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Review

Microbial enzymatic activities for improved fermented meats

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Fermented sausages constitute typical products that have been produced for centuries and transmitted from generation to generation. The introduction of microbial starters for the manufacturing of such sausages contributed to a significant improvement in the uniformity and safety of the final product. In fact, the knowledge of the fermentation microbiology and also on the enzymes involved, their catalytic characteristics and their mode of action has experienced a rapid increase in the last decades and has prompted relevant technological evolution with a decisive contribution to the final sensory quality. The enzymes and main biochemical reactions taking place during fermentation and ripening, especially those closely related to flavour and texture development, are reviewed in this manuscript. The enzyme activities of microbial starters and their effects on the quality of fermented meats are described.

Introduction

The origin of fermented meats is lost in ancient times. Fermented sausages were already manufactured by Ancient Romans and Greeks and then its production and consumption expanded throughout Europe in the Middle Age. The processing conditions were adapted to the environmental conditions so that drying was applied in the Mediterranean area while smoking was applied in Northern Europe (Toldrá, 2002). Today, a wide variety of semidry- and dry-fermented sausages are produced and the final characteristics and quality depend on a large number of variables related to the raw materials, microbial population and processing conditions.

A key step in sausages processing is fermentation. Traditionally, fermentation was depending on the development and growth of desirable indigenous flora, either naturally present or reinforced by back-slopping (Toldrá, 2002) (addition of an extract from a previous ripened fermented sausage). The major microflora in such traditional sausages was based on lactic acid bacteria (Lebert, Leroy, & Talon, 2007). The use of microbial starters prompted a rapid development of fermented sausages at industrial level, with standardized quality and substantially improved safety (Toldrá, 2006). This manuscript is presenting a review on main microbial enzymes present in microorganisms isolated from fermented meats and/or currently used as starter cultures, their main characteristics and how they contribute to the development of main sensory characteristics. It was reported that part of lipolysis and proteolysis, specially the initial protein breakdown, appeared to be carried out by endogenous meat enzymes (Molly *et al*., 1997). However, the description of muscle enzymes or any further chemical reactions related to aroma generation are out of the focus of this review.

Main microorganisms used as starter cultures

The selection of starter cultures is based on technological relevant traits but most especially in some requirements like the absence of amino acid decarboxylase activity, lack of toxicogenic activity or antimicrobial resistance, stability under processing conditions, salt tolerance, and bacteriocin production. The natural microflora in traditional sausages constitutes a good reference for the isolation of bacteria to be used as starter because they are well adapted to the meat environment and can control the microbiota in the product (Cocconcelli & Fontana, 2008). In recent years, modern molecular methods for the analysis of the nucleic acids have been applied during the traditional fermentation of sausages and have allowed a better detection, identification and characterisation of microorganisms (Cocolin, Dolci, & Rantsiou, 2008). In fact, fermented sausages are mostly produced today with starter cultures consisting of lactic acid bacteria alone or combined with coagulase-negative staphylococci (CNS) and yeasts or molds. The use of LAB is essential for carbohydrate fermentation and lactic acid generation with subsequent pH drop. These microorganisms provide a good number of enzymes involved

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Table 1. Examples of purified enzymes isolated from microorganisms used as starter cultures in fermented meats.

Microorganism	Enzyme	Main biochemical characteristics	Main biochemical actions	Main sensory effects	References
Lactic acid bacteria					
L. sakei	Dipeptidase	Opt pH 7.6	Exopeptidase, broad dipeptide specificity except with Pro and Gly at the N terminus.	Increase Val, Met and Leu in dry sausages.	Montel *et al.*, 1995
L. sakei	Major aminopeptidase	Opt pH 7.5, Opt T^a 37 °C	Exopeptidase, no active against basic amino acids in the N terminus.	Increase free amino acids for flavour development.	Sanz & Toldrá, 1997
L. sakei	Tripeptidase	Opt pH 7.0, Opt T^a 40 °C, Activated by Mn^{2+}	Exopeptidase	Breakdown of hydrophobic tripeptides.	Sanz *et al.*, 1998
L. sakei	X-prolyl-dipeptidylpeptidase	Opt pH 7.5, Opt T^a 55 °C	Exopeptidase, almost exclusively hydrolyse X-Pro from the N terminus of peptides	Increase free amino acids for flavour development.	Sanz & Toldrá, 2001
L. sakei	Arginine aminopeptidase	Opt pH 5.0, Opt T^a 37 °C, Activated by NaCl	Exopeptidase, hydrolyse exclusively basic amino acids in the N terminus.	Increase basic amino acids for flavour development.	Sanz & Toldrá, 2002
L. sakei	Catalase	pH 7.0	catalase	Antioxidant properties.	Hertel *et al.*, 1998
L. plantarum	Lipase		Lipase, highly heat resistance, extracellular.		Silva Lopes *et al.*, 2002
L. plantarum	Lipase	Opt pH 9.3, Opt T^a 37 °C	lipase	Accelerate maturation in dry sausages.	Andersen *et al.*, 1995
Coagulase negative staphylococci					
S. xylosus	Lipase	Opt pH 7.0, Opt T^a 30 °C, Inhibited by NaCl 5%	Generation of free fatty acids.	Under the conditions found in fermented sausages, the lipase activity is scarce.	Kenneally *et al.*, 1998
S. xylosus	Superoxide dismutase	Mn^{2+}	Detoxify superoxide radicals into water and oxygen	Antioxidant properties	Barriere, Bruckner *et al.*, 2001
S. xylosus	Catalases	Two enzymes	Dismutation of hydrogen peroxide into water and oxygen	Antioxidant properties	Barriere *et al.*, 2002
S. carnosus	β-oxidation and thioesterase activities		β-oxidation is involved in the synthesis of methyl ketones	Contribute to the cured aroma in fermented sausages	Engelvin *et al.*, 2000
S. carnosus	Catalase and superoxide dismutase	Synthesised at low aeration, presence of nitrite and nitrate and low pH	Dismutation of hydrogen peroxide into water and oxygen. Detoxify superoxide radicals into water and oxygen.	Antioxidant properties. Decrease the level of volatiles arising from lipid oxidation	Barriere, Leroy-Setrin *et al.*, 2001
S. carnosus	Decarboxylase	Constitutive enzyme	Final step of the β -decarboxylation process leading to CO_2 and methyl ketone.	Increase the levels of methyl ketones contributing to the cured aroma	Fadda, Lebert *et al.*, 2002
S. warneri	lipase	Opt pH 9.0, Opt T^a 25 °C, Broad substrate specificity.	Lipase	Increase free fatty acid in sausages	Talon *et al.*, 1995
Yeasts and molds					
Debaryomyces hansenii	Glutaminase	Opt pH 8.5, Opt T^a 45 °C	L-glutamine amidohydrolase	Neutralize acidity (by ammonium generation) and flavour enhancer (L-glutamate generation)	Durá *et al.*, 2002
D. hansenii	Prolyl aminopeptidase	Opt pH 7.5, Opt T^a 45 °C,	Exopeptidase, specificity against N-terminal proline substrates.	Increase free amino acids for flavour development	Bolumar *et al.*, 2003a

Table 1 (*continued*)

Microorganism	Enzyme	Main biochemical characteristics	Main biochemical actions	Main sensory effects	References
D. hansenii	Arginyl aminopeptidase	Opt pH 7.0, Opt T[a] 37 °C	Exopeptidase, maximum specificity for basic amino acids but able to hydrolyse other amino acids.	Increase free amino acids for flavour development	Bolumar *et al.*, 2003b
D. hansenii	Protease B	Opt pH 8.0	Endopeptidase, able to hydrolyse sarcoplasmic proteins	Protein degradation during meat fermentation	Bolumar *et al.*, 2005
D. hansenii	Protease A	Opt pH 5.5	Endoprotease	Accelerates proteolysis in acidic and salty meat products	Bolumar *et al.*, 2008
D. hansenii	Protease D	Opt pH 7.5	Endoprotease		Bolumar *et al.*, 2008
Penicillium chrysogenum	Protease EPg222	Opt pH 6.0, Opt T[a] 45 °C	Endoprotease. Active against myofibrillar proteins	Increase the aroma and reduce the hardness in dry-fermented sausages	Benito *et al.*, 2002

in relevant biochemical changes like the enzymatic breakdown of carbohydrates, proteins and lipids that affect colour, flavour and texture. It must be taken into account that these enzyme activities will be influenced by processing parameters like the temperature of fermentation and ripening, the length of ripening time, the content of salt, nitrate and nitrite, the type and content of carbohydrates, the changes in pH and water activity, and the presence of spices (Stahnke & Tjener, 2007). The technological evaluation and control of such complex system requires a deep knowledge of the multiple interactions involved but very especially of the microbial enzymatic activity closely related to proteolysis and lipolysis mechanisms (Demeyer & Toldrá, 2004).

Microbial enzymes purified from microorganisms isolated from fermented meats

A summary of the enzymes purified from microorganisms used as starter cultures in fermented meats including their main biochemical characteristics and sensory effects in meat products is shown in Table 1. Several peptidases have been purified and characterised in lactic acid bacteria essentially from *Lactobacillus sakei* (Montel, Seronie, Talon, & Hebraud, 1995; Sanz & Toldrá, 1997, 2001, 2002; Sanz, Mulholland, & Toldrá, 1998). In general, these endo and exo-peptidases contribute to increase the concentration of free amino acids that affect flavour development. In addition, several lipases have been purified from *Lactobacillus plantarum* accelerating the maturation process (Andersen, Ostdal, & Blom, 1995; Silva Lopes *et al.*, 2002). On the other hand, a catalase enzyme was detected in *L. sakei* which has antioxidant capacity able to prevent the reduction of quality in fermented meats such as rancidity (Hertel, Schmidt, Fischer, Oellers, & Hammes, 1998). The catalase production is a relevant technological property of starter cultures for fermented meats (Leroy, Verluyten, & De Vuyst, 2006).

The sensory quality of fermented meats has been improved by using coagulase-negative staphylococci (CNS) and different enzymes have been purified and characterised (Table 1). So, several antioxidant enzymes have been detected in *Staphylococcus xylosus* and *Staphylococcus carnosus*. Their antioxidative properties were responsible of the decrease in the levels of volatiles compounds derived from lipid oxidation reactions and avoided the loss in sausage quality (Barriere, Bruckner, Centeno, & Talon, 2002; Barriere, Bruckner, & Talon, 2001; Barriere, Leroy-Setrin, & Talon, 2001). Also, the enzymes involved in the β-oxidation of fatty acids have been purified from *S. carnosus* (Engelvin, Feron, Perrin, Molle, & Talon, 2000; Fadda, Lebert, Leroy-Setrin, & Talon, 2002). These enzymes enhanced the cured aroma in fermented sausages by increasing the concentration of methyl ketones. Lipases, enzymes responsible for the increase in free fatty acids in fermented sausages, have also been purified and characterised in *S. xylosus* and *S. warnieri* (Talon, Dublet, Montel, & Cantonnet, 1995). However, other authors reported a scarce lipase activity under the real conditions found in fermented sausages (Kenneally, Leuschner, & Arendt, 1998).

Molds and yeasts have been studied by their capacity to enhance the appearance and flavour of meat products including a contribution to the cured colour in fermented sausages by the reduction of nitrites. Different enzyme activities have been purified and characterised from yeast and molds isolated from meat products (Table 1). Several endo and exo-peptidases have been purified from *Debaryomyces hansenii* contributing to enhance the content of free amino acids and peptides in meat products (Bolumar, Sanz, Aristoy, & Toldrá, 2003a, 2003b, 2005, 2008). In addition, a glutaminase was purified from *D. hansenii* that was able to neutralize the acid pH of fermented sausages and generate L-glutamate that can act as flavour enhancer (Durá, Flores, & Toldrá, 2002). Another peptidase was purified from *Penicillium chrysogenum* isolated from dry cured

Table 2. Effects of starter cultures (lactic acid bacteria and Coagulase negative staphylococci) in fermented meats.

Origin	Type of extract	Meat product or extract where applied	Conditions of use	Main effects	Reference
Lactic acid bacteria					
Lactobacillus and staphylococcus	*L. sakei, S. carnosus, S. saprofiticus and S. warnieri*	Applied to dry-fermented sausage	Sausage manufactured and ripened for 40 d.	Lipolysis occurred in control sausages and inoculated batches. Lipolysis was highest with *S. warnieri*	Montel *et al.*, 1993
L. curvatus and L. sakei	Whole cells, cell extracts and combination of both	Applied to myofibrillar extracts	Incubation at 37 °C during 96 h. Samples analysed by SDS-PAGE, peptide and amino acid analyses.	*L. sakei* produced a greater release of free amino acids. Proteolysis was promoted when cell suspensions were provided with cell extracts.	Fadda *et al.*, 1999a
L. plantarum	Whole cells, cell extracts and combination of both	Applied to sarcoplasmic and myofibrillar extracts	Incubation at 37 °C during 96 h. Samples analysed by SDS-PAGE, peptide and amino acid analyses.	Whole cells generated hydrophylic peptides from sarcoplasmic and myofibrillar proteins. Pronounced hydrolysis of muscle proteins required enzymes from whole cells and those in cell extracts.	Fadda *et al.*, 1999b
L. curvatus and L. sakei	Whole cells, cell extracts and combination of both	Applied to myofibrillar extracts	Incubation at 37 °C during 96 h. Samples analysed by SDS-PAGE, peptide and amino acid analyses.	*L. sakei* cell-free extract contributes to initial hydrolysis of myofibrillar proteins. Whole cells from *L. sakei* and *L. curvatus* generated peptides	Sanz *et al.*, 1999a
L. casei	Whole cells, cell extracts and combination of both	Applied to sarcoplasmic and myofibrillar extracts	Incubation at 37 °C during 96 h. Samples analysed by SDS-PAGE, peptide and amino acid analyses.	Whole cells degraded sarcoplasmic proteins. CFE also produced a partial hydrolysis of sarcoplasmic proteins. High generation of free amino acids when using whole cells and cell extracts together.	Sanz *et al.*, 1999b
Lactobacillus, Pediococcus, Carnobacterium	Inoculated strains of lactic acid bacteria and staphylococci	Grown in a media including melted pork fat, containing oleic, linoleic or linolenic acids.	Incubated at 25 °C during 20 d.	All the strains had no prooxidant activity. *S. carnosus* and *S. xylosus* inhibited the oxidation of linolenic acid. *L. plantarum* and *P. pediococcus* limited oxidation of linoleic acid.Carnobacterium had no antioxidant properties.	Talon, Walter, & Montel, 2000
L. plantarum and L. casei	Strains of *L. plantarum* and *L. casei*	Inoculated in a sausage-like system including meat and additives.	Incubated at 25 °C during 4 d.	Degradation of sarcoplasmic and myofibrillar proteins. Maximum generation of free amino acids in the presence of the mixed starter cultures	Fadda, Oliver *et al.*, 2002
L. sakei and Bacillus pumilus	Cell-free extracts of both strains and/or in combination with papain.	Applied to dry-fermented sausage	Sausage manufactured with starter cultures. Ripened for 22 d.	*L. sakei* extract reduced the content of free amino acids. The sensory quality of dry sausages was not improved	Herranz *et al.*, 2006
Lactobacillus and *Staphylococcus*	Different strains of Lactobacillus and Staphylococcus	Applied to fermented sausages	Sausage manufactured and ripened for 15 d.	No differences in the free fatty acid content between control and inoculated sausages. The lipolysis is attributed to muscle endogenous enzymes	Zuber & Horvat, 2007
Lactobacillus and *Staphylococcus*	Different strains of Lactobacillus and Staphylococcus	Applied to fermented sausages	A laboratory scale fermented sausage ripened for 14 d.	The most suitable strains were *L. sakei* DVL17 and *S. xylosus* AVS5. Discrepancies in the results obtained in *in vitro* analysis and *in situ* for proteolytic and lipolytic assays	Villani *et al.*, 2007
L. curvatus and S. xylosus	Strains of *L. Curvatus* and *S. xylosus*	Applied to dry-fermented sausage	Sausage manufactured and ripened for 38 d.	No clarification of the effect of starter cultures on proteolysis and lipolysis	Casaburi *et al.*, 2007

L. curvatus and S. xylosus	Strains of *L. Curvatus* and *S. xylosus*	Applied to dry-fermented sausage	Sausage manufactured and ripened for 65 d.	Effects on the sensory characteristics	Casaburi *et al.*, 2008
L. curvatus, L. sakei, Str. Griseus, P. pentosaceus	Strains of *L. curvatus, L. sake, Str. Griseus, P. pentosaceus*	Applied to USA rapid fermented sausage production.	After drying for 18 d, the sausages were heat processed	Few differences in individual free amino acids. Absence of effect of starter culture on proteolysis.	Candogan *et al.*, 2009
Coagulase negative staphylococci					
S. warnieri, S. saprophyticus and K. varians	Inoculated	Grown in a media including fat and additives.	Incubation at 22 °C during 3 d, and 15 °C during 27 d.	*S. saprophyticus* was the most lipolytic	Talon *et al.*, 1993
Staphylococcus	19 different strains	Grown in a dry sausage model including meat, fat and additives.	Models incubated for 2 d at 22 °C and then 25d at 12 °C	*S. carnosus* and *S. xylosus* had low lipolytic and proteolytic activities and were able to reduce nitrate. Highest dry cured odour obtained with *S. carnosus* and *S. xylosus*	Montel *et al.*, 1996
S. xylosus	Strains of *S. xylosus*	Grown in a medium plus linoleic acid	Incubation at 25 °C during 2 and 4 d. Volatile extracted by DH-GC-MS	The inoculated control had low levels of volatiles from lipid oxidation. Antioxidant capacity in fermented meat products	Barriere, Centeno *et al.*, 2001
S. xylosus	Strains of *S. xylosus*	Grown in a medium plus myofibrillar or sarcoplasmic proteins. Applied to dry-fermented sausage	Plates incubated at 37 °C during 48 h. Sausage manufactured and ripened for 33 d.	Sarcoplasmic and myofibrillar proteins were hydrolysed	Mauriello *et al.*, 2002
L. sakei, K. varians,	Inoculated	Applied to porcine whole loin muscle	Brine cured loin product fermented for 10 d and then cooked	No differences in proteolysis and lipolysis against the non-inoculated loin.	Scannell, Kenneally and Arendt (2004)

Table 3. Effects of starter cultures (yeasts and molds) in fermented meats.

Origin	Type of extract	Meat product or where applied	Conditions of use	Main effects	Sensory	Reference
D. hansenii	Whole cells, cell extracts and combination of both	Applied to sarcoplasmic extracts	Incubation at 27 °C during 96 h. Evaluation of proteolytic activity	High sarcoplasmic proteolysis by both extracts. Whole cells generated higher quantities of free amino acids than cell-free extract.	–	Santos *et al.*, 2001
D. hansenii	Internal inoculation of *D. hansenii*	Applied to dry-fermented sausage	Sausage manufactured with starter cultures. Ripened for 35 d.	*D. hansenii* produced a lower ammonia content. No effect on sarcoplasmic proteins. *D. hansenii* did not contribute to a major proteolysis	–	Durá *et al.*, 2004
D. hansenii	Internal inoculation of *D. hansenii*	Applied to dry-fermented sausage	Sausage manufactured with starter cultures. Ripened for 35 d.	*D. hansenii* inhibited the generation of volatiles from lipid oxidation and increased ethyl ester formation.	Overall quality improved with a low content of *D. hansenii.*	Flores *et al.*, 2004
D. hansenii and L. sakei	Cell-free extracts of both microorganism together and another extract with only *D hansenii.*	Applied to dry-fermented sausage	Sausage manufactured with starter cultures. Ripened for 35 d.	The *D. hansenii* extract accelerated lipolysis. The use of both extracts increased the generation of volatiles from lipid oxidation and carbohydrate fermentation	Both extracts increased the overall quality of dry sausages. The extract with both microorganisms increased the aroma.	Bolumar *et al.*, 2006
D. hansenii and Y. lipolytica	Surface inoculated	Applied to dry-fermented sausage	Sausage manufactured with a starter culture. Ripened for 301 d.	Increased proteolysis and lipolysis. *D. hansenii* had higher proteolysis and less lipolysis activity than *Y. lipolytica.*	–	Patrignani *et al.*, 2007
P. chrysogenum	Inoculation of *P. chrysogenum* Pg222	Applied to a culture medium including sarcoplasmic or myofibrillar proteins	Incubation at 25 °C during 4 d with 5% NaCl	Hydrolysis of main myofibrillar proteins down to free amino acids	Contribution to proteolysis during ripening	Benito *et al.*, 2003
P. chrysogenum	Purified Protease EPg222	Applied to dry-fermented sausage	Sausage manufactured with starter cultures. Ripened for 145 d.	Higher values of NPN and volatiles from amino acid catabolism	Higher aroma intensity and lower hardness	Benito *et al.*, 2004, 2005
P. chrysogenum and D. hansenii	Surface inoculation	Applied to sterile loins	Loins ripened for 105 d.	*D. hansenii* did not have proteolytic activity. *P. chrysogenum* showed high proteolytic activity on myofibrilar proteins	Not evaluated	Martín *et al.*, 2002
P. aurantiogriseum	Extracts of *P. aurantiogriseum* and Pronase E	Applied to dry-fermented sausage	Sausage manufactured with starter cultures. Ripened for 26 d.	Pronase E alone increased free amino acids and biogenic amines. Both extracts increased volatile from amino acid catabolism.	Both extracts increased the odour, flavour and texture of dry sausages	Bruna *et al.*, 2000a
Penicillium	Mold inoculated	In PDA medium for 5 d at 25–30 °C	Evaluation of proteolytic activity by NPN index, free amino acids index and ammonium index.	Molds reflected proteolytic activity. *P. chrysogenum* showed the highest proteolytic activity.	–	Ockerman, Céspedes-Sánchez, Ortega-Mariscal, Martín-Serrano *et al.*, 2001
Penicillium	Mold inoculated	In PDA medium for 5 d at 25–30 °C	Evaluation of lipolytic activity by peroxide index and TBA index and ammonium index.	*P. camemberti* had the highest acidity index. *P. chrysogenum* had the highest TBA index.	–	Ockerman, Céspedes-Sánchez, Ortega-Mariscal, & León-Crespo, 2001
Mucor racemosus	Superficial inoculation and intracellular extracts	Applied to dry-fermented sausage	Sausage manufactured with starter cultures. Ripened for 22 d.	Superficial inoculo and intracellular extracts produced an increase in volatile and ammonia	Increased the sensory quality of sausages	Bruna *et al.*, 2000b

ham (Benito *et al*., 2002). This enzyme was active against myofibrillar proteins producing a decrease in hardness and an increase in aroma when it was used in fermented sausages.

On the other hand, many enzyme activities have been detected in starter cultures isolated from meat products although they have not been fully purified and characterised. Several enzyme activities like β-galactosidase, esterase-lipase, aminopeptidase and phosphatase activities, were detected in *S. xylosus*, *S. carnosus* and *K. varians* isolated from Greek dry-fermented sausages (Papamanoli, Kotzekidou, Tzanetakis, & Litopoulou-Tzanetaki, 2002). Mauriello, Casaburi, Blaiotta, and Villani (2004) detected proteolytic, lipolytic, nitrate reductase, decarboxylase and antioxidant activities in coagulase negative staphylococci isolated from Italian fermented sausages that were mainly classified as *Staphylococcus* and one isolate to *Kocuria* spp. In the last years, Casaburi, Blaiotta, Mauriello, Pepe, and Villani (2005) and Casaburi, Villani, Toldrá, and Sanz (2006), detected proteolytic, lipolytic, nitrate reductase, decarboxylase and antioxidant activities in *Staphylococci*. In general, the strains *S. carnosus* and *Staphylococcus simulans* were able to hydrolyse sarcoplasmic but not myofibrillar proteins (Casaburi *et al*., 2005). However, in other staphylococci they detected no protease activity but low aminopeptidase and high esterase activity (Casaburi *et al*., 2006).

Effects of microbial enzyme activity in fermented meats

The effect of the enzymes from starter cultures has been widely studied in fermented meat products as can be observed in Tables 2 and 3. The effect of *lactobacillus* and *staphylococcus* has been studied inoculating the starter cultures in dry-fermented sausages (Candogan, Wardlaw, & Acton, 2009; Casaburi *et al*., 2007; Casaburi *et al*., 2008; Herranz, Fernández, de la Hoz, & Ordoñez, 2006; Montel, Talon, Berdagué, & Cantonnet, 1993; Zuber & Horvat, 2007) (Table 2). There were many differences in the processing conditions applied and therefore, the effects obtained by the starter cultures were different among the studies. While Montel *et al*. (1993) reported a highest lipolysis in sausages inoculated with *S. warnieri*, other authors did not find any lipolytic effect in sausages inoculated with different strains of lactobacilli and staphylococci (Zuber & Horvat, 2007). In addition, an absence of significant effect of *Lactobacillus curvatus* and *S. xylosus* on proteolysis and lipolysis was reported (Casaburi *et al*., 2007) although the sensory characteristics were observed to improve in long ripened sausages (Casaburi *et al*., 2008). Recently, Candogan *et al*. (2009) reported an absence of effect of *L. curvatus*, *L. sakei*, *Streptococcus Griseus* and *Penicillium pentosaceus* in American dry sausages ripened for a short time and finally heat processed.

Other studies have been performed using sausage-like systems (Table 2) where a degradation of sarcoplasmic and myofibrillar proteins and an increase in free amino acids were reported in the presence of *L. plantarum* and *Lactobacillus casei* (Fadda, Oliver *et al*., 2002). However, differences in the results obtained using the sausage system and *in vitro* assays have been observed (Villani *et al*., 2007).

Many studies have been performed directly on the substrates for proteolysis and lipolysis (Table 2). They have been performed with several lactobacilli like *L. sakei*, *L. curvatus*, *L. plantarum* and *L. casei*, applied to sarcoplasmic and myofibrillar extracts using the whole cell, cell free extract and combinations of both (Fadda *et al*., 1999a, 1999b; Sanz *et al*., 1999a, 1999b). These authors found a high proteolytic activity, especially exopeptidase activity, in the assayed strains. This fact would confirm the complementary role of microbial peptidases in relation to initial proteolysis by endogenous muscle endopeptidases. In 2000, Talon *et al*., studied the lipolytic activity of several strains of *Lactobacillus*, *Pediococcus* and *Carnobacterium* using a media with pork fat containing oleic, linoleic or linolenic acids and they reported differences in the lipolytic activity among the studied strains.

Several studies using exclusively staphylococci have been done in model systems (Table 2). Talon, Montel, Gandemer, Viau, and Cantonnet (1993) studied the effect of *S. warnieri*, *S. saprofiticus*, and *K. varians* in a media with fat and additives and they observed that *S. saprofiticus* exerted the highest lipolytic activity. However, the effect of staphylococci was determined in a dry sausage model where *S. carnosus* and *S. xylosus* demonstrated low lipolytic and proteolytic activities but they were responsible of an increase in sausage odour (Montel, Reitz, Talon, Berdagué, & Rousset-Akrim, 1996). Barriere, Centeno *et al*. (2001) reported an antioxidant capacity of *S. xylosus* when it was assayed in a media with linoleic acid. Moreover, an activity against sarcoplasmic and myofibrillar proteins was detected in *S. xylosus* when it was assayed in a media with proteins and also in fermented sausages (Mauriello, Casaburi, & Villani, 2002). In 2004, Scannell *et al*., inoculated with *L. sakei* and *K. varians* a porcine whole loin muscle that was afterwards brine cured and they observed an absence of differences in proteolysis and lipolysis against the non-inoculated batch.

The effects of enzyme activity from yeasts and molds in meat products have been demonstrated directly in meat products and also in culture media and extracts (Table 3). The effect of *D. hansenii* in sarcoplasmic protein extracts was studied and the whole cells showed a high proteolytic activity that resulted in an increase in the concentration of free amino acids (Santos *et al*., 2001). Also, the proteolytic activity of *P. chrysogenum* was determined in sarcoplasmic and myofibrillar extracts indicating a positive contribution to the ripening process (Benito, Córdoba, Alonso, Asensio, & Nuñez, 2003). In addition, several strains of *Penicillium* showed proteolytic and lipolytic activity in a culture media (Ockerman, Céspedes-Sánchez, Ortega-Mariscal, Martín-Serrano *et al*., 2001).

Moreover, the effects of yeasts or molds have been assayed directly in meat products. Several studies have

revealed the effect of *D. hansenii* inoculated in fermented sausages (Bolumar *et al*., 2006; Durá, Flores and Toldrá, 2004; Flores, Dura, Marco, & Toldrá, 2004; Patrignani *et al*., 2007). The sensory characteristics of the fermented sausages were improved in the presence of *D. hansenii* (Flores *et al*., 2004) and also the aroma was increased when cell-free extracts of *D. Hansenii* were used in combination with cell-free extracts of *L. sakei* (Bolumar *et al*., 2006). In addition, *D. hansenii* showed a relevant proteolytic activity in dry-fermented sausages although its lipolytic activity was lower than the one produced by *Yarrowia lipolytica* (Patrignani *et al*., 2007). Also, the use of *D. hansenii* in combination with *P. chrysogenum* in dry cured loins produced a high proteolytic activity of *P. chrysogenum* while *D. hansenii* did not showed proteolytic activity (Martín *et al*., 2002). The effect of *P. chrysogenum* was also evaluated in dry-fermented sausages and it resulted in an increase in non protein nitrogen values (NPN) and volatiles from amino acid catabolism. In addition, sensory changes like an increase in aroma intensity and lower hardness were observed (Benito, Rodríguez, Córdoba, Andrade, & Córdoba, 2005; Benito, Rodríguez, Martin, Aranda, & Córdoba, 2004). Other molds, such as *Penicillium aurantiogriseum* (Bruna, Fernández, Hierro, Ordoñez, & de la Hoz, 2000a) and *Mucor racemosus*, have been also evaluated for their effect in dry-fermented sausages and they were reported to improve the sensory quality of the sausages (Bruna, Fernández, Hierro, Ordoñez, & de la Hoz, 2000b).

The selection of starter cultures has been focused on those cultures that generate high quantities of aroma compounds to improve the sensory properties and accelerate the ripening process. The strains should have amino acid converting enzymes and specific peptide uptake mechanisms to produce volatile aroma compounds (Leroy *et al*., 2006). Many of them were staphylococci (Stahnke, 1999; Stahnke, Holck, Jensen, Nilsen, & Zanardi, 2002), although aroma generation has been proved to be affected by many processing factors (Olesen, Meyer, & Stahnke, 2004; Olesen & Stahnke, 2003, 2004; Sondergaard & Stahnke, 2002; Tjener, Stahnke, Andersen, & Martinussen, 2004a, 2004b). Other starter cultures with effect on the aroma of meat products were yeast. However, the role of yeast in aroma development in dry-fermented sausages has not been clarified as controversial results were obtained by Olesen and Stahnke (2000) who reported a little effect of *D. hansenii* on volatile compounds even though its effect on aroma development was clearly established (Bolumar *et al*., 2006; Flores *et al*., 2004).

In summary, from the reported results, the main contribution of LAB would be in the latest steps of proteolysis, mainly generating small peptides and free amino acids. CNS and kocuria would contribute to some lipolysis and antioxidant activity while yeast and molds would be mainly focused on proteolysis and amino acid transformation. However, the effect of the starter cultures on the sensory characteristics of the fermented meat products has not always been completely clarified. Although the predominance of the starter culture during fermentation and ripening has been associated to major biochemical changes. Of course, this contribution is affected by many other factors such as the ingredients used, the processing technology and the activity of the endogenous muscle enzymes. Therefore, it is essential to elucidate and clarify the microbial metabolism during sausage processing to determine the final effect on the sensory properties of meat products.

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