSA, Merck) and LM17–agar (AES Chemunex, Barcelona, Spain), respectively, after incubation of 48-72 h at 30 °C. Yeasts and moulds were counted in Yeast-Glucose-Chloramphenicol agar (YGC, Merck) incubated for 5 days at 25 ºC.

Physicochemical and compositional analysis

The pH was determined with a portable pH-meter (Crison pH25. Crison 133 Instruments S.A., Alella, Spain). Water activity (a_w) was measured at 25 °C using an S3TE Aqualab equipment (Decagon Devices, Inc. Pullman, Washington, USA). The fat, protein and moisture contents were determined according to the AOAC official method 2007.04 (Anderson, 2007) with a FoodScan™ device (FOSS Analytic, Hillerod, Denmark), which uses the near-infrared spectrophotometer system.

Proteolysis analysis

For proteolytic analysis, 10 g of ground sample was homogenized with 20 ml of 2% trichloroacetic acid (TCA) (Hughes *et al,*. 2002) using an Ultra-Turrax (IKA T18 basic) and then centrifuged at 27000 $\times g$ for 20 min at 4 °C. The supernatant was filtered (Whatman No1, Whatman International Ltd. Maidstone, England) and the obtained 2% TCA-soluble extract was used for the determination of non-protein nitrogen content (NPN) by Kjeldahl and quantification of individual free amino acids (FAA) by HPLC. For individual FAA analysis, the most relevant amino acids were analysed. Samples were deproteinised by mixing equal volumes of 24% (w/v)

TCA, these were allowed to stand for 10 min before centrifuging at 14400 $\times g$ (Microcentaur, MSE, UK) for 10 min. Supernatants were removed and diluted with 0.2 M sodium citrate buffer, at pH 2.2 to give approx. 250 nmol of each amino acid residue. Samples were then diluted 1:2 with the internal standard norleucine, to give a final concentration of 125 nmol/ml. Amino acids were quantified using a Jeol JLC-500/ V amino acid analyser (Jeol Ltd., Garden city, Herts, UK) fitted with a Jeol Na$^+$ high performance cation exchange column. Results were expressed in mg/100 g of dry matter.

Sensory analysis: Quantitative Descriptive Analysis (QDA)

A Quantitative Descriptive Analysis (QDA) was applied to provide quantitative description of products based on the perceptions of a group of trained assessors (Stone, Sidel, Oliver, Woolsey & Singleton, 1974; Stone and Sidel, 1993). Six selected and trained assessors (ASTM, 1981; ISO 8586-1, 1993 and ISO 8586-2, 1994) with a minimum of ten years of experience in tasting meat products undertook the sensory analysis on 1 mm-thick slices of dry fermented sausages at the end of the storage period. The generation of the descriptors was carried out by open discussion in two previous sessions. The descriptors retained were: acid odour (intensity of a sharp acid odour), ripened cheese odour (intensity of odour characteristic of cheese ripened for a long period of time), cooked odour (intensity of odour sensation elicited by pork fat cooked in water at 100 °C and refrigerated at 3 ± 2 °C (Ferrini *et al,.* 2012)), acid flavour (basic taste sensation elicited by an acid), bitterness (basic taste sensation elicited by L-Tryptophan), saltiness (basic taste sensation elicited by NaCl), umami (basic taste sensation elicited by sodium glutamate), ripened cheese flavour (flavour characteristic of cheese ripened for a long period of time), adhesiveness (textural property rated by the degree to which the surface of the dry fermented sausage slice adheres to the palate when compressed with the tongue), hardness (force required to bite through the sample), crumbliness (textural property characterised by easiness with which a sample can be separated into smaller particles during chewing), and pastiness (feeling of paste detected in hams with a high proteolytic index). Overall sensory quality (scoring of the sensory quality of the

sample by reference to the standard of quality for a product) was also assessed. A non-structured scoring scale was used, where 0 meant absence of the descriptor and 10 meant high intensity of the descriptor. Sensory evaluation was undertaken separately in three sessions/sampling time and a complete block design was used, where each taster assessed all the treatments in each session. A total of 9 sensory sessions were carried out. Samples were coded with three random numbers and were presented to the assessors balancing the first order and the carry over effects (MacFie, Bratchell, Greenhoff & Vallis, 1989). The average score of the six experts for each sample and session was recorded and used in the statistical analysis.

Data analysis

Analysis of variance (ANOVA) and the post-hoc Tukey test at a $p<0.05$ significance level were carried out using Statistica 7.0 software (Statsoft, Tulsa, UK). For determining statistical differences among batches in composition, physicochemical parameters, technological microbiota, NPN and Total FAA, the model included the batch and the sampling time as fixed factors. Analysis of individual FAA was carried out at each sampling time and, as a consequence, the model included as main factor the batch.

Data from quantitative descriptive sensory analysis was evaluated using SAS for Windows version 9.1 (SAS, 2005). For the sensory attributes, the average scores of the panel for each fermented sausage from 2 replicates and 2 sampling points (t2 and t3) was used. The model included treatment, replicate and sensory session as fixed effects. In all cases, the interaction between fixed effects was tested and dropped from the model since it was not significant.

RESULTS AND DISCUSSION

Microbiota, physicochemical parameters and composition

The technological microbiota, pH, a_w, and composition (moisture, protein and fat contents) were not significantly affected by the addition of the enzyme preparations ($p>0.05$) (Figure 2 and 3).

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Yeasts and moulds kept *ca.* 3 log cfu/g throughout the study. Initial LAB and GCC+ counts correspond to the added starter culture levels (both *ca.* 6.6 log cfu/g). LAB increased 2.5 logs during fermentation and maintained at ca. 9 log cfu/g during the rest of the study. GCC+, described as less competitive than LAB (Stollewerk, Jofré, Comaposada, Ferrini & Garrriga, 2011), remained at initial levels until the end of ripening and slightly decreased (1.7 logs) during the 2 months of refrigerated storage. pH decreased from 5.6 to 5.0 during fermentation and ripening and was 5.1 in the final product. As expected in dry fermented sausages (Corral, Salvador & Flores, 2013), progressive dehydration decreased a_w and water content at the end of ripening and drying to 0.906 and 38.53%, respectively. Consequently, the fat and protein contents increased to 23.46 and 25.62%, respectively, at the end of drying and remained at the same levels during subsequent refrigerated storage due to vacuum-package and refrigeration storage (38.2 ± 0.1 % of fat and 40.7 ± 1.2 % of protein as dry matter). Levels of fat and protein are in the range of *salchichón* type sausages although they are highly dependent on the manufacturer/brand (Beriain, Chasco & Lizaso, 2000).

[insert Figure 2 and Figure 3]

Proteolysis analysis

Intensity of proteolysis, assessed by nonprotein nitrogen content (NPN) is shown in Table 1. After fermentation (t1), values significantly increased in all the batches although the highest increase was observed in DBP-Lo and DBP-Hi. Subsequently, they remained at similar levels (p>0.05, *ca.* 10 – 11%) in all the batches except for DBP-Lo and DBP-Hi (p<0.05), which increased up to 13.3 and 17.0 % (1.7 and 2.2 fold increase compared to the meat batter (t0), respectively, at the end of storage. The same trend was observed by total FAA, with increases from t0 to t3 ranging from 5-fold (in Control and CPG-Lo) to 12-fold (in DBP-Hi).

During the whole experiment, levels of individual free amino acids, increased, especially during the drying/ripening 32-day period (t2, Figure 4). Only levels of ARG decreased in all batches

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from t0 to t1 and remained at low levels (< 4 mg/100g) during the rest of the study. ARG has been described to be metabolized by several species of LAB, which use the amino acid for survival and convert it to flavour compounds (Chen, Liu, Sun, Kong, & Xiong, 2015; Gallego et al. 2018). Regarding the Control batch, the predominant amino acids in the dry product were HIS (99,47 mg/100 g), GLU (86,70 mg/100g), LEU (40,42 mg/100g), ALA (39,40 mg/100g), LYS (34,55 mg/100g), THR (30,35 mg/100g), VAL (28,99 mg/100g) and PHE (26,81 mg/100g), showing concentrations higher than 20 mg/100g. The high levels of Glu agree with results obtained for other fermented sausages with a fermentation step at 20-27ºC, which independently of the formulation showed a higher production of GLU than fermented sausages produced at low temperatures (Gallego et al., 2018, Aro Aro et al., 2010). In this regard, differences in the profiles of different fermented sausages have been related with the applied starter culture/s and the processing conditions (i.e. time and temperature of fermentation and drying), that determine the activity of enzymes of muscular and bacterial origin (Dominguez et al., 2016, Gallego et al., 2018). In contrast to the present results, low levels of HIS were detected in the mentioned studies. Conversely, Henriksen & Stahnke (1997) found high levels of HIS in Danish salami and related this fact to the presence of histidine dipeptides in skeletal muscle, the concentration depending on muscle type and possibly to the proportion of beef meat in the salami.

1   Table 1. Changes in non-protein nitrogen (NPN) and total free amino acids (FAA) throughout manufacturing and storage of dry fermented sausages.

Batch	t0	t1	t2	t3	Fold increase[1]
Non-Protein Nitrogen (NPN), in % of total N					
Control	7.9 ± 0.6	7.5 ± 0.4^d	9.6 ± 0.6^c	10.4 ± 1.0 ^{bc}	1.3
CPG-Lo	-	8.9 ± 0.4^c	10.2 ± 0.7^c	9.2 ± 1.1^c	1.2
CPG-Hi	-	9.0 ± 0.3^c	10.4 ± 0.7^c	10.6 ± 0.3^c	1.3
DBP-Lo	-	11.6 ± 0.1^b	14.8 ± 0.5^b	13.3 ± 1.7^b	1.7
DBP-Hi	-	12.7± 0.3^a	16.2 ± 0.1^a	17.0 ± 0.2^a	2.2
FL-Lo	-	9.1 ± 0.0^c	10.1 ± 0.3^c	11.2 ± 1.1^{bc}	1.4
FL-Hi	-	9.4 ± 0.4^c	10.7 ± 0.3^c	10.1± 1.9^c	1.3
Total Free Amino Acids (FAA), in mg/100g dry matter					
Control	92.1 ± 0.3	97.2 ± 0.4^e	380.0 ± 7.5^c	487.9 ± 3.3^{cd}	5.3
CPG-Lo	-	129.4 ± 12.2^d	414.7 ± 68.6^c	446.2 ± 30.5^d	4.8
CPG-Hi	-	133.0 ± 3.6^d	404.7 ± 22.9^c	519.3 ± 23.9^{cd}	5.6
DBP-Lo	-	184.0 ± 12.5^b	740.9 ± 38.0^b	814.7 ± 85.7^b	8.8
DBP-Hi	-	226.9 ± 3.9^a	903.6 ± 16.7^a	1089.1 ± 16.0^a	11.8
FL-Lo	-	162.6 ± 4.3^c	474.9 ± 9.7^c	621.4 ± 63.4^c	6.7
FL-Hi	-	173.3 ± 10.5^{bc}	492.3 ± 58.5^c	588.7± 104.7^{cd}	6.4

2   Data represent mean values of 2 independent experiments performed in duplicate ± standard deviation.
3   Small letters in a column indicate significant differences (p<0.05) between batches.
4   -: not determined
5   [1]Fold increase throughout the process, i.e., from the meat batter to the end of storage

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8   Nevertheless, LEU, PHE, VAL, LYS, GLU and ALA, some of the FAA detected in the evaluated

9   *salchichón*, were shown to be the most predominant in other meat products (foal sausage

10   (Domínguez et al., 2016; Lorenzo & Franco, 2012), foal "cecina" (Aro et al., 2010; Lorenzo,

11   Fonseca, Gómez, & Domínguez, 2015) and dry cured lacón (Garrido, Domínguez, Lorenzo,

12   Franco, & Carballo, 2012). Those FAA, specifically compounds originated by the degradation of

13   VAL, LEU and ILE have been linked to the ripened flavour of fermented foods (Herranz 2006).

14   Taking into account the added enzymatic preparations, FAA levels changed in parallel with the

15   dose rate, except for the FL batches, where greater levels ($p > 0.05$) were found in FL-Lo than in

16   FL-Hi samples at the end of storage (t3). The greatest increase over the Control was in DBP-Hi

17   sausages, where significant differences were observed for all FAA at t1 (except PRO and ARG),

18   t2 (except ARG) and t3 (2- to 3-fold amounts). Elevated levels of FAA (except for ARG) were

19   also observed in the FL-Lo, FL-Hi and DBP-Lo batches over the Control at t1, t2 and t3 (Figure

20   4). For both CPG samples (CGP-Lo and CPG-Hi), in contrast, significantly higher levels than in

21   the Control were observed for THR, GLY, ALA, VAL, MET, LEU, TYR and HIS at t1, but not

22   at t2 and t3.

23   [Insert Figure 4]

24   These results can be explained by the fact that Debitrase DBP20 had the highest proteinase

25   activity and therefore, DBP batches had the highest NPN and total FAA levels. It has been

26   reported that products derived from *Aspergillus* spp have high levels of Pep N activity and

27   products from *L. lactis* have highest levels of PepX activity (Kilcawley et al., 2002a). This

28   explains why Debitrase™ DBP 20, containing *L. lactis* together with *Aspergillus oryzae* is the

29   product with the highest effect on protein breakdown. Furthermore, Debitrase DBP20 was shown

30   to have high levels of peptidase A, which generates GLU and to have significant levels of proline-

31   specific activities (Kilcawley et al., 2002a; Kilcawley, Wilkinson, & Fox, 2002b). FlavoGard®, a

32   flavour adjunct culture of *L. lactis* subsp. *cremoris* described by its manufacturers to have high

33   aminopeptidase activity and Accelerzyme CPG®, a purified enzyme preparation containing a

34   specific carboxypeptidase (Kilcawley et al., 2012), did not remarkably increase levels of NPN but

35   modified FAA total levels and profile. In this regard, the enzyme preparations used in the present

36   study increased the amount of aroma flavour formation precursors, anticipating a varying flavour

37   in fermented sausages. We observed high amounts of HIS and GLU. Sofrza et al. (2006) pointed

38   out that GLU and ASP helped umami taste in Parma ham. In addition, Zhao et al. (2005) observed

39   that LEU, ILE, ARG, HIS and PHE contributed to bitter taste in Jinhua ham.

40   **Sensory Analysis**

41   The sensory comparison of Control dry fermented sausages with those formulated with the

42   enzyme preparations CPG, DBP and FL is shown in Table 2. The addition of CPG-Hi increased

43   ripened cheese flavour and overall sensory quality compared to Control. No significant difference

44   was found between control and FL batches. In contrast, the addition of DBP resulted in significant

45   differences in some attributes. DBP-Hi samples scored higher ripened cheese odour and lower

46   cooked odour. Ferrini et al. (2012) also observed a lower cooked flavour in fermented sausages

47   with stronger flavour and antioxidant ingredients. In a similar way, the higher ripened cheese

48   odour could mask the detection of cooked odour, which is considered as not appropriate for this

49   kind of fermented sausages.

50 Table 2. Sensory attributes and overall liking of dry fermented sausages.

Descriptor	Treatments			p	RMSE
	Control	CPG-Lo	CPG-Hi		
Odor					
Acid	3.3	3.6	3.4	0.4708	0.3913
Ripened cheese	1.0	1.6	1.7	0.1859	0.7105
Cooked	1.6	1.0	0.7	0.2181	1.0714
Flavour					
Acid	3.9	3.8	3.6	0.4977	0.4868
Bitterness	0.4	0.6	0.6	0.3807	0.2933
Saltiness	2.9	3.0	2.8	0.0975	0.2343
Umami	1.8	1.8	2.1	0.2292	0.3684
Ripened cheese	1.3[b]	1.6[b]	2.5[a]	0.0475	0.5436
Texture					
Adhesiveness	1.2	0.9	0.7	0.0529	0.3866
Hardness	3.2	3.7	3.7	0.1836	0.5093
Crumbliness	5.3	4.8	4.7	0.0652	0.4153
Pastiness	0.3	0.3	0.2	0.2543	0.1504
Overall Sensory Quality	5.1[b]	5.3[ab]	5.8[a]	0.0451	0.3971
	Control	DBP-Lo	DBP-Hi		
Odor					
Acid	3.3	3.0	3.2	0.4623	0.5950
Ripened cheese	1.0[b]	1.8[ab]	2.4[a]	0.0211	0.7756
Cooked	3.5[a]	0.9[b]	0.7[b]	0.0007	0.9799
Flavour					
Acid	4.1[a]	3.4[a]	2.8[b]	0.0004	0.4528
Bitterness	0.7[b]	1.1[b]	1.9[a]	0.0011	0.5229
Saltiness	2.9	2.8	2.8	0.8966	0.2542
Umami	1.9[b]	2.0[b]	2.6[a]	0.0315	0.2834
Ripened cheese	1.2[b]	1.9[b]	3.1[a]	<0.0001	0.6210
Texture					
Adhesiveness	1.3[b]	1.6[b]	2.3[a]	0.0002	0.4197
Hardness	3.5[a]	3.1[ab]	2.7[b]	0.0120	0.4234
Crumbliness	5.2	5.7	6.0	0.0890	0.5827
Pastiness	0.8[b]	1.4[b]	2.9[a]	<0.0001	0.6583
Overall Sensory Quality	5.0[b]	5.8[a]	5.9[a]	0.0481	0.3718
	Control	FL-Lo	FL-Hi		
Odor[1]					
Acid	2.6	3.9	3.5	0.2814	1.0075
Ripened cheese	0.9	2.8	2.0	0.2125	1.3265
Flavour					
Acid	3.3	4.0	3.8	0.5260	0.7273
Bitterness	0.3	1.2	0.6	0.2754	0.7388
Saltiness	2.7	3.5	3.0	0.3934	0.8636
Umami	1.5	1.2	1.8	0.3536	0.6335
Ripened cheese	0.9	2.7	1.5	0.2158	1.3688
Texture					
Adhesiveness	1.8	1.8	1.0	0.7186	1.6075
Hardness	3.3	3.9	3.8	0.2065	0.5968
Crumbliness	4.9	4.7	4.4	0.7508	0.8338
Pastiness	1.0	0.2	0.0	0.0621	0.4619
Overall Sensory Quality	5.0	5.7	5.3	0.1514	0.7813

51 [1]Cooked odour was not evaluated in FL samples because it was not detected in none of the evaluated
52 samples
53 RMSE:Root Mean Sum of Errors; Values are average scores of six experts; Within a row, different
54 letters indicate significant differences (p<0.05) between the different treatments;
55 CPG: Accelerzyme CPG,DBP: Debitrase DBP 20, FL: FlavoGard. Lo and Hi indicate High and Low
56 dose levels of enzyme preparation.
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58   Neither of these compounds were strongly associated with the DBP samples. Ripened cheese

59   odour and flavour, bitter taste, umami, adhesiveness and pastiness were also higher ($p<0.05$) in

60   the DBP-Hi samples than in the Control. In contrast, both acid taste and hardness were lower

61   ($p<0.05$) in the DBP-Hi samples. All these results were in agreement with proteolysis results,

62   which showed a higher increase of NPN and FAA levels during fermentation, ripening and

63   specially at the end of storage in DBP sausages than in Control, CPG or FL. Ripened cheese odour

64   and flavor was developed, in part, as in cheese, as a result of proteolysis and glycolysis of lactic

65   ingredients, and to some extent to lipolysis of pork fat.

66   The overall sensory quality of sausages at the end of the storage was higher in sausages formulated

67   with enzyme preparations CPG and DBP, but not in FL. In CPG batch, this score was higher in

68   high dose batch. In DBP there was an increase in overall sensory quality between control and

69   DBP-Lo, but no increase was observed between DBP-Lo and DBP-Hi probably because the

70   improvement in some positive attributes (acid, umami, ripened cheese) was compensated by the

71   increase in bitterness, pastiness, adhesiveness and decrease in hardness.

72

73   **CONCLUSIONS**

74   The application of the three evaluated enzyme preparations (i.e. Accelerzyme CPG®,

75   FlavoGard® and Debitrase™ DBP20) developed for cheese on dry fermented sausages did not

76   negatively affect physicochemical and microbial parameters. Conversely, sensory analysis

77   revealed that flavour attributes were improved by CPG and DBP which could be suitable as

78   flavour developers in dry fermented sausages with a high content of lactic ingredients.

79

80   **FUNDING**

84

DECLARATION OF CONFLICTING INTERESTS

The Authors declare that there is no conflict of interest

87

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216   **Figure captions**

217   Figure 1. Schematic representation of the production process of dry fermented sausages with and

218   without exogenous commercial enzymes or cellular preparations. The sampling time

219   points are indicated as t0=after mixing, t1=after fermentation, t2=after ripening/drying

220   and t3=after storage.

221   Figure 2. Development of pH and technological microbiota (Lactic Acid Bacteria (LAB) and

222   Gram-Positive Catalase Positive cocci (GCC+)) in dry fermented sausages during

223   fermentation (F), drying and refrigerated storage. Data represent mean values and

224   standard deviation of all sausage batches.

225   Figure 3. Development of a_w and compositional parameters humidity, protein content and fat

226   content in dry fermented sausages during fermentation (F), drying and refrigerated

227   storage. Data represent mean values and standard deviation of all sausage batches. Note

228   that values expressed as dry matter were 38.2 ± 0.1 % for fat and 40.7 ± 1.2 % for protein

229   throughout the study.

230   Figure 4. Changes in individual free amino acids (mg/ 100g dry matter) in dry fermented sausages

231   in the meat batter (t0), after fermentation (t1), drying (t2) and refrigerated storage (t3).

232   Data represent mean values and standard deviation of 2 independent experiments

233   performed in duplicate.