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Changes in the Components of Dry-Fermented Sausages during Ripening

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ABSTRACT: Several chemical changes occur during the ripening of dry-fermented sausages that determine the flavor and odor of the end product. The phenomena that take place during fermentation, that is, both acidification of the sugars by lactic acid bacteria and reduction of nitrates and nitrites to nitric oxide by micrococci have been known for several years. However, the chemical changes involved in this process, and, particularly, the agents responsible have not yet been established, although they have been attributed to changes in the majority components (proteins and lipids) and to the ingredients added (spices and condiments) in the preparation of the original mixture.

The typical flavor and odor of dry-fermented sausages cannot be attributed to volatile substances alone, but to a large number of volatile and nonvolatile compounds present in the product in suitable proportions. Microbial growth in the sausage together with activity of the meat endogenous enzymes are undoubtedly partially responsible for the development of a number of aromatic and sapid compounds. However, lipid autooxidation reactions are also an important source of these substances, and it is not yet known which of these processes is more important in sausage ripening. Much research has focused on the break up of triglycerides into free fatty acids, diglycerides, and monoglycerides during ripening and the progressive increase in the amounts of different carbonyl oxidation products. Carbonyl compounds probably play a significant role in determining the flavor because, in general, these have very low perception thresholds, in the ppm and ppb range. Similarly, the protein breakdown to yield peptides and amino acids has been studied extensively, the latter being substrates of several microbial and chemical reactions that generate many flavor compounds.

KEY WORDS: dry-fermented sausages, ripening, proteolysis, lipolysis.

I. INTRODUCTION

Dry-fermented sausages can be defined as meat products that are manufactured by selecting, chopping, and mincing meat and fat, with or without offal, adding condiments, spices and certain authorized additives, and ripened, cured, and in some cases smoked. Dry-fermented sausages are classified according to the following criteria: composition, caliber, degree of mincing of the ingredients, spices and condiments added, smoked or not, duration of the ripening period, etc.

One could say that throughout the world there are almost as many kinds of dry-fermented sausages as there are geographical regions or even producers. Although their manufacture always involves a combination of fermentation and dehydration processes, there are clear regional differences. In Mediterra-

nean countries, Portugal, Hungary, and the Balkans most sausages are made with spices and are dried in air, whereas in central and northern Europe fermented sausages are smoked, which involves a less intense curing process. In the U.S. semicured sausages are common. These are rapidly fermented at high temperatures followed by a short drying period.[1]

Nowadays, dry-fermented sausage manufacture is a very important part of the meat industry of continental Europe with the strongest influence in Germany and Mediterranean countries. In the European Union (EU), fermented sausage production amounted to 689,000 tonnes in 1988. In Spain, according to figures published by the Ministry of Agriculture and Fisheries, dry-fermented sausage production exceeded 166,000 tonnes in 1992 representing approximately one-fifth of the national production of meat products and corresponding to a value of 625 million Euros. These figures illustrate the importance of these food products in both EU and our country.

II. DRY-FERMENTED SAUSAGE MANUFACTURE

The manufacture of dry-fermented sausages is usually considered to entail three main steps: formulation, fermentation, and ripening/drying. In the formulation stage, the ingredients are prepared to be stuffed into casings. During fermentation two basic microbiological reactions proceed simultaneously and influence each other: the formation of nitric oxide by nitrate and nitrite-reducing bacteria and the reduction of pH via glycolisis and lactic acid bacteria (LAB). These two first steps are well known, and several reviews have been published in which the mechanisms and interactions of the phenomena taking place are thoroughly discussed.[1–3] In the final ripening/drying phase, the flavor, odor, and texture of the product are developed. During this phase many biochemical and chemical reactions occur that are not yet well understood, although several new advances have been made since the above-mentioned publications. Although dry-cured ham is not dealt with in this article, some references are made on this product because many changes are parallel in both dry ham and fermented sausages. Furthermore, some excellent reviews on dry ham have been published recently.[4,5] Therefore, in the present review the formulation and fermentation processes of dry-fermented sausages are described briefly and more attention is paid to the ripening phase.

A. Formulation

In this stage, the meat and the pork fat are minced, at cool temperatures, to a specific size. Then, other ingredients such as condiments, curing salts, and starter culture are added and the mixture is kneaded, usually in a vacuum machine. The mixture is then refrigerated for about 24 h to facilitate interaction between its components. After this period, the sausage casings are filled with the mixture in machines under vacuum and the sausages enter the fermentation stage.

Table 1 shows the typical ingredients of a sausage mixture for "salchichón" (a salami-like sausage).[6] This name describes sausages added of black pepper (whole or minced) with a diameter of 40 to 60 mm.[7] Other kinds include "fuet", which differ from the latter in that they have a diameter of approximately 25 mm, and "chorizo" in which different condiments and spices are added to the mixture (garlic and paprika or red hot pepper).[7]

During the mincing process, the muscle fibers are broken, to a greater or lesser extent, and the myofibrillar proteins, which comprise almost 80% of the cell contents, are exposed to the action of the salt. The salt facilitates electrostatic processes involved in the formation of the film of protein that surrounds the fatty particles and favors protein-fat and protein-protein interactions. The ions interact with

TABLE 1
Formula of a Typical Fermented Sausage Mix

Ingredients	Weight percentage
Pork meat	56
Beef meat	12
Pork fat	25
Salt	2.5
Dextrine	1.8
Lactose	1.0
Dextrose	0.8
Sodium glutamate	0.25
Nitrates	0.0085
Nitrites	0.0065
Sodium ascorbate	0.046
Black pepper	0.14

From Ref. 6.

the proteins and reduce the electrostatic attraction between oppositely charged ions of nearby groups, promoting unfolding and dissociation. Gradually, the conditions are established for the formation of a three-dimensional lattice structure necessary to generate the texture of these products.

B. Fermentation

Once the casings have been filled, the sausages are kept in ripening cabinets under conditions of controlled temperature, relative humidity, and air flow. Here, the sausages are stored for 1 to 2 days in a controlled temperature of 18 to 26°C and a relative humidity of 90%.

During fermentation, several critical microbiological changes take place. Before fermentation, the microbial load in the sausages ranges from 10^5 to 10^6 cfu/g. The initial microbial population is always very varied and is similar to that found in the fresh meat, that is, it comprises lactobacilli, micrococci, enterbacteria, *Pseudomonas* spp, *Achromobacter* spp., *Flavobacterium* spp, *Bacillus* spp., etc. and also molds and yeasts. However, the conditions that inhibit the bacteria responsible for the spoilage of fresh meat, especially the Gram-negative bacteria (predominantly consisting of pseudomonas) have already been established.[8,9] The a_w is reduced from 0.99 to 0.96 by adding curing salts and other solutes (e.g., sugars), and specific inhibitory effects of nitrates and low oxygen tension also come into play. Therefore, in naturally produced dry-fermented sausages (without starters), the typical microbiota (LAB and *Micrococcaceae*) of these products is soon established and boosts the growth of starters when these are added.

The most commonly LAB isolated from conventional dry-fermented sausages or those used in starter cultures are *Lactobacillus sake*, *Lb. curvatus* and *Lb. plantarum*, *Pediococcus pentosaceus*, and *P. acidilacti*.[10] The nitrate and nitrite-reducing bacteria present in the dry-fermented sausages belong to the *Staphylococcus* and *Micrococcus* genera (*St. carnosus*, *St. xylosus*, *M. varians*, etc.).[11] These related microorganisms or species play an important role in dry-fermented sausage fermentation. Figure 1 shows the changes in microbial load during the periods of fermentation and ripening.

The LAB present in the dry-fermented sausages are mainly homofermentative. A typical homofermentative LAB produces approximately 1.8 mol of lactic acid per mole of metabolized hexose and approximately 10% of byproducts.[12] This produces a decline in pH (from an initial value of 5.8 to 6.2 to values close to 5.0 or below), which has a number of beneficial effects on the manufacturing process and the quality and shelf-life. These are as follows:

- It facilitates the conservation process by selecting the characteristic microbial flora that inhibits undesirable microbial growth.
- It helps to develop the texture Because it reduces the water-retention capacity of the meat proteins and thus favors the drying

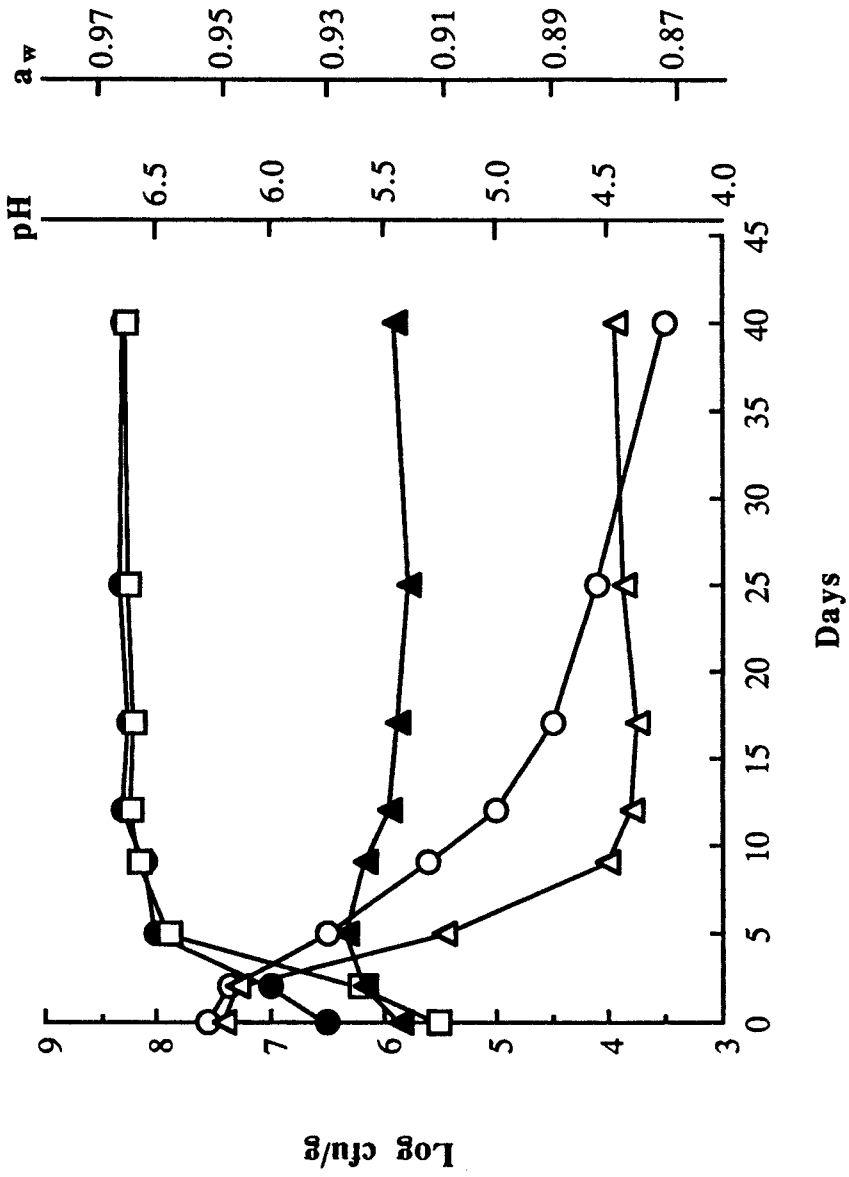

FIGURE 1. Changes observed during ripening of dry-fermented sausages: total viable (●), lactobacilli (□), and *Micrococcaceae* (▲) counts, p (△), and water activity (○).

process. It also accelerates the gelation mechanism of the myofibrillar proteins.
- It controls the enzymic reactions that contribute to the flavor and odor.
- It favors the reduction reactions necessary for color formation.

In the U.S. where most fermented sausages are fermented at very high temperatures (30 to 45°C) for short periods (approximately 24 h), pediococci, with optimum temperatures above 30°C, are preferentially used as starter culture instead of lactobacilli.[13] The rapid acidification that these bacteria bring about is essential to inhibit unwanted bacterial growth that can develop as a consequence of the high temperatures. However, in Europe much lower fermentation temperatures are used (20 to 26°C), and lactobacilli are preferred in order to obtain a much slower reduction in pH that does not inhibit the *Micrococcaceae*, which actively contribute to color formation during the ripening process.

Throughout fermentation, the NaCl, temperature (20 to 26°C), and the decline in pH all provoke insolubilization of the sarcoplasmic and myofibrillar proteins.[14] Insolubilization progresses during ripening due to progressive dehydration. Although the pH no longer declines and, instead, near the end of this process begins to rise and the temperature is lower during the ripening phase (11 to 15°C), dehydration is prolonged due to the low relative environmental humidity producing a reduced protein-water interaction that prevents solubilization. This brings about a rise in NaCl concentration that, because of its high ionic strength, contributes to the denaturization of the meat proteins, especially the myofibrillar ones, producing important structural changes. The proteins dissolved as a result of the mincing and the addition of salt produce filament-shaped aggregates that interact and contribute to the stability of the gel.

Micrococcaceae are responsible for reducing the nitrates and nitrites to nitric oxide during the fermentation phase. The number of these bacteria rises markedly in this phase (Figure 1), sometimes even before the rise in lactobacilli,[15] and this level is maintained and then decreases during ripening.[16,17] These microorganisms only constitute the majority bacteria in dry-fermented sausages prepared with large amounts of nitrates and low levels of carbohydrates,[18,19] due to their sensitivity to decreases in pH.[1,20] For this reason, fermentation is a critical process in conventional dry-fermented sausage manufacturing and must occur slowly in order to permit *Micrococcaceae* growth. If the *Micrococcaceae* do not grow, reduction of nitrates and nitrites is impeded. The reactions in which the *Micrococcaceae* are involved are recorded in Figure 2.

Nitrite has a number of functions in the curing of meat: it is involved in development of the color[21,22] and odor,[23,24] it has a conserving effect,[25,26] and also acts as an antioxidant.[27–29]

The characteristic color of dry-fermented sausages is produced by the interaction between the meat pigments and the products resulting from reduction of the nitrates and nitrites added. Nitrate in itself does not produce the cured color,[30] but it must first be reduced to nitrite to achieve this effect. This reaction only takes place in the presence of the enzyme nitrate reductase that is produced by the *Micrococcaceae*. The nitrite formed is reduced again to nitric oxide, which finally reacts with the myoglobin of the meat. In addition to microorganisms, a number of reducing agents are involved in the reduction of nitrite to nitric oxide: additives such as ascorbic acid, the cysteine/cystine redox system, and possibly other enzymic redox systems such as that involving cytochrome-c and NADH.[31]

The effect of nitrite on taste and odor formation was first studied by Brooks et al.,[32] who found a difference in the taste associated with use of this compound. Several authors[33–35] in sensory tests found that the sensory quality of products manufactured with the addition of nitrites was better than equiva-

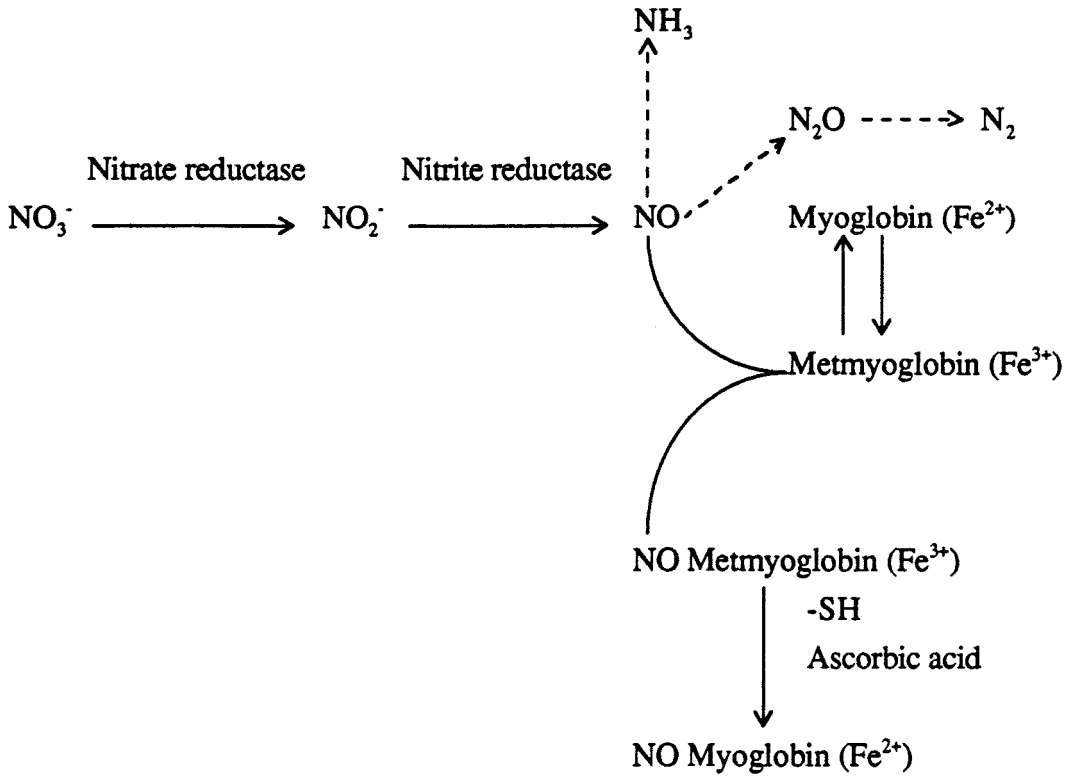

FIGURE 2. Diagram showing the changes of the heme pigments color during the fermentation of sausages. (Adapted from Ref. 20.)

lent products to which they had not been added. Therefore, it appears that primarily the reaction of compounds present in the meat with nitrite or nitric oxide is responsible for the cured taste and odor.[36] However, these reactions are not yet completely understood,[37] and specific compounds responsible for the characteristic taste and odor of cured meat have not yet been identified.[36] Nevertheless, several authors[36,39] compared the volatile fraction of cured and noncured cooked meat and found a lower concentration of carbonyl compounds in the former, probably because of lipid oxidation having ceased.

Nitrite also has an important conserving effect on meat products. Whereas even at high doses nitrate does not seem to affect the microorganisms growth, at relatively low doses nitrite inhibits the growth of some species of bacteria, especially of *Clostridium botulinum*.[30] This protective effect of the nitrite is dependent on the conditions present in the sausage. Nitrite alone has no effect, but when accompanied by low pH and salt it inhibits bacterial growth.[40,41]

Nitrite has also been shown to be an effective antioxidant and has been found to retard oxidation in cooked ground beef when used at the 50 mg/kg level and completely eliminate oxidation at the 2000 mg/kg level.[28] Similar results have been observed with nitrite (156 mg/kg) inhibiting warmed-over flavor in cooked meat.[29] It is also observed that nitrite has higher antioxidant power than antioxidants commonly used as a preservative.[42] This effect protects the fat from becoming rancid and therefore contribute to the taste and odor of the product. Several studies have attempted to explain the antioxidant mechanism of nitrite. Westerberg[43] found that when

nitrite reacts with iron, it maintains the heme group in the Fe^{2+} form, reducing the amount of iron present as Fe^{3+}, which is a catalyst of oxidation. Other studies have shown the situation to be more complex than this and another series of mechanisms have been proposed. Pearson et al.[44] suggest that nitrite could stabilize the membrane lipids and inhibit the action of prooxidant compounds in the muscle tissue.

Finally, during the fermentation phase dehydration processes of the sausage begin due to the conditions present in the ripening cabinet. This dehydration process contributes to forming the characteristic texture of the dry-fermented sausage and is accompanied by a reduction in a_w (Figure 1), another of the factors that strongly influences the stability of the final product.

III. RIPENING PHASE

A. Lipid Breakdown

Usually, lipids are the major component of dry-fermented sausages. The fat content of these products ranges from 25 to 55%, and their sensory characteristics are closely related with lipid breakdown and transformation during ripening.[45,46] The fats undergo two principal kinds of changes: hydrolysis and oxidation.

Hydrolysis of fats, or lipolysis, occurs as a consequence of the action of glycerol ester hydrolases (EC 3.1.1.3.) that break the ester bonds of the tri-, di-, and monoglycerides producing diglycerides, monoglycerides, and free fatty acids. These enzymes belong to the esterases that act only at the water-lipid interphase.[47,48] These lipases are derived from two different sources, either from muscle or adipose tissue[49] or are generated by microorganisms.[50]

Pork fat, commonly used to manufacture dry-fermented sausages, is mainly composed of triglycerides with the following average composition of fatty acids: myristic 1.5%, palmitic 25%, stearic 14%, palmitoleic 3%, oleic 43%, linoleic 11%, and linolenic 1%.[51] These fatty acids are arranged in a specific manner in the triglyceride molecule. Most of the stearic acid, about 60%, is in the sn-1 position, between 60 and 80% of the palmitic acid is in the sn-2 position, and approximately 50 to 60% of the octadecenoic acids are in the sn-3 position.[52] The fat in pork muscle tissue is mainly composed of triglycerides (62 to 80%) and has a smaller proportion of phospholipids (16 to 34%). It contains about of 40% of saturated fatty acids, 50% of monounsaturated, and 10% polyunsaturated fatty acids.

Lipases, especially bacterial lipases, can be classified into two groups according to their positional specificity. The first group of lipases are nonspecific and thus release fatty acids from any of the three positions of the triglyceride molecule. Therefore, these can induce the complete break-up of the triglycerides into free fatty acids and glycerol. The second group of lipases preferentially hydrolyze the fatty acids in positions sn-1 and sn-3 of the triglycerides, yielding free fatty acids, diglycerides, and monoglycerides. Especially the 2-monoglycerides and sometimes the 1,2 or 2,3-diglycerides are unstable, and in these the acyl group migrates to form 1-monoglycerides and 1,3-diglycerides.[48] As a consequence, prolonged interaction of the substrate with the enzyme can induce complete rupture of the molecule into free fatty acids and glycerol.[53]

The research by Alford et al.[54] shows that the lipases present in sausages preferentially hydrolyze the external positions of the triglyceride molecule. The accumulation of diglycerides and the kind of fatty acid released indicate that only one of the outer positions is attacked. Demeyer et al.[46] found that linoleic acid was the fatty acid most released followed by oleic, stearic, and palmitic acids, respectively. Therefore, they confirmed the specificity of the lipases for position sn-3 of

the triglycerides. Moreover, they suggested that the differences observed for linoleic and oleic acids could be related with enzyme specificity for the fatty acid structure. Previously, Alford et al.[54] had proposed both kinds of specificity, positional and structural, in microbial lipases. However, research into the possible existence of specificity for fatty acids should take heed of the substrates and conditions used to ensure that reactions are due to changes in the structure of the acyl groups and not to external factors.

1. Microbial Lipolysis

Classically, *Micrococcaeae* are considered to be the main microorganisms reponsible for lipid breakdown in dry-fermented sausages.[46,50,55–57]

Strains of micrococci with very varied lipolytic activity have been isolated from dry-fermented sausages. Some strains are strongly lipolytic, whereas others show very little lipolytic activity.[58] Selgas et al.[59] studied the extracellular and intracellular lipolytic activity *in vitro* of six strains of micrococci isolated from Spanish dry-fermented sausages and only three were lipolytic. In general, there was more extracellular than intracellular lipolytic activity. This is important because the micrococci load progressively decreases after 15 to 20 days of ripening.[17,60] Therefore, the extracellular enzymes remain in the medium and can catalyze hydrolysis of the long chain triglycerides, including those with 16 and 18 carbon atoms.[56]

Other studies[57] carried out on fat-enriched media showed that *Micrococcus varians*, a species that is available commercially as a starter culture,[61] degraded triolein and pork fat, although after a long incubation period (30 days) it only produced lipases when agitated. In consequence, the authors concluded that this microorganism did not actively participate in lipolysis during sausage ripening.

With respect to the genus *Staphylococcus*, Delarras[62] in his work on *Micrococcaceae* isolated from meat products, found these bacteria to have a better lipolytic activity than the genus *Micrococcus*. Talon et al.[57] also studied the lipolytic activity *in vitro* of several species, including *S. saprophyticus*, *S. warneri*, and *S. carnosus*, commonly used as starter cultures in French sausages. They observed a high lipolytic activity in the first two, the highest by *S. warneri*, hydrolyzing tributyrin, triolein, and pork fat, indicating that these could be involved in the release of fatty acids during ripening. This lipolytic activity was confirmed by Montel et al.,[63] who produced sausages inoculated with these two microbial species. In contrast, *S. carnosus* showed very little lipolytic activity.[57,64] Another species, *S. xylosus*, had a lipolytic action on tributyrine but very little activity on triolein and pork fat.[57,65,66]

Lipolytic activity has also been detected in LAB.[67–69] However, Nordal and Slinde[70] did not observe any significant lipolytic behavior when LAB were used as starter cultures in the manufacture of salami- type sausages. The lipolytic capacity of LAB was initially studied in lactobacilli isolated from milk products.[71–73] In these studies, they were found to hydrolyze triglycerides with short chain fatty acids, especially tributyrin, but not to attack triglycerides with long chain fatty acids. These results are in accordance with those reported by Sanz et al. [74] for lactobacilli isolated from Spanish dry-fermented sausages. Moreover, according to these authors the extracellular lipases attack mono-, di-, and triglycerides with short chain fatty acids, whereas the intracellular ones show little activity against triglycerides. They did not observe any either extracellular or intracellular lipolytic activity against fatty acids with more than six carbon atoms. Therefore, there was a maximum lipolytic activity against tributyrin, and this activity decreased with increasing the length of the fatty acid chain.[75] However, despite the fact that Sanz et al.[74]

detected extracellular lipolytic activity, Papon and Talon[67] demonstrated in different strains of lactobacilli that lipolytic capacity is associated to the cell and has not been detected significantly in supernatant fluids. In a later study that Papon and Talon[76] carried out on *Lactobacillus curvatus*, the lipases were located in the fraction obtained after cell rupture by ultrasound followed by centrifugation at 30000 g (the soluble cell fraction) and not in the membrane or the cell wall.

Many molds and yeasts isolated from dry-fermented sausages are lipolytic,[48,77] and therefore can attack the fatty tissue and contribute to the development of taste and odor. Some authors[78,79] have demonstrated that dry-fermented sausages inoculated with yeasts have a greater lipolytic activity that is manifest by a stronger odor.

In summary, a review of the research into lipolytic activity reveals that this has been found in both *Micrococcaceae* and LAB, but to a greater extent in the former. Nevertheless, most studies were made *in vitro* experiments, and the true activity of these microorganisms in dry-fermented sausages is unknown.

2. Endogenous Lipolysis

As mentioned previously, the lipolytic processes that take place in dry-fermented sausages have been mainly attributed to the presence of microbial lipases.[2,45,46,66,80] Nevertheless, in the first stages of ripening, fats can be degraded by tissular lipases.[49,81] Recent research[82] in dry-fermented sausages manufactured aseptically reported a similar increase in the level of fatty acids in batches inoculated with lactobacilli and/or micrococci as in those manufactured aseptically, suggesting that the release of fatty acids could be due partially to the meat endogenous lipases. In an attempt to determine the effect of different starter cultures on the biochemical processes taking place in dry-fermented sausages, Montel et al.[63] aseptically prepared several batches of sausages, some of which were inoculated and others not. When they studied the lipolysis they found that the noninoculated batch showed only slightly lower levels than the inoculated batches over the ripening period, and therefore, like García et al.,[82] these authors also attributed lipolysis to the endogenous lipases. In a later study, Hierro et al.[83] found a greater increase in the level of fatty acids in dry-fermented sausages produced aseptically and inoculated with only one lipolytic strain of *Staphylococcus* and without LAB. However, aseptic batches and those inoculated with both LAB and lipolytic *Micrococcaceae* presented very similar final fatty acid values, and the latter had only slightly higher levels than the batch produced aseptically. When these authors compared this slight increase they estimated that around 70% of lipolytic activity was due to endogenous lipases. This study also estimated the amount of each fatty acid released. The results indicated that unsaturated fatty acids were released in the largest quantities linoleic > oleic ≈ palmitoleic, followed by the saturated fatty acids myristic > palmitic ≈ stearic. In general, the inoculated batches released fatty acids at a similar rate, but the aseptic batch was different with a clearly higher release of C-18:2 and C-18:1. This fact indicates that both microbial and meat endogenous enzymes preferentially hydrolyze the outer fatty acid of the triglyceride molecule as previously described[48,54] or have a preference for the polar lipid fraction (mainly phospholipids), because it has higher levels of polyunsaturated fatty acids than the neutral one.[84] Positions sn1 and sn3 are the ones most frequently occupied by unsaturated fatty acids; nearly 30% of the dominant octadecenoic acids (oleic and linoleic) are esterified in the sn1 position and about 50 to 60% of the same fatty acids appear in sn3.[85] The higher C-18:2 and C-18:1 release rate in the aseptic batch probably indicates that meat enzymes have a greater speci-

ficity for the sn3 position or polar lipids than microbial enzymes. Similar results to those recorded by Hierro et al.[83] have been reported previously by Molly et al.[86] These authors showed that polyunsaturated fatty acids are released from the polar lipid fraction, and their specific release is higher than for monounsaturated and saturated fatty acids, although it seems that the pattern of lipolysis is similar when endogenous and microbial effects are compared.

In other meat products (dry hams), lipolytic phenomena have been attributed to an endogenous enzymic system,[4,87–90] because there are only moderate levels of microorganisms, not exceeding 10^4 to 10^5 cfu/g inside the ham.[91,92] During the processing of dry hams, lipolytic activity has been recorded in the muscle and adipose tissues.[93–95] The latter authors studied the effect of curing salts and the a_w on the activity of muscle and adipose lipases from samples of pig *Biceps femoris*. The acid lipase was strongly activated as the NaCl concentration increased and the a_w decreased and could have been actively involved in muscular lipolysis over the entire curing process. However, basic and neutral lipases were strongly inactivated with reduced a_w values and the latter was greatly inhibited by an increase in NaCl concentration. In experiments on hams the activity of these two enzymes increased during the first 2 months of the curing process and then sharply dropped when the drying process began, remaining active until the end of the process. On the other hand, acid lipase remained active during the entire processing procedure.[4,96] As the pH of dry-fermented sausages ranges from 4.8 to 6.0 throughout the ripening period, the acid lipase could develop important lipolytic activity in these products.

Similarly, in studies on dry hams lipases of subcutaneous adipose tissue were less stable than muscular lipases.[96] Neutral lipase would be the enzyme mainly responsible for lipolysis in adipose tissue, as changes in the NaCl concentration between 1 and 10 g/l do not affect this enzyme and its activity increases slightly when the a_w drops to between 0.98 and 0.62.[94] Probably, this enzyme does not make a very important contribution to lipolysis in dry-fermented sausages because it has an optimum pH around neutral and, as mentioned previously, dry-fermented sausages have an acid pH. Moreover, the NaCl concentration in these products of 22 to 25 g/l[97] also might adversely affects the activity of neutral lipase.

There is still some uncertainty about the possible role of muscular and adipose esterases in the ripening processes of dry-fermented sausages and dry hams. Esterases are more sensitive to changes in NaCl concentration and a_w than lipases.[94] However, in experiments on hams esterases of both muscle and adipose tissue showed considerable activity over the entire process.[96] Nevertheless, they do not play an important role in lipolytic processes because of the absence of suitable substrate[89,90] among other factors.

B. Oxidative Phenomena

During lipid oxidation many volatile and nonvolatile substances are formed that change the taste and odor of the food products. In many products these modifications are not desirable because they produce a rancid flavor. Nevertheless, a certain degree of oxidation can make an important contribution to the characteristic taste and odor of some products such as dry-fermented sausages and dry hams. The substrates of these reactions are mainly unsaturated fatty acids. In the free form these are generally oxidated more rapidly than when they form part of triglycerides or phospholipids.

Lipid peroxides, also called hydroperoxides, formed during the propagation phase are the primary products of oxidation. These compounds have no odor or taste and therefore do not participate in the flavor or odor of the food products. However, being unstable they

are rapidly broken down into byproducts that are responsible for the organoleptic changes induced by phenomena of autoxidation.[98,99] Each unsaturated fatty acid produces specific hydroperoxides that decompose to also form specific aldehydes (Table 2).

The final products of lipid oxidation (aldehydes, alcohols, ketones, furanes, etc.) are highly volatile and have a low olfactory threshold and therefore acquire an important role in the development of the flavor and odor of the food products in which they are present. Their spectra and the amounts in which they are present determine whether they generate desirable or undesirable flavors and, in consequence, the degree of acceptance of the product by the consumer.

The intensity of the autoxidative reactions that take place in the meat depend on a number of factors, particularly on the level of polyunsaturated fatty acids present in a muscular system.[100] The phospholipid content in the meat is relatively small compared with the triglyceride fraction, although the former are especially important because of their susceptibility to oxidation. This is due to their high unsaturated fatty acid content (especially linoleic and arichidonic acids) and to their increased contact with tissular oxidation catalysts such as trace metals or metaloporphyrins.[101,102] Phospholipids are the main constituent of cellular and intracellular membranes and therefore present a large area that is susceptible to attack. However, because triglycerides are located inside the adipocytes only a very reduced area is exposed to oxidation.

In fact, lipid oxidation in dry-fermented sausages is influenced by a wide range of factors: composition of the mixture, the degree of mincing of the meat, the pH, the addition of ingredients such as NaCl, nitrites, spices, antioxidants, etc. With respect to the compo-

TABLE 2
Hydroperoxides and Aldehydes (with Single Oxygen Function) That May Be Formed in Autoxidation of Some Unsaturated Fatty Acids[a]

Fatty acid	Methylene group involved	Isomeric hydroperoxides formed from the structures contributing to the intermediate free radical resonance hybrid	Aldehydes formed by decomposition of the hydroperoxides
Oleic	11	11-Hydroperoxy-9-ene	Octanal
		9-Hydroperoxy-10-ene	2-Decenal
	8	8-Hydroperoxy-9-ene	2-Undecenal
		10-Hydroperoxy-8-ene	Nonanal
Linoleic	11	13-Hydroperoxy-9,11-diene	Hexanal
		11-Hydroperoxy-9,12-diene	2-Octenal
		9-Hydroperoxy-10,12-diene	2,4-Decadienal
Linolenic	14	16-Hydroperoxy-9,12,14-triene	Propanal
		14-Hydroperoxy-9,12,15-triene	2-Pentenal
		12-Hydroperoxy-9,13,15-triene	2,4-Heptadienal
	11	13-Hydroperoxy-9,11,15-triene	3-Hexenal
		11-Hydroperoxy-9,12,15-triene	2,5-Octadienal
		9-Hydroperoxy-10,12,15-triene	2,4,7-Decatrienal

[a] Only the most active methylene groups in each acid are considered.

sition of the mixture, apart from a high triglyceride content, which is mainly derived from fat, pork contains a significantly high proportion of phospholipids, corresponding to between 16 and 34% of the fat of the muscle tissue[51] compared with only about 4% in beef.[103] As previously mentioned, phospholipids are highly susceptible to oxidation. Moreover, the high unsaturated fatty acid content in beef (approximately 55%) and pork (approximately 60%) also favors oxidation.

When the cells become damaged as a consequence of chopping and mincing the meat, the phospholipids and triglycerides are exposed to oxygen, enzymes, heme pigments, and metallic ions, which facilitates their rapid oxidation.[44,104]

With respect to the pH, the rate of oxidation increases with pH values lower than 7.0.[105,106] In fresh meat lipid oxidation is inhibited by enzymic reductor systems in the mitochondria.[107] However, because of the low pH present in the dry-fermented sausage of approximately 4.8 to 5.0 these enzymic systems show little activity.[108,109] On the other hand, lipid oxidation catalyzed by heme and non-heme iron is also affected by the pH. Non-heme iron has a greater catalytic activity at pH 5.5, whereas oxidation of heme is favored by alkaline pH.[110]

The increased NaCl concentration during ripening as a consequence of dehydration also contributes to lipid oxidation. The prooxidant effect of NaCl is partially attributed to its ability to displace the iron ions from the macromolecules so that these can participate in the oxidation reactions. This hypothesis is supported by the finding that this action is inhibited by EDTA.[111] The use of KCl reduces oxidation phenomena.[112,113] However, because of its bitter taste it should only be added in small amounts.

The addition of nitrite also influences oxidation reactions, because, as mentioned in Section II.B, this has antioxidant effects. Other ingredients with antioxidant properties include certain spices, tocopherols, and ascorbates. In the case of smoked sausages, it is noteworthy that smoke contains antioxidant substances such as, for example, certain phenolic derivatives.

C. Proteolytic Phenomena

1. Microbial Proteolysis

A large proportion of the proteins are hydrolyzed during ripening. Initially, protein hydrolysis is mainly attributed to microbial proteases,[114] especially to those produced by the LAB.[68,115,116] However, it is not known to what extent LAB and *Micrococcaceae* participate in proteolytic changes during the ripening of dry-fermented sausages and dry hams.[75]

Bacteria cannot directly use the proteins present in the medium. First, the proteins must be hydrolyzed to peptides and amino acids that, being smaller, can be transported into the cell. Once inside the cytoplasm, the peptides are broken down to form free amino acids by the action of intracellular peptidases.[117] A number of studies in the last few years have focused on the proteolytic system of the microorganisms used in the manufacture of milk products,[118–120] although little is known about the microbial proteolytic activity in meat products.

Reuter[121,122] isolated lactobacilli from dry-fermented sausages. These had an ability to hydrolyze peptides in addition to a mild proteinase activity that produced a considerable increase in the free amino acids in culture media and meat suspensions. Cantoni et al.[123] found that lactobacilli made an important contribution to proteolysis because of their decarboxylase activity. Martin[116] attributed a greater proteolytic activity to lactobacilli than to micrococci and suggested that these were mainly responsible for the presence of amino acids. Similarly, Montel et al.[124] *in vitro* experiments showed that species from the genera *Lactobacillus* and *Pediococcus* produced a rise in the free amino acids as a result of intracellular peptidase ac-

tivity. In sausages to which *L. plantarum* and *L. casei* strains, subsp. *tolerans*, were added during manufacture, and which had been previously isolated from this kind of product, a maximum proteolytic activity was observed at 40°C. This enzymic activity is affected by the salt concentration. Thus, at 30°C, the temperature at which fermentation takes place in many dry-fermented sausages, proteolytic activity is reduced by 80% when the salt concentration rises from 3 to 5%. This phenomenon is even more pronounced at NaCl concentrations of 7.5%.[125]

Despite the probable existence of proteinases bound to the cell wall of the lactobacilli, these microorganisms and LAB in general are considered to be weakly proteolytic compared with other groups of bacteria (*Bacillus, Proteus, Pseudomonas*, coliforms).[75] According to Law and Kolstad,[126] there is no direct evidence that these microorganisms play a significant role in generating the flavor compounds of fermented meat products. It is not certain whether its enzymes are involved in meat proteolysis. Several authors[70,127] have shown that different LAB used as starter cultures lack any kind of proteolytic activity. Bermell et al.[128] reached similar conclusions working with *L. curvatus* and *P. pentosaceus* isolated from dry hams.

Research into the genus *Micrococcus* has mainly focused on micrococci species isolated from cheeses. In some strains extracellular proteinases with an optimum activity at alkaline pH and loss of activity at an acid pH[129,130] and intracellular proteinases, endopeptidases, aminopeptidases, and dipeptidases[131] have been found. With respect to the *Micrococcaceae* present in dry-fermented sausages, it has not yet been established to what extent these are involved in proteolysis. Several authors[123,132–134] claim that micrococci play an active role in proteolytic phenomena producing an increase in free amino acids. In *in vitro* studies on strains of micrococci isolated from Spanish dry-fermented sausages, Selgas et al.[59] showed the existence of intra- and extracellular proteolytic activity, and in general more of the latter than the former. However, Montel et al.[124] in studies on species of the genus *Staphylococcus* found these bacteria to have little aminopeptidase activity and no proteinase capacity. Also, Hammes et al.[64] isolated strains of *S. carnosus* with a very low proteolytic activity, and Bermell et al.[128] did not detect any proteolytic activity in *S. xylosus* isolated from dry hams.

Many species of molds (*Penicillium*, *Aspergillus*) have been found to have proteolytic activity, and some proteinases were also identified (an acid proteinase and a neutral metaloprotease) in strains of *Penicillium* isolated from cheese. Also, even some of the broken peptidic bonds of the caseines have been determined.[135–137] The few studies carried out on the proteolytic activity of molds isolated from dry-fermented sausages have all shown this activity to exist,[77,138] although, with the exception of the work by Grazia et al.,[139] they do not quantify it. The latter authors determined levels of soluble nitrogen and ammonia in salami and concluded that molds play a very limited role in the proteolysis of dry-fermented sausages. In a more recent study, Trigueros et al.[140] selected different strains of mold from the surface of dry-fermented sausages belonging to the genera *Penicillium* and *Mucor* and studied their proteolytic activity on myofibrillar and sarcoplasmic proteins *in vitro*. These authors concluded that some strains have an important proteolytic activity and therefore can be potentially selected for inoculation of sausages. In a later study, a number of these strains were studied in dry-fermented sausages,[141] and significant differences were found between inoculated and control batches.

2. Endogenous Proteolysis

During dry-fermented sausage ripening proteins undergo a series of changes as a con-

sequence of the action of different proteolytic enzymes. As previously mentioned, some of these enzymes are of microbial origin, whereas others are derived from the tissues.

Toldrá et al.[97] studied the activity of cathepsins B, D, H, and L in model systems under the conditions present in dry-fermented sausages and dry hams. In the former, cathepsin H and D activities were strongly inhibited by pH and salt concentration, and all of these were inhibited by low a_w. Nitrate and ascorbic acid had little influence on these activities. These authors simulated the first steps of the manufacturing process of dry-fermented sausages. They concluded from their results that cathepsins B, L, and D are mainly active in the first stages of the manufacturing process (kneading and fermentation), whereas only cathepsin L was significantly active during the drying stage. In contrast, cathepsin H was strongly inhibited by acidity.

Verplaetse et al.[142] also studied proteolytic activity in dry-fermented sausages to which microbial growth inhibitors (antibiotics) or protease inhibitors (pepstatin and leupeptin) were added during the manufacturing process. They found that the latter inhibited the breakdown of actin and myosin to a greater extent than when microbial growth was inhibited by addition of antibiotics. This suggests that endogenous proteases are more important than bacterial ones in proteolytic phenomena. Cathepsin D type muscle proteinases (endopeptidases) are mainly active during the fermentation phase, whereas bacterial and muscular exopeptidases protagonize the drying stage.[143] In a study on proteolysis in dry-fermented sausage ripening, Garriga et al.[144] did not find any correlation between non-protein soluble nitrogen and the microbial flora and proposed that tissular cathepsins were involved, especially in the first stages of the process.

In a recent study in dry-fermented sausages manufactured aseptically, Hierro[145] observed a similar increase in the level of free amino acids in batches inoculated with lactobacilli and/or proteolytic micrococci and in those manufactured aseptically, indicating that the release of amino acids was due exclusively to the action of endogenous proteases. Similarly, individual analysis of the free amino acids also pointed to the muscular proteases as being responsible for proteolysis. As the control batch was not inoculated with any microorganism and the other batches were inoculated with different starter cultures, one would expect each amino acid to increase to a different extent. However, the profiles obtained were relatively similar. In other studies[6,146,147] on dry-fermented sausages inoculated with starter culture in the conventional manufacturing process, microorganisms were found to play only a limited role in the proteolysis of sausages.

Experiments carried out by Toldrá et al.,[97] who simulated the different stages of dry ham processing *in vitro*, showed cathepsin H to be less active at the beginning of the process (salting phase), followed by a much greater activity in later stages. Cathepsins B and L maintained a high activity during the entire maufacturing process, compared with cathepsin D, the activity of which was barely detectable. In more recent research on dry hams, Toldrá et al.[148] observed cathepsin B, H, and L activity that was even present, although only at lower levels, at the end of the process (15 months). In contrast, the role of cathepsin D was limited to the first few months, especially the first 5 months. These authors concluded that cathepsins B and L are the most important in the ripening of dry hams.

In research into other proteolytic enzymes, namely, the calpains, Sárraga et al.[149] detected activity of these enzymes in fresh hams and after salting, although significantly less after salting and no calpain activity was observed during the later stages. The early disappearance of this enzymic activity during the curing process could be explained by the fact that these, in particular calpain I, are relatively unstable enzymes.[150,151] As a result of

the combined effect of this with the relatively long curing period of dry hams and the addition of curing salts,[152] calpains are unlikely to play a significant role in these processes.

D. Sources and Reactions Potentially Responsible for the Flavor of Meat Products

The flavor and odor of food products is partially due to the presence of volatile compounds, but also to nonvolatile compounds with sapid and textural properties or that act as potentiators or synergists.[153]

Although raw meat has little aroma and only a slight bloody taste, it is a large store of compounds with sapid and tactile properties and precursors and flavor enhancers.[154,155]

Volatile compounds in meat are mainly derived from carbohydrates, lipids, and proteins. Oxidation and degradation of lipids contribute to the volatile fraction,[156] and Maillard reaction products formed by the interaction of reducing sugars and amino acids or peptides comprise a large group of volatile substances.[157,158] These are present in small amount,s yet produce a wide range of olfactory sensations.[159,160] Around 1000 compounds have been identified in the volatile fraction of beef, pork, chicken, and lamb.[159] Qualitatively, there is no significant difference between the compounds present in the meat from different animal species, although quantitative differences do exist. Thus, mutton contains high levels of sulfur-containing compounds compared with other species due to the high content of sulfurous amino acids it has been compared to beef or pork.[161]

Nonvolatile compounds contribute to taste of the meat products. These mainly consist of inorganic salts, nucleotide metabolites, sugars, organic acids, amino acids, and peptides and are often present in relatively high quantities.[156,162]

Flavor enhancers and synergists also play a role in the development of sensory properties.[163,164] These compounds do not, themselves, have aromatic or sapid properties but instead reinforce the effect of the compounds that do.[165] Some L-amino acids (such as glutamic acid) and certain 5′-nucleotides (such as 5′-ribonucleotides that contain 6-hydroxipurine) have this effect. The most important of these are glutamic and inosinic acids and monosodium glutamate.[165]

There are a number of potential precursors of substances responsible for the flavor and odor of dry-fermented sausages. Lipids are the source of a number of aromatic and sapid compounds (aldehydes, ketones, alcohols, etc.) due to hydrolytic and autooxidative phenomena.[2,46,54] A large proportion of the proteins initially present in sausages are also hydrolyzed during ripening, resulting in an increase in the non-proteic nitrogen, mainly in the form of free amino acids,[166] which can later undergo further transformations to produce other compounds with sapid and aromatic properties. In addition to this, other equally important sources are the spices and other condiments that are added during sausage manufacture. On the one hand, their volatile components directly contribute to the flavor and odor of the final product, and, on the other hand, their autooxidant properties can modulate autooxidative reactions.[167,168]

1. Products of Lipid Oxidation and Their Effects on the Sensory Properties of Dry-Fermented Sausages

Of all the compounds produced by lipid oxidation, carbonyls are the most important in the development of the sapid and aromatic properties of dry-fermented sausages and also the most abundant in the volatile fraction.[36,169] Edwards et al.[7] demonstrated the existence of a correlation between the caliber of the dry-fermented sausages and the presence of carbonyl compounds. Hence, in "fuet"-type sausages (caliber 20 to 40 mm) the levels of aldehydes and ketones were higher than in

those classified as "salchichón" (diameter 40 to 60 mm) probably due to the smaller diameter of the "fuets" permitting greater diffusion of atmospheric oxygen to the inside.

The concentration of aldehydes increased significantly as ripening progressed in dry-fermented sausages[46,169–172] and dry hams.[173] Aldehydes can produce a wide range of flavors and odors. The low perception thresholds of some of these means that they can even be potent aromatic substances when present in trace amounts.[174] The role of saturated aldehydes is to potentiate the odor, whereas 2-enals and 2,4-dienals give the taste and odor sweet, fruity, and fatty properties.[175]

Branched aldehydes (2-methylpropanal, 2-methylbutanal, 3-methylbutanal) are produced by reactions other than lipid autooxidation (see Section III.D.2.a), whereas the short chain ones (formaldehyde, acetaldehyde, propanal) mainly derive from carbohydrate metabolism. The latter do not seem to make a significant contribution to the odor of dry-fermented sausages.[176,177] The saturated and unsaturated high-molecular-weight aldehydes are mainly derived from lipid oxidation reactions by the disintegration of hydroperoxides generated from polyunsaturated fatty acids.[159,178] These compounds appear to have a significant effect on the aroma, and it is likely that given the different fatty acid composition of the lipids in pork and beef the difference in the kind and amount of carbonyls produced via this mechanism is probably the main factor responsible for the different aromatic properties of the dry-fermented sausages produced using pork alone or those that also contain beef.[170]

Several ketones have been identified in the volatile fraction of dry-fermented sausages and dry hams.[169,171] The methyl ketones with an odd number of carbon atoms predominate in food products.[159] Nevertheless, both methyl ketones with an odd number and those with an even number of carbon atoms have been found in dry-fermented sausages and dry hams (from propanone to 2-nonanone) produced by lipid oxidation[169,172] and the β-oxidation of free fatty acids by molds.[179] One of the ketones identified is 3-hydroxi-2-butanone (acetoine) produced by fermentation of sugars by LAB.[180] In cooked meats the presence of this ketone produces a buttery flavor.[181]

Alcohols are mainly generated as reaction products of lipid oxidation.[159] The aldehydes derived from fatty acids and amino acids are reduced to the corresponding alcohols by alcohol dehydrogenases.

$$R—CH_2—OH + NAD^+ \rightleftarrows R—CHO + NADH + H^+$$

Alcohol formation is favored by a greater NADH compared to NAD^+ concentration. Nevertheless, in most cases aldehydes with more than five carbon atoms are only reduced slowly.[182]

In general, primary unbranched alcohols produce grassy or woody aromas and make an overall contribution to the odor.[183] Secondary alcohols such as 1-penten-3-ol give the products a strong grassy odor, whereas 1-octen-3-ol gives them a mushroomy odor. Secondary alcohols can be formed by certain organisms via β-oxidation of free fatty acids and hydrolysis to the corresponding β-keto acid. Via a process of decarboxylation these generate methyl ketones that on reduction yield the correspondent secondary alcohols.[12] Branched alcohols such as methylpropanol and 2- and 3-methylbutanol that are highly volatile are generated by the catabolism of branched amino acids.[184] Ethanol detected in the volatile fraction of dry-fermented sausages and dry hams is derived from different sources, of which the main one is the fermentation of sugars, but can also be produced by the catabolism of lipids and amino acids.[169]

Several hydrocarbons have been identified in the volatile fraction of dry-fermented sausages[169] and dry hams.[183–185] N-alkanes are produced by the autooxidation of lipids.

Branched alkanes, present in large amounts in dry hams[183] and in dry-fermented sausages,[169] are also quite often detected in fresh meats.[159] These could originate from the oxidation of branched fatty acids usually present in small amounts in animal tissues or even from the unsaponifiable fraction of vegetable products used in animal feeds.[186,187] Aromatic hydrocarbons such as toluene, ethylbenzene, pseudocumene, xylenes, and others have also been identified.[169,185,188] Toluene could be produced by the catabolism of phenylalanine,[189] whereas 1,2-dimethylbenzene (*o*-xylene) is probably derived from steroids or skatole;[190] both compounds can also be derived from animal feeds[191] due to their presence in certain plants.[186] Animal feed can also be a source of other aromatic hydrocarbons such as 1,3-dimethylbenzene (*m*-xylene) and 1,4-dimethylbenzene (*p*-xylene). All these compounds can accumulate in animal lipids, and they have been found in fresh meat, in dorsal fat, and in cured meat products.[169,171,188,190] Many of these hydrocarbons have also been identified by Wittkowski[192] in the smoke aromas used to process certain meat products. In any case, a number of authors[159,193] are of the opinion that saturated and unsaturated hydrocarbons play an insignificant role in the development of the flavor and odor of meat.

2. The Importance of Amino Acids and Peptides in the Flavor of Dry-Fermented Sausages

Amino acids and peptides form part of the nonvolatile fraction that contributes to the sapid and textural properties of dry-fermented sausages. Amino acids and peptides are present in the four principal tastes: sweet, salty, acid, and bitter and also in the "umami" taste.[194,195] The term "umami", a Japanese word meaning deliciousness, was first used to define the characteristic flavor of monosodium glutamate (MSG) and the 5′-ribonucleotides IMP (5′-inosine monophosphate) and GMP (5′-guanosine monophosphate).[156,195,196] Some authors[156,197–199] consider it to be a new basic taste. The term "umami" is used to describe agreeable slightly sweet and sour tastes that produce so-called "mouth satisfaction".[156,182,199]

Some L-amino acids such as glycine, alanine, serine, threonine, proline, and hydroxiproline contribute to the sweet taste, whereas others, such as histidine, arginine, methionine, valine, leucine, isoleucine, phenylalanine, and tryptophan, are responsible for the bitterness.[200] Several peptides have also been associated with the bitter taste such as carnosine and anserine dipeptides.[195] Almost all peptides with hydrophobic L-amino acids have a bitter taste, indicating that the bitterness is associated with the hydrophobic nature of the side chains of its amino acids.[194] This is not an uncommon problem in cheeses.[201] Sodium salts of glutamic and aspartic acids contribute to the salty taste of meat.[195] The acidity is provided by aspartic and glutamic acids, histidine, and asparagine.[195] Dipeptides with an acidic taste have two acid amino acids, one acid and one neutral amino acid or one acid and one aromatic amino acid. The acidity is associated with the hydrogen ions of the amino acids.

"Umami" substances are associated with the structure of the L-amino acids with five carbon atoms and with the 5′-monophosphate ribonucleotides. The most important ones in meat are glutamic acid, monosodium glutamate, IMP, GMP, and certain peptides (such as the dipeptides with a glutamic acid in the N-terminal position).[195]

In fact, the role that these compounds play in determining the taste of dry-fermented sausages has not been well established. However, they are known to contribute to the taste of products in which proteolytic enzymes are involved in the manufacturing process. Therefore, the taste of some traditional Japanese food products such as sake and soya sauce is due to the amino acids released during fermentation.[194] The taste of cheese has also

been associated with the amino acids and peptides released by the proteolytic enzymes such as quimosine and those released by LAB during ripening. Toldrá et al.[97] report that the improvement in the flavor of dry hams could be correlated with an increase in the levels of the free amino acids. In a study on the flavor of meat broth, Nishimura and Kato[202] showed that the increase in free amino acids and peptides contributed to enhancing the meat's flavor. In a later work, Pereira-Lima[203] reported that the broth flavor of the watery extracts of beef is strongly related to an increase in the nonproteic nitrogen substances, especially to the levels of the free amino acids glutamic acid, asparagine, lisine, and methionine and the dipeptides carnosine and anserine.

a. Transformations of the Amino Acids

The free amino acids undergo a number of chemical transformations producing different compounds, including amines, keto acids, organic acids, and ammonia, that affect the sensory characteristics of the food and in some cases even have undesirable properties.

Biogenic amines are low-molecular-weight organic bases with biological activity. They are generated as a result of the normal metabolic processes that take place in living organisms and therefore are present in food products. Normally, they are produced by decarboxylation of amino acids. This requires the availability of amino acid precursors, the presence of microorganisms capable of decarboxylating amino acids, and, finally, favorable conditions for the growth of these microorganisms and the development of their decarboxylase activity.[204–206] Although the bacterial decarboxylases are not highly specific, the different bacterial strains and species have a characteristic activity.[207] Tyramine, tryptamine, and phenylethylamine are produced as a result of enzymic decarboxylation of the amino acids tyrosine, tryptophan, and phenylalanine, respectively.[208] Similarly, lysine, histidine, and ornithine gave rise to cadaverine, histamine, and putrescine, respectively.[115] Putrescine acts as a precursor of spermine and spermidine.[209]

Biogenic amines can be generated in dry-fermented sausages and other meat products because other proteolytic phenomena occur that lead to the release of amino acids. During the fermentation/ripening stage, the microflora typically found in these products can generate biogenic amines or even contaminant microorganisms with amino acid-decarboxilation activity can develop. Thus, tryptamine, phenylethylamine, putrescine, cadaverine, histamine, tyramine, spermidine, and spermine have been detected in dry-fermented sausages and other meat products.[115,210]

The presence of high levels of biogenic amines can affect the product's flavor and can also constitute a risk for the consumer. In fact, high levels of these amines have been related with toxicological problems; for example, histamine and tyramine can cause hypertensive crises in patients receiving treatment with monoamine oxidase inhibitors (MAOI). According to Blackwell and Mabbit,[211] the minimum amount required to produce symptoms of this interaction is 6 mg of tyramine. Phenylethylamine[212] and tyramine[213] have been associated with migranes. In healthy individuals, putrescine and cadaverine diamines are not considered to be toxic, although they can potentiate the toxicity of histamine.[214] Similarly, in the presence of nitrites, putrescine and cadaverine can bring about formation of nitrosamines.[215] It is very difficult to determine the exact toxicity threshold of biogenic amines because the toxic dose is strongly dependent on the efficacy of the detoxification mechanisms of different individuals.[216] Nevertheless, quantities of histamine of around 100 mg can induce a mild toxicity, and 1000 mg can induce severe toxicity, whereas between 10 and 80 mg of tyramine can cause inflammation and doses of 100-mg migranes.[217,218]

The works by Teuber,[219] and Voigt and Eitenmiller[220] show that contaminating mi-

croflora is more responsible for high levels of biogenic amines than starter cultures. Nevertheless, different species of microorganisms used in the food industry can produce biogenic amines.[64,221,222] A large number of *Enterobacteriaceae* and a number of lactobacilli, pediococci, and enterococci are especially active in the formation of biogenic amines. Histidine decarboxylase activity is present in the genera *Escherichia*, *Salmonella*, *Clostridium*, *Bacillus*, and *Lactobacillus*,[220] but very few of the bacteria isolated from food products have resulted to be producers of toxicologically significant levels of histamine. Hammes et al.,[64] in experiments on *Staphylococcus carnosus* strains, did not detect either histamine, putrescine, or cadaverine formation, nor were any cadaverine-producing LAB detected[221] and only a few cases of putrescine formers, *Lactobacillus brevis*[223] and one lactobacilli isolated from the oral cavity.[224] In a work by Dainty et al.,[221] lactobacilli of the subgenera *Streptobacterium* and *Leuconostoc* strains were used to inoculate beef that was later vacuum packed and refrigerated. Neither putrescine nor cadaverine production was detected in any of the strains studied either in the pure cultures or in the packaged meat. However, in studies on inoculated and vacuum-packed meat tyramine was associated with the growth of LAB.[225] In dry-fermented sausages these microorganisms are assumed to be the source of tyramine,[115,222,227] and its formation is associated more with the fermentation stage than with the following drying stage.

The quality of the raw material strongly effects the levels of biogenic amines present in dry-fermented sausages.[228] Tschabrun et al.[229] reduced the histamine content in dry-fermented sausages by using very fresh meat. Therefore, selection of the raw material appears to be one of the most critical points of control for reducing the level of biogenic amines in the final product.

The processing temperature is another important factor to take into account. This should be optimum for the growth of the starter cultures, which should not consist of amine producers, so that these can compete with bacteria with decarboxylase activity.[228] Hudson-Arnold and Duane-Brown[230] found that the optimum pH for decarboxylation of amino acids to occur is low, between 2.5 and 6.5. Therefore, the a_w levels and low pH that prevail in fermented meat products together with the presence of amino acids potentiate amine formation.[231]

Another important pathway in the formation of monoamines in food products is the amination of aldehydes.[232] Via this pathway hexylamine is produced from hexanal and ethylamine from acetaldehyde.[233]

Amino acids can also be broken down by deamination via different pathways.[12,234]

i. Oxidative Deamination

Several bacteria have flavinodependent L- and D-amino acid oxidases that catalyze oxidative deamination but play a relatively secondary role and are not considered as part of the main metabolism of the amino groups. These enzymes are relatively nonspecific, and one particular oxidase can attack up to 10 different amino acids. In consequence, the corresponding keto acid and ammonia are generated:

$$R\text{—}CHNH_2COOH + H_2O + \text{Enz-FAD} \rightarrow \alpha\text{-keto acid} + NH_3 + \text{Enz-FADH}_2$$

Reduced forms of the L-amino acid oxidases can directly react with molecular oxygen forming hydrogen peroxide and regenerating the oxidized forms of the enzymes.

$$\text{Enz-FADH}_2 + O_2 \rightarrow \text{Enz-FAD} + H_2O_2$$

Many bacteria have $NAD(P)^+$-dependent dehydrogenases that, starting with amino acids, produce the corresponding keto acid

together with NH_4^+ ions. Oxidative deamination of glutamic acid is a reversible reaction catalyzed by glutamate dehydrogenases.

$$\text{L-glutamate} + \text{NAD}^+(\text{NADP}^+) + H_2O \rightleftarrows \alpha\text{-ketoglutarate} + NH_4^+ + \text{NADH(NADPH)}$$

Most microorganisms have two glutamate dehydrogenases, one of which is specific for NAD^+ and the other for $NADP^+$. The former enzyme primarily brings about the catabolism of glutamate, whereas the latter catalyzes its synthesis.

Another enzyme in this group is alanine dehydrogenase, which is present in some members of the *Bacillus* and *Clostridium* genera.

$$\text{L-alanine} + \text{NAD}^+(\text{NADP}^+) + H_2O \rightleftarrows \text{pyruvate} + NH_4^+ + \text{NADH(NADPH)}$$

ii. Nonoxidative Deamination

Nonoxidative deamination is another of the pathways via which amino acids are catabolized. This kind of reaction is facilitated by the presence of substitutions in the β carbon atom. Therefore, amino acids such as serine, threonine, aspartic acid, and histidine are all subject to this deamination. The enzymes that catalyze these reactions are called deaminases.

Aspartase (aspartic acid deaminase), which is present in a number of microorganisms, transforms aspartate into fumarate and ammonia.

$$\text{aspartate} \underset{\text{aspartase}}{\overset{\text{aspartase}}{\rightleftarrows}} \text{fumarate} + NH_3$$

The reaction is reversible and provides a potential mechanism for the assimilation of ammonia.

Serine and threonine deaminases (often called dehydratases because they first eliminate water and then they deaminate) catalyze the deamination of the respective amino acids resulting in the formation of pyruvate and α-ketobutyrate, respectively.

$$\text{serine} \xrightarrow{\text{serine dehydrase}} \text{pyruvate} + NH_3$$

$$\text{threonine} \xrightarrow{\text{threonine dehydrase}} \alpha\text{-ketobutyrate} + NH_3$$

The reaction begins with the removal of water to generate an imino acid via β-elimination. In the second stage of the reaction in which there is no enzymic involvement, water reacts with the imino acid resulting in the release of ammonia and the corresponding α-keto acid. Cysteine desulfhydrase has a similar mechanism of action to the previous one, although in this case instead of giving off water, hydrogen sulfide is released. The reactions catalyzed by dehydratases and desulfhydrases are mainly irreversible and therefore do not participate in the assimilation of ammonia.

Amino acids can also undergo transaminations. The α-amino group is transferred to the α carbon atom from an α-keto acid, usually α-ketoglutarate, and the corresponding α-keto acid from the amino acid resulting in amination of the α-ketoglutarate.

$$\text{amino acid 1} + \text{keto acid 2} \rightleftarrows \text{keto acid 1} + \text{amino acid 2}$$

These reactions are catalyzed by known enzymes such as amino transferases or transaminases that are present in high quantities in bacteria such as *E. coli*, *B. subtilis*, and *E. faecalis* and others.

Branched aldehydes form part of the volatile fraction of a wide range of cured products such as bacon,[235] dry ham,[171,188] and dry-fermented sausages.[169,236] These compounds can be of microbial origin, and certain microorganisms can generate these substances from amino acids.[171] One example is the transami-

nation-decarboxylation of leucine that results in the formation of 3-methylbutanal,[237] as MacLeod and Morgan[238] demonstrated in *Streptococcus lactis* var. *maltigenes* (Figure 3, reaction A). However, the main pathway by which branched aldehydes are produced is oxidative deamination-decarboxylation of amino acids by Strecker degradation,[183] which involves the interaction of α-dicarbonyl compounds and α-amino acids (Figure 3, reaction B), generating an aldehyde with one less carbon atom than the amino acid involved in the initial reaction. This results in the generation of 3-methylbutanal, 2-methylbutanal, and phenylacetaldehyde from leucine, isoleucine, and phenylalanine, respectively. Although this reaction usually takes place at temperatures of around 90°C and in alkaline medium, very different conditions to those present in dry-fermented sausage ripening and the curing of hams, it is possible that a rise in the amount of free amino acids together with a low a_w could favor this reaction.[188] The presence of some of these aldehydes in dry hams has been attributed to this reaction.[183,188,239] The possibility of this reaction developing in dry-fermented sausages would be restricted by the conditions present and the short ripening period to which these products are exposed.

Another pathway by which these aldehydes can be generated is through the biosynthesis of amino acids. In this way, 2-methylpropanal and 3-methylbutanal are produced as byproducts of valine and leucine synthesis (Figure 3, reaction C), and similarly butanal and 2-methylbutanal are produced by isoleucine synthesis.[182]

Some esters have been identified in commercial salamis and "salchichón" by several authors.[7,172,236,240,241] The origin of these compounds in dry-fermented sausages is not completely clear, and very little research has focused on this area. Edwards et al.[240] analyzed the volatile fraction of three different brands of Spanish "salchichón" and one German salami and found that esters were the major compounds in the Spanish "salchichón", whereas none of these compounds were detected in the German salami. These authors made two batches of "salchichón" (one not inoculated and the other inoculated with a mixture of lactobacilli and micrococci previously isolated from commercial dry-fermented sausages) using an aseptic procedure. They also produced a nonaseptic batch using the same ingredients as used previously, therefore with the environmental microbiota. The noninoculated batch of sausages was completely free of esters after 20 days of ripening, whereas several of these compounds were detected in the products made with the same ingredients but without an aseptic procedure (environmental contamination). Therefore, it seems likely that these microorganisms participate in the ester formation.

Stahnke,[242] in a recent study, attempted to determine the participation of *Staphylococcus xylosus* in the production of aromatic compounds in dry-fermented sausages. For that two batches were prepared, one inoculated with *S. xylosus* and another noninoculated (control batch) to which antibiotics and fungicides had been added to inhibit microbial growth. When the compounds in the headspace of both batches were analyzed, the dry-fermented sausages manufactured with *S. xylosus* had higher levels of esters, some of which were not even detected in the control sausages. According to Stahnke,[242] the aldehydes were possibly oxidized to the corresponding acids and later esterified with ethanol by microbial action; it is not known whether the aldehydes are oxidized to acids by microbial enzymes or by endogenous enzymes. In cheeses, which usually contain short chain free fatty acids, ethyl esters of butanoic, hexanoic, octanoic, and decanoic acids have been detected.[243–245] Hosono et al.[244] showed that esterases are produced by different species of the *Streptococcus, Lactobacillus*, and *Pseudomonas* genera present in cheeses as either starter microorganisms or contaminants. Nevertheless, LAB produce almost 10 times less esterases than pseudo-

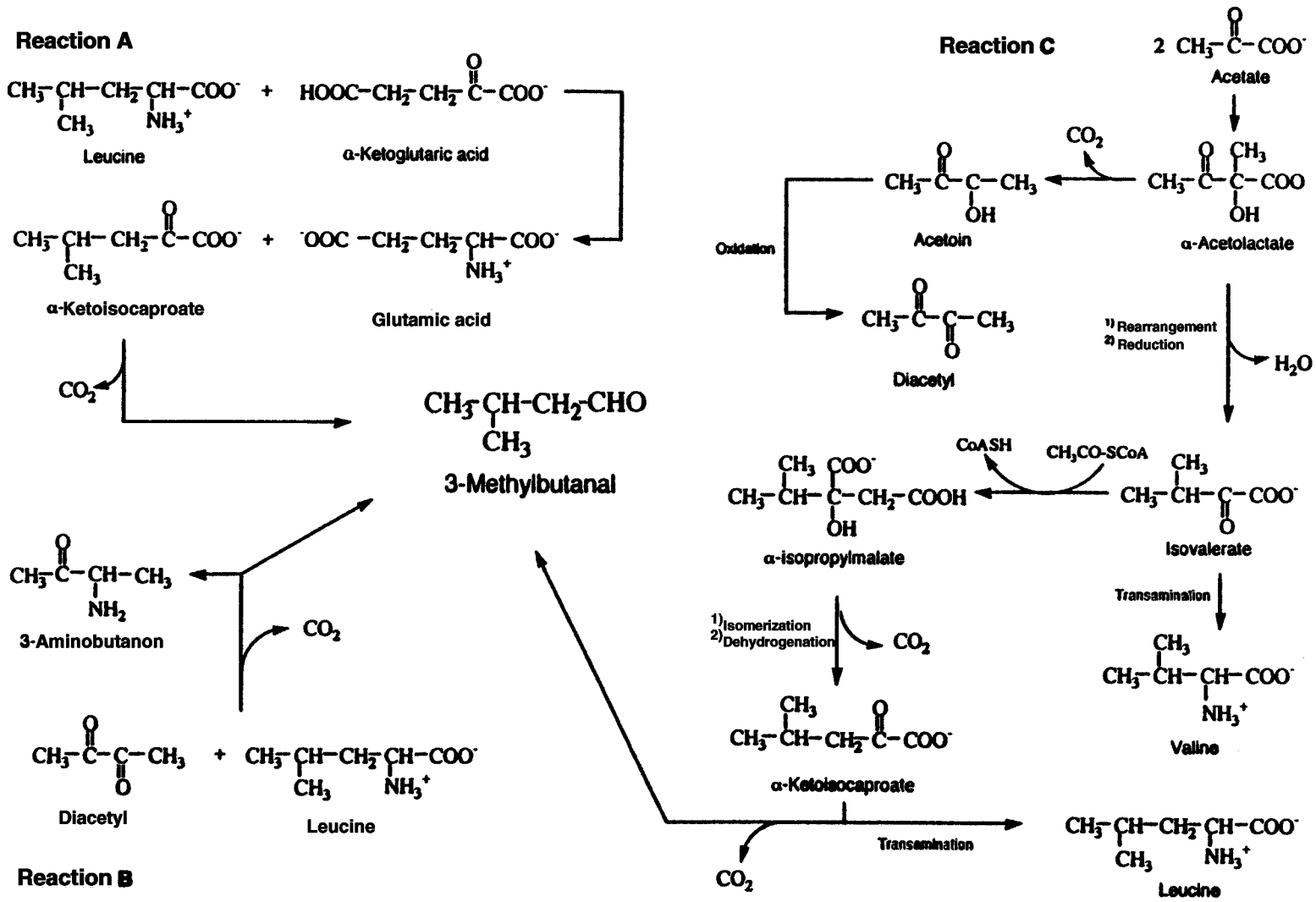

FIGURE 3. Different pathways of branched aldehydes generation. (Copy of scheme 1 from Hinrichsen and Andersen, Volatile compunds and chemical changes in cured pork: role of three halotolerant bacteria, *J. Agric. Food Chem.*, 42, 1537, 1994.)

monas. Because of this, Stahnke[242] remarked that esters produced in the batch inoculated with *S. xylosus*, in which the LAB reached values of almost 10^7 cfu/g, were probably not only due to the action of this organism.

Several sulfurous compounds such as dimethyl disulfide and dimethyl trisulfide have been detected in the headspace from dry-fermented sausages and dry hams.[169,188] Thioles are generated by Strecker degradation of sulfurous amino acids such as cysteine, cystine, and methionine. These thioles are easily oxidized to disulfides that can be transformed to trisulfides.[182] Dimethyl disulfide is a compound with exceptional aromatic activity (olfactory threshold of 0.01 mg/l) that contributes to the flavor of hen. Bodrero et al.[246] demonstrated that this group of compounds play an important role in the odor of beef.

Little is known about the possibility of Maillard reactions taking place in cured meat. Maillard reaction or nonenzymic browning is a combination of complex reactions that take place in both technological treatments and also during storage of food products that contain protein and carbohydrate reducers or carbonyl compounds. These reactions generate a number of volatile compounds, including ketones, aldehydes, furanes, pyrazines, alcohols, etc.[247] They are favored, although only to a limited extent, by the conditions present during the curing of hams: a temperature range between 15 and 25°C, a pH of around 6.0, a reduction in a_w to approximately 0.85 due to salting and dehydration, and a ripening period longer than 12 months.[191,239] However, Berger et al.[248] did not detect the products of Maillard reaction in the volatile fraction obtained from salami.

3. The Role of Spices in the Flavor and Odor of Dry-Fermented Sausages

Spices include a wide range of ingredients that comprise 0.5 to 2% of most fermented sausages. In the meat industry, spices include a variety of aromatic plant substances, including fruit (pepper, paprika, cumin, coriander, etc.), seeds (mustard, nutmeg, etc.), flowers (cloves, saffron, etc.), leaves (oregano, rosemary, thyme, etc.), and bulbs (garlic, onion, etc.). The addition of spices is very useful in the manufacture of dry-fermented sausages. This is mainly done to give the product a characteristic flavor and odor and occasionally, as is the case of paprika in "chorizo", to give it a specific color.

In the manufacture of dry-fermented sausages, spices can be used in their natural form (whole, coarsely ground, or as powder) or as extracts (essential oils and oleoresins). Essential oils are mainly obtained by steam distillation and oleoresins by using organic solvents. Although essential oils consist exclusively of volatile compounds, oleoresins contain fats, waxes, and sapid compounds.

Research into the composition of spices, especially the essential oils, became possible from the 1960s onward with the development of new analytical techniques. Volatile compounds are mainly analyzed by a combination of gas chromatography and mass spectrometry (GC-MS) and gas chromatography combined with Fourier transform infrared spectroscopy (GC-FTIR). However, these techniques were not able to solve all the problems. Despite the important data they furnish (Kovats indices, mass spectra, infrared spectra), this information is difficult to correctly interpret, for example, the incorrect interpretation of terpene data is a prime example. This is due to the fact that most monoterpene and sesquiterpene hydrocarbons have very similar spectra and can only be differentiated by their retention times and infrared spectra. Research in this field is highly complex because of the very small number of reference products and the instability of the terpenes that are both qualitatively and quantitatively abundant in the essential oils of spices and aromatic products, all of which can sometimes lead to misinterpretation.

Pepper (*Peper nigrum*) is probably the most important spice in the manufacture of salami, "salchichón", and "fuet"-type fermented sausages. The compounds mainly responsible for its hot flavor are

- Piperine, which comprises from 3 to 8% of black pepper; this is the component in black pepper most responsible for its hot flavor
- Piperine isomers: chavicine, isochavicine, and isopiperine
- Piperiline, piperetine, piperanine, and piperoleine A and B

On exposure to light (350 nm) piperine is transformed into isochavicine, which is flavorless, explaining the loss of the hot flavor of old black pepper.

Salzer[249] considers that the monoterpene hydrocarbons in pepper essential oils are responsible for the overall smell and sensation, whereas the sesquiterpenes give it its flavor. Mono- and sesquiterpene hydrocarbons comprise approximately 90% of the essential oils of this spice.[250,251] The most important ones are β-caryophyllene, which comprises between 9.5 and 31%, and limonene, which comprises around 17%. There are wide variations in some compounds according to the geographic origin or the plant of origin (see Table 3 from Richard[252]).

Some of the most exhaustive research into the composition of the essential oils in black pepper was carried out by Debrauwere and Verzele.[256,257] These authors recorded a terpene hydrocarbon content of 88.9% (77.3% monoterpenes and 18.2% sesquiterpenes) and 10% oxygenated terpenes. They identified 26 hydrocarbons, 3 of which had not been described previously (β-cubebene, α-guaiene, and γ-cadinene).[256] The most abundant other

TABLE 3
Composition of Black Pepper Essential Oil (Major Compounds)

	Concentration (%)		
Compound	India (1)	India (2)	Borneo and Sarawak (3)
δ-3-Carene	5.4	tr–15.5	20.2
p-Cymene	1.3	0.2–2.8	0.8
Limonene	17.5	16.4–24.4[a]	17.0
α-Phellandrene	1.7	0.5–27.4	
β-Phellandrene	4.0[b]		
α-Pinene	9.0	1.1–16.2	5.8
β-Pinene	10.4	4.9–14.3	10.4
Sabinene	19.4	0.2–13.8	
β-Bisabolene	2.0	0.1–5.2	
β-Caryophillene	14.7	9.4–30.9	28.1
α-Copaene	0.5	tr–3.9	2.4
δ-Elemene			2.6
α-Humulene	0.5	1.0–2.1	1.4
α-Selinene		tr–3.3	
β-Selinene		0.2–4.6	
1-Terpinen-4-ol	1.0	tr–0.5	0.1

[a] Together with β-phellandrene.
[b] Together with 1,8-cineole.

From: (1) Ref. 253; (2) Ref. 254; (3) Ref. 255.

oxygenated compounds identified that were present at levels lower than 0.1% were linalol, 1-terpinen-4-ol, α-terpineol, and cis- and trans-thujan-4-ol.

Garlic (*Allium sativum*) is another important ingredient used in the manufacture of some dry-fermented sausages, especially "chorizo". Together with onion this bulb has one of the most pungent and penetrating smells.

The volatile compounds of sliced raw garlic have been isolated by solvent extraction and also by steam distillation and 27 compounds have been obtained, of which 3 were recognized as artifacts of GC. The main equipment used to analyze the volatile fraction was GC-MS, although the identification of thermolabile compounds has led to the use of other spectroscopic techniques as well.

With the exception of propanal and trimethylamine, all the other compounds contain sulfur. These include four thiols, three sulfides, seven disulfides, three trisulfides, and six dialkyl thiosulfinates. The two vinyl dithienes detected appear to be artifacts of GC formed by dehydration of allicin. Di-2-propenyl sulfide is also a product of the thermal decomposition of allicine. The majority components are 2-propenyl 2-propenethiosulfinate (around 800 mg/kg), di-2-propenyl disulfide (57 mg/kg), 2-propenol (54 mg/kg), dimethyl trisulfide (24 mg/kg), methyl 2-propenyl trisulfide (15 mg/kg), methyl 2-propenyl disulfide (12 mg/kg), and di-2-propenyl-trisulfide (10 mg/kg).[258]

Allicin (2-propenyl 2-propenethiosulfinate or diallyl thiosulfinate) is the main component of the volatile fraction of garlic. However, this compound is not usually present in whole cells unless the precursor alliin is present (*S*-allylcysteine sulfoxide) in which case it is formed by the action of the enzyme alliinase when the garlic is sliced or crushed.[259]

Members of the genus *Capsicum* in the *Solenaceae* family amount to around 200 varieties, which are not only used as spices but also as important components of the diet in tropical, subtropical, and Mediterranean regions. The different crops have a variable morphology (form and size) and also different flavors and odors. The main species of this genus are *Capsicum annuum* var. *grossum* Sendt, which includes all those denominated paprika and *Capsicum frutescens*, or chilles that include all the Tabasco and Jalapeño peppers.

The main compound responsible for the hot flavor is capsaicin, although this is also associated with other analagous compounds such as dihydrocapsaicin, homocapsaicin, and nordihydrocapsaicin. The variety, the geographical location, growth and processing conditions, degree of ripening, and its location inside the fruit are the most important factors that influence the capsaicin content.[260]

Apart from the capsaicinoids *Capsicum* spp., also present is a wide range of volatile compounds. Buttery et al.[261] identified 23 compounds in the volatile fraction of sweet peppers; the main classes included carbonyl compounds and aliphatic alcohols (11), aromatic compounds (6), and monoterpenes (3). Among these trans-β-ocimene, limonene, methyl salicilate, and linalol were also present in significant amounts. However, 2-(2-methylpropyl)-3-methoxypyrazine is considered to be the main compound responsible for the aroma in these kinds of peppers. This compound can be produced by bacterial action on the plant roots.[262]

Paprika is used in dry-fermented sausage manufacture not only for its sapid and aromatic qualities but also because it gives some of these products their characteristic color. Carotenoids are the main pigments responsible for the color of paprika, the main component of which is capsantin (which represents approximately 35% of the carotenes). Other compounds are present in smaller quantities such as β-carotene, violaxantine, capsorrubine, etc.[263,264]

In dry-fermented sausages a large variety of volatile compounds derived from the spices have been identified by GC combined with MS. Berger et al.[248] identified monoterpene hydrocarbons as the quantiatively dominant compounds in salami and obtained a

similar distribution to that presented by the essential oils of pepper.[256] In experiments on French fermented sausages made with pepper and garlic, Croizet et al.[172] also detected the presence of seven terpenes and six sulfurous compounds derived mainly from these two species. Mateo and Zumalacárregui[265] analyzed the volatile fraction of "chorizo" obtained by distillation in a Lickens-Nikerson apparatus. Of all the 126 compounds detected, only 115 were identified. The sulfurous compounds from the garlic and acetic acid were the major components. Edwards et al.[7] detected in "chorizo" sulfurous compounds and 3-hexenol derived from garlic and paprika, respectively.

An important factor to consider in determining the profile of the volatile compounds of dry-fermented sausages is the form in which the black pepper is added. Edwards et al.[7] showed that in dry-fermented sausages made with ground black pepper the terpenes were the major components and reached much higher levels than in dry-fermented sausages that had been made with whole peppercorns. In the latter products, compounds produced by lipid oxidation and/or microbial growth were much more important.

IV. ACCELERATED RIPENING OF DRY-FERMENTED SAUSAGES

Traditionally, it has been generally accepted that for all ripened food (cheese, dry ham, dry and semidry sausages, etc.) there must be a certain lag between the initial product manufacturing and the final aged product. However, while some maturation time is inevitable, present understanding of some phenomena occurring during ripening has led to experimentation into means of shortening it by speeding up the reactions that generate flavor and modify texture because the cost of the ripening of these kind of products is very high due to the time of this process. Thus, the acceleration of ripening would result in a reduction of the storage time, and it would increase the profit margin and the competitivity of the end product.

The first attempts in this area were made to accelerate the cheese ripening, starting in the early 1970s,[266] most of the work was directed toward the addition of free enzymes obtained from various sources to the cheese milk of curd. On this basis, from the early 1990s some attempts to enhance the flavor/accelerate the ripening of dry-fermented sausages have been made. The means used for that has been the addition of either lipases and proteases.

Zalacain et al.[267,268] studied the addition of lipase from *Candida cylindracea*, observing a higher free fatty acid release, but no clear improvement of the sensory quality was obtained. Also, Zalacain et al.[269,270] studied the effect of the lipase (Lipozyme) from *Rhizomucor miehei*, concluding that the use of this lipase might have some advantages because a light enhancing of the sensory quality was observed due to the acceleration of lipolytic phenomena. Fernández et al.[271,272] used pancreatic lipase, detecting a flavor improvement when 60 to 90 units were used, although results were not as good as expected.

Naes et al.[273] and Blom et al.[274] analyzed the effect of a serine proteinase from *Lactobacillus paracasei* subsp. *paracasei* NCDO 151, concluding that the production time of Northern-type dry-fermented sausages may be reduced by 30 to 50% with the addition of a proteinase extract in combination with the starter culture *Lactobacillus sake* L45. Zapelena et al.[275] studied the use of a proteinase (Neutrase) from *Bacillus subtilis*, concluding that sausages added with proteinase showed a slight improvement on the overall acceptability significantly correlated with some flavor parameters. Melendo et al.[276] used bromelain to improve the tenderness of a type of Spanish dry-fermented sausage (chorizo), although high proteinase concentrations gave rise to an excessive sausage softening. Several amounts

of pronase E from *Streptomyces griseus*,[146] aspartyl proteinase from *Aspergillus oryzae*,[147] and papain[6] were used by our research group with the same aim. A later study was additionally made with these proteinases, in which the dosis of each enzyme was adjusted according to the results of the previous experiments. It was textually stated,[6] together to results from pancreatic lipase addition,[271,272] that:

> In an attempt to either accelerate the ripening or potentiate the flavor of dry-fermented sausages, we have explored the addition, in several amounts, of three proteinases (pronase E from *Streptomyces griseus*, aspartyl proteinase from *Aspergillus oryzae* and papain from *Carica papaya*) and pancreatic lipase. The results demonstrate that it is possible to accelerate the proteolysis and lipolysis phenomena, respectively. When proteinases were added, final products ranged from sausages in which, in comparison with conventional sausages, no important chemical changes (NPN,* PTN,* SSN,* TVBN* and free amino acids and amines) were observed (if low amount of enzymes were added) to final products in which great increases in the above mentioned nitrogen fractions were produced (if high level of enzymes were added) in such a way that, in some batches, the enzime provoked an excessive softness (spreadable texture). Similarly, when pancreatic lipase was added a range of increased level of free fatty acids was obtained according to the amounts of enzyme added from normal (similar to that observed in conventional dry sausages) to very high level of free fatty acid in the product, in which it might be observed an "oily exudate".
>
> These results mean that it is possible to accelerate the proteolysis and lipolysis phenomena by the addition of the corresponding enzyme in the appropiate amount. However, the sensory analysis demonstrated that solely, and only in some cases, a slight increase in the flavor was obtained, that is, it can be possible to obtain a very drastric degradations of proteins and fat that, in turn, produced spectacular increases of the degradation products (amino acids and free fatty acids, respectively), but these effects do not bear a noticeable increase in the flavor. Thus, it seems to be that the addition of proteinases and lipases alone are not useful for shortening the ripening period. In our opinion, a long time is necessary for ripening in order to allow the transformation of free amino acids and fatty acids through microbial (oxidative deaminations, decarboxylations, etc.) and/or chemical (Strecker and Maillard reactions, autooxidations of fat, etc.) methods to yield aromatic compounds (aldehydes, ketones, lactones, alcohols, esters, etc.), which have been proven to be the main compounds responsible of the flavor of dry-fermented sausages.
>
> Thus, to shorten the ripening of sausages, the addition of proteinases and lipases may be useful to provide substrates, which must be transformed in aromatic compounds. Therefore, it is also necessary, besides the addition of proteinases and lipases, to create conditions or to add either an efficient starter or other kind of enzymes, so that the above-mentioned volatiles may be formed in a shorter time than the usual from free amino acids and fatty acids generated by the enzymes.

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REFERENCES

1. **Lücke, F. K.,** Fermented sausages, in *Microbiology of Food Fermentations*, Wood, B. J. B., Ed., Applied Science Publishers, London, Vol. 2, 1984, 41.
2. **Palumbo, S. A. and Smith, J. L.,** Chemical and microbiological changes during sausage

* **NPN:** non-protein nitrogen; **PTN:** phosphotungstic nitrogen; **SSN:** sulfosalicilic nitrogen; **TVBN:** total volatile basic nitrogen.

fermentation and ripening, in *Enzymes in Food and Beverage Processing*, Ory, L. and Angelo, J. St., Eds., ACS Symposium Series, 47, 1977, 279.

3. **Liepe, H. U.,** Starter cultures in meat production, in *Biotechnology*, Rehm, H. J. and Reed, G., Eds., Verlag Chemie, Basel, Vol. 5, 1982, 400.
4. **Toldrá, F. and Flores, M.,** The role of muscle proteases and lipases in flavor development during the processing of dry-cured ham, *CRC Crit. Rev. Food Sci. Nutr*., 38, 331, 1998.
5. **Arnau, J. and Monfort, J. M.,** El jamón curado: tecnología y análisis de consumo, in *Special Symposium of the 44th Inter. Cong. Meat Sci. Technol*, Estrategias Alimentarias-Eurocarne, Barcelona, Spain, 1998.
6. **Díaz, O., Fernández, M., García de Fernando, G. D., de la Hoz, L., and Ordóñez, J. A.,** Proteolysis in Dry-fermented sausages: the effect of selected exogenous proteases, *Meat Sci*., 46, 115, 1997.
7. **Edwards, R. A., Ordóñez, J. A., Dainty, R. H., Hierro, E. M., and de la Hoz, L.,** Characterization of the headspace volatile compounds of selected Spanish dry-fermented sausages, *Food Chem.*, 64, 461, 1999.
8. **Dainty, R. H., Shaw, B. G., and Robert, T. A.,** Microbial and chemical changes in chill-stored red meats, in *Food Microbiology: Advances and Prospects*, Roberts, T. A. and Skinner, F. A., Eds., Academic Press Inc., London, 1983, 151.
9. **Mcmeekin, T. A.,** Microbial spoilage of meats, in *Developments in Food Microbiology*, 1, Davies, R., Ed., Applied Science Publishers, London, 1982, 1.
10. **Hammes, W.P., Rölz, I., and Bantleon, A.,** Mikrobiologische untersuchung der auf dem deutschen markt vorhandenen starterkulturpreparate für die rohwurstreifung, *Fleischwirtsch*., 65, 629, 1985.
11. **Fischer, U. and Schleifer, K. H.,** Presence of staphylococci and micrococci in dry sausage, *Fleischwirtsch*., 60, 1046, 1980.
12. **Gottschalk, G.,** *Bacterial Metabolism*, Springer-Verlag, New York, 1986.
13. **Deibel, R. H., Wilson, G. D., and Niven, C. F.,** Microbiology of meat curing. IV. A lyophilized *Pediococcus cerevisae* starter culture for fermented sausages, *Appl. Microbiol*., 9, 239, 1961.
14. **Wardlaw, F. B., Skelley, G. C., Johnson, M. G., and Acton, J. C.,** Changes in meat components during fermentation, heat processing and drying of a summer sausage, *J. Food Sci*., 38, 1228, 1973.
15. **Rozier, J.,** Mécanismes de la maturation du saucisson sec, *Rec. Med. Vet.*, 145, 1069, 1969.
16. **De Ketelaere, A., Demeyer, D., Vandekerckhove, P., and Vervaeke, I.,** Stoichiometry of carbohydrate fermentation during dry sausage ripening, *J. Food Sci*., 39, 297, 1974.
17. **Selgas, M. D., Sanz, B., and Ordóñez, J. A.,** Selected characteristics of micrococci isolated from Spanish dry-fermented sausages, *Food Microbiol*., 5, 185, 1988.
18. **Ayroulet, M. and Fornaud, J.,** Mikrobiologische wechselwirkungen bei der rohwurstherstellung, *Fleischwirtsch.*, 56, 1331, 1976.
19. **Sarra, P. G., Cabras, M., and Dellaglio, F.,** Aspetti microbiologici nella maturazione della coppa piacentina, *Ind. Aliment.*, 21, 477, 1982.
20. **Nychas, G. J. E. and Arkoudelos, J. S.,** Staphylococci: their role in fermented sausages, *J. Appl. Bacteriol. Symp. Suppl*., 167S, 1990.
21. **Eakes, B. D., Blumer, T. N., and Monroe, R. J.,** Effect of nitrate and nitrite on colour and flavor of country-style hams, *J. Food Sci*., 40, 973, 1975.
22. **Giddings, G. G.,** The basis of color in muscle food, *J. Food Sci*., 42, 288, 1977.
23. **Cross, C. K. and Ziegler, P.,** A comparation of the volatile fractions of cured and uncured meat, *J. Food Sci*., 30, 610, 1965.
24. **Bailey, M. E. and Swain, J. W.,** Influence of nitrite in meat flavor, in *Proc. Meat Ind. Res. Conf*., American Meat Institute Foundation, Chicago, 1973, 29.
25. **Hauschild, A. H. W., Hilsheimer, R., Jarvis, G., and Raymond, D. P.,** Contribution of nitrite to the control of *Clostridium botulinum* in liver sausage, *J. Food Prot*., 45, 500, 1982.
26. **Pierson, M. D. and Smoot, L. A.,** Nitrite, nitrite alternatives, and the control of *Clos-*

tridium botulinum in cured meats, *CRC Crit. Rev. Food Sci. Nutr.*, 17, 141, 1982.

27. **Mac Donald, B., Gray, J. I., and Gibbins, L. N.,** Role of nitrite in cured meat flavor: antioxidant role of nitrite, *J. Food Sci.*, 45, 893, 1980.
28. **Sato, K. and Hegarty, G. R.,** Warmed-over flavor in cooked meats, *J. Food Sci.*, 36, 1098, 1971.
29. **Fooladi, M. H., Pearson, A. M., Coleman, T. H., and Merckel, R. A.,** The role of nitrite in preventing development of warmed-over flavor, *Food Chem.*, 4, 283, 1977.
30. **Wirth, F.,** La reducción y el no empleo de las sustancias de curado en los productos cárnicos, *Fleischwirtsch. español*, 1, 3, 1993.
31. **Tóth, L.,** Nitrite reactions during the curing of meat products, *Fleischwirtsch.*, 63, 208, 1983.
32. **Brooks, J., Haines, R. B., Moran, T., and Pace, J.,** The function of nitrate, nitrite and bacteria in the curing of bacon and hams, *Food Invest. Special Report*, London, 49, 1940.
33. **Mottram, D. S. and Rhodes, D. N.,** Nitrite and the flavor of cured meat, in *Proc. Int. Symp. Nitrite Meat Prod.*, Zeist, Pudic, Wageningen, 1973, 161.
34. **Wasserman, A. E.,** Nitrite and the flavor of cured meat. II. Discussion, in *Proc. Int. Symp. Nitrite Meat Prod.*, Zeist, Pudic, Wageningen, 1973.
35. **Noel, P., Briand, E., and Dumont, J. P.,** Role of nitrite in flavor development in uncooked cured meat products: sensory assessment, *Meat Sci.*, 28, 1, 1990.
36. **Mottram, D. S., Croft, S. E., and Patterson, R. L. S.,** Volatile components of cured and uncured pork: the role of nitrite and the formation of nitrogen compounds, *J. Sci. Food Agric.*, 35, 233, 1984.
37. **Gray, J. I. and Pearson, A. M.,** Cured meat flavor, in *Advances in Food Research*, C.O. Chichester, Mrak, E. M. and Schweigert, B. S., Eds., Academic Press Inc., Orlando, Vol. 29, 1984, 1.
38. **Mac Donald, B., Gray, J. I., Kakuda, Y., and Lee, M. L.,** Role of nitrite in cured meat flavor: chemical analysis, *J. Food Sci.*, 45, 889, 1980.
39. **Ramarathnam, R., Rubin, L., and Diosady, L. L.,** Studies of meat flavor. I. Qualitative and quantitative differences in uncured and cured pork, *J. Agric. Food Chem.*, 39, 344, 1991.
40. **Roberts, T. A.,** The microbiological role of nitrite and nitrate, *J. Sci. Food Agric.*, 26, 1755, 1975.
41. **Vösgen, W.,** Curado. ¿Son necesarios o superfluos el nitrito o el nitrato como agentes curantes?, *Fleischwirtsch. español*, 2, 25, 1993.
42. **Greene, B. E. and Price, L. G.,** Oxidation-induced color and flavor changes in meat, *J. Agric. Food Chem.*, 23, 164, 1975.
43. **Westerberg, D. O.,** Cured meat flavor and the role of nitrite in its development, in *Proc. Recip. Meat Conf.*, 45, 1973.
44. **Pearson, A. M., Love, J. D., and Shorland, F. B.,** Warmed over flavor in meat, poultry and fish, in *Advances in Food Research*, Chichester, C. O., Mrak, E. M., and Schweigert, B. S., Eds., Academic Press Inc., Orlando, Vol. 23, 1977, 1.
45. **Cantoni, C., Molnar, M. R., Renon, P., and Giolitti, G.,** Ricerche sui lipidi degli insaccati stagionatti, *Ind. Conserve*, 41, 188, 1966.
46. **Demeyer, D., Hoozee, J., and Mesdom, H.,** Specificity of lipolysis during dry sausage ripening, *J. Food Sci.*, 39, 293, 1974.
47. **Whitaker, J. R.,** *Principles of Enzymology for the Food Sciences*, Marcel Dekker Inc., New York, 1972.
48. **Kilara, A.,** Enzyme-modified lipid food ingredients, *Proc. Biochem.*, 20, 35, 1985.
49. **Wallach, D. P.,** Isolation and characterization of four lipolytic preparations from rat skeletal muscle, *J. Lipid Res.*, 9, 200, 1968.
50. **Cantoni, C., Molnar, M. R., Renon, P., and Giolitti, G.,** Lipolytic micrococci in pork fat, *J. Appl. Bacteriol.*, 30, 190, 1967.
51. **Rhee, K. S.,** Fatty acids in meats and meat products, in *Fatty Acids in Foods and their Health Implications*, Chow, Ch. K., Ed., Marcel Dekker Inc., New York, 1992, 65.

52. **Brockerhoff, H.,** Fatty acid distribution patterns of animal depot fats, *Comp. Biochem. Physiol*., 19, 1, 1966.

53. **Macrae, A. R.,** *Microbial Enzymes and Biotechnology*, Fogarty, W. H., Ed., Applied Science Publishers, New York, 1983, 225.

54. **Alford, J. A., Smith, J. L., and Lilly, H. D.,** Relationship of microbial activity to changes in lipids of foods, *J. Appl. Bacteriol*., 34, 133, 1971.

55. **Dineva, B., Piperova, L., Tsvetkov, T. S., Krustev, A., and Brankova, R.,** Changes in lipid fractions during ripening of fast-ripened sausages manufactured using starter cultures, in *Proc. 31st Eur. Meet. Meat Res. Work*., 1985, 359.

56 **Selgas, M. D., Sanz, B., and Ordóñez, J. A.,** Selection of micrococci strains for their use as a starter culture for dry-femented sausages, in *Proc. 32nd Eur. Meet. Meat Res. Work*., Gent, 1986, 251.

57. **Talon, R., Montel, M. C., and Cantonnet, M.,** Lipolytic activity of *Micrococcaceae*, in *Proc. 38th Int. Congr. Meat Sci. Technol*., Clermont-Ferrand, 1992, 843.

58. **Soncini, G., Bianchi, M. A., Paleari, C., Traldi, C., and Cantoni, C.,** Criteria for selection of starters for raw cured meat products, *Technol. Aliment*., 9, 10, 1982.

59. **Selgas, D., García, L., García de Fernando, G., and Ordóñez, J. A.,** Lipolytic and proteolytic activity of micrococci isolated from Dry-fermented sausages, *Fleischwirtsch*., 73, 1164, 1993.

60. **Palumbo, S. A., Zaika, L. L., Kissinger, J. C., and Smith, J. L.,** Microbiology and technology of the pepperoni process, *J. Food Sci*., 41, 12, 1976.

61. **Jessen, B.,** Starter cultures for meat fermentations, in *Fermented Meats*, Campbell-Platt, G. and Cook, P. E., Eds., Blackie Academic and Professional, Suffolk, 1995, 130.

62. **Delarras, C.,** Etude de l'activite lipolytique des *Micrococcaceae*, *Rev. Francaise Corps Gras*, 29 annee 4, 165, 1982.

63. **Montel, M. C., Talon, R., Berdagué, J. L., and Cantonnet, M.,** Effects of starter cultures on the biochemical characteristics of French dry sausages, *Meat Sci*., 35, 229, 1993.

64. **Hammes, W. P., Bosch, I., and Wolf, G.,** Contribution of *Staphylococcus carnosus* and *Staphylococcus piscifermentans* to the fermentation of protein foods, *J. Appl. Bacteriol. Symp. Suppl*., 79, 76S, 1995.

65. **Nielsen, H. J. S. and Kemmer, M. K. B.,** Lipolytic activity of meat starter cultures, in *Proc. 35th Int. Congr. Meat Sci. Technol.,* Copenhagen, 1989, 318.

66. **Nieto, P., Molina, I., Flores, J., Silla, M. H., and Bermell, S.,** Lipolytic activity of microorganisms isolated from dry-cured ham, in *Proc. 35th Int. Congr. Meat Sci. Technol.,* Copenhagen, 1989, 323.

67. **Papon, M. and Talon, R.,** Factors affecting growth and lipase production by meat lactobacilli strains and *Brochothrix thermosphacta*, *J. Appl. Bacteriol*., 64, 107, 1988.

68. **Guo, S. L. and Chen, M. T.,** Studies on the microbial flora of Chinese-style sausage. I. The microbial flora and its biochemical characteristics, *Fleischwirtsch.*, 71, 1425, 1991.

69. **Naes, H., Chrzanowska, J., Nissen-Meyer, J., Pedersen, B. O., and Blom, H.,** Fermentation of dry sausage. The importance of proteolytic and lipolytic activities of lactic acid bacteria, in *Proc. 37th Int. Congr. Meat Sci. Technol.,* Kulmbach, 1991, 914.

70. **Nordal, J. and Slinde, E.,** Characteristics of some lactic acid bacteria as starter cultures in dry sausage production, *Appl. Environ. Microbiol*., 40, 472, 1980.

71. **Fryer, T. F., Reiter, B., and Lawrence, R. C.,** Lipolytic activity of acid lactic bacteria, *J. Dairy Sci.*, 50, 388, 1967.

72. **Stadhouders, J. and Veringa, H. A.,** Fat hidrolysis by lactic acid bacteria in cheese, *Neth. Milk Dairy J*., 27, 77, 1973.

73. **Stadhouders, J.,** Dairy starter cultures, *Milchwissensch*., 29, 329, 1974.

74. **Sanz, B., Selgas, D., Parejo, I., and Ordóñez, J. A.,** Characteristics of lactobacilli isolated from Dry-fermented sausages, *Int. J. Food Microbiol*., 6, 199, 1988.

75. **Kröckel, L.,** Bacterial fermentation of meats, in *Fermented Meats*, Campbell-Platt, G. and Cook, P. E., Eds., Blackie Academic and Professional, Suffolk, 1995, 69.

76. **Papon, M. and Talon, R.,** Cell location and partial characterization of *Brochothrix thermosphacta* and *Lactobacillus curvatus* lipases, *J. Appl. Bacteriol*., 66, 235, 1989.

77. **Geisen, R., Lücke, F. K., and Kröckel, L.,** Starter and protective cultures for meat and meat products, *Fleischwirtsch*., 72, 894, 1992.

78. **Nestorov, N., Dineva, B. Krastev, D., and Brankova, R.,** Changes in some biochemical indices characterizing the technological properties of fermented sausages manufactured using starter cultures, in *Proc. 30th Eur. Meet. Meat Res. Work*., Bristol, 1984, 340.

79. **Miteva, E., Kirova, E., Gadjeva, D., and Radeva, M.,** Sensory aroma and taste profiles of raw-dried sausages manufactured with a lipolytically active yeast culture, *Nahrung*, 30, 829, 1986.

80. **Papon, M., Talon, R., and Montel, M. C.,** Les flores lipolytiques des viandes et produits carnes, *Viandes Prod. Carnés*, 11, 49, 1990.

81. **Ferrer, J. and Arboix, P.,** The "Salchichón de Vich" (Vich sausage). II. Evolution of chemical parameter during the curing process and valoration of its organoleptic quality, in *Proc. 32nd Eur. Meet. Meat Res. Work*, Gent, 1986, 279.

82. **García, M. L., Selgas, M. D., Fernández, M., and Ordóñez, J. A.,** Microorganisms and lipolysis in the ripening of Dry-fermented sausages, *Int. J. Food Sci. Technol*., 27, 675, 1992.

83. **Hierro, E., de la Hoz, L., and Ordóñez, J. A.,** Contribution of microbial and meat endogenous enzymes to the lipolysis of dry-fermented sausages, *J. Agric. Food Chem*., 45, 2989, 1997.

84. **Foegeding, E. A., Lanier, T. C., and Hultin, H. O.,** Characteristics of edible muscle tissues, in *Food Chemistry*, Fennema, O. R., Ed., Marcel Dekker Inc., New York, 1996, 879.

85. **Christie, W. W. and Moore, J. H.,** Comparison of the structures of triglycerides from various pig tissues, *Biochim. Biophys. Acta*, 210, 46, 1970.

86. **Molly, K., Demeyer, D., Civera, T., and Verplaetse, A.,** Lipolysis in a Belgian sausage: relative importance of endogenous and bacterial enzymes, *Meat Sci*., 43, 235, 1996.

87 **Hayman, L. W. and Acton, J. C.,** Flavors from lipids by microbiological action, in *Lipids as a Source of Flavor*, Supran, M. K., Ed., A.C.S. Symp. Series, 75, 1978, 94.

88. **Paleari, M. A., Piantoni, L., and Caloni, F.,** Prove sull-impiego della lipasi nella preparazione dei salumi, *Ind. Aliment*., 30, 1072, 1991.

89. **Motilva, M. J., Toldrá, F., Nieto, P., and Flores, J.,** Muscle lipolysis phenomena in the processing of dry-cured ham, *Food Chem*., 48, 121, 1993.

90. **Motilva, M. J., Toldrá, F., Aristoy, M. C., and Flores, J.,** Subcutaneous adipose tissue lipolysis in the processing of dry-cured ham, *J. Food Biochem*., 16, 323, 1993.

91. **Lücke, F. K.,** Procesos microbiológicos en la elaboración de embutidos secos y jamones crudos, *Fleischwirtsch. español*, 2, 39, 1987.

92. **Francisco, J. J., Gutiérrez, L. M., Menes, I., García, M. L., Díez, V., and Moreno, B.,** Flora microbiana del jamón crudo curado, *Anal. Bromatol*., XXXIII, 259, 1981.

93. **Cantoni, C., d'Aubert, S., Bianchi, M. A., and Gianpaolo, L.,** La lipolisi nel grosso di copertura dei prosciutti, *Atti Soc. It. Sci. Vet*., 24, 504, 1970.

94. **Motilva, M. J. and Toldrá, F.,** Effect of curing agents and water activity on pork muscle an adipose tissue lipolytic activity, *Z. Lebensm. Unters. Forsch*., 196, 228, 1993.

95. **Toldrá, F.,** Desarollo de las características de textura y flavor: contribución enzimática, *in El jamón curado: tecnología y análisis de consumo, Special Symposium of the 44th Int. Congr. Meat Sci. Technol*., Arnau, J. and Monfort, J. M., Eds., Estrategias Alimentarias-Eurocarne, Barcelona, Spain, 1998, 41.

96. **Toldrá, F., Motilva, M. J., Rico, E. and Flores, J.,** Enzyme activities in the processing of dry cured ham, in *Proc. 37th Int. Congr. Meat Sci. Technol*., Kulmbach, 1991, 954.

97. **Toldrá, F., Rico, E., and Flores, J.,** Activities of pork muscle proteases in model cured meat systems, *Biochimie*, 74, 291, 1992.

98. **De Man, J. M.,** Chemical and physical properties of fatty acids, in *Fatty Acids in Foods and their Health Implications*, Chow, Ch. K., Ed., Marcel Dekker Inc., New York, 1992, 17.

99. **Keeney, M.,** *Secondary degradation products in lipids and their oxidation*, Schultz, H. W., Day, E. A., and Sinnhuber, R. O., Eds., AVI Publishing Comp. Westport, Conn., 1962.

100. **Allen, C. E. and Foegeding, E. A.,** Some muscle characteristics and interactions in muscle foods — a review, *Food Technol*., 35, 253, 1981.

101. **Hornstein, I. and Crowe, P. F.,** Meat flavor: lamb, *J. Agric. Food Chem*., 11, 147, 1963.

102. **Love, J. D. and Pearson, A. M.,** Lipid oxidation in meat and meat products — a review, *J. Am. Oil Chem. Soc.*, 48, 547, 1971.

103. **Wilson, B. R., Pearson, A. M., and Shorland, F. B.,** Effect of total lipids and phospholipids on warmed-over flavor in red and white muscles from several species as measured by TBA analysis, *J. Agric. Food Chem*., 24, 7, 1976.

104. **Asghar, A., Gray, J. I., Buckley, D. J., Pearson, A. M., and Booren, A. M.**, Perspectives on warmed-over flavor, *Food Technol*., 42, 102, 1988.

105. **Yasosky, J. J., Aberle, E. D., Peng, I. C., Mills, E. W., and Judge, M. D.,** Effects of pH and time of grinding on lipid oxidation of fresh ground pork, *J. Food Sci*., 49, 1510, 1984.

106. **Tichivangana, J. Z. and Morrissey, P. A.,** Myoglobin and inorganic metals as prooxidants in raw and cooked muscle systems, *Meat Sci*., 15, 107, 1985.

107. **Kwoh, T. L.,** Catalysis of lipid peroxidation in meats, *J. Am. Oil Chem. Soc*., 48, 550, 1971.

108. **Rhee, K. S., Dutson, T. R., and Smith, G. C.,** Enzymic lipid peroxidation in microsomal fractions from beef skeletal muscle, *J. Food Sci*., 49, 675, 1984.

109. **Rhee, K. S., Dutson, T. R., and Savell, J. W.,** Enzymic lipid peroxidation in beef muscle microsomes as affected by electrical stimulation and posmortem muscle excision time, *J. Food Biochem*., 9, 27, 1984.

110. **Lee, Y. B., Hargus, G. L., Kirkpatrick, J. A., Berner, D. L., and Forsythe, H.,** Mechanism of lipid oxidation in mechanically deboned chicken meat, *J. Food Sci.*, 40, 964, 1975.

111. **Kanner, J., Harel, S., and Granit, R.,** Oxidative processes in meat and meat products: quality implications, in *Proc. 38th Int. Congr. Meat Sci. Technol*., Clermont-Ferrand, 1992, 111.

112. **Zipser, M. W., Kwon, T. W., and Watts, B. M.,** Oxidative changes in cured and uncured frozen cooked pork, *J. Agric. Food Chem*., 12, 105, 1964.

113. **Rhee, K. S., Smith, G. C., and Terrell, R. N.,** Effect of reduction and replacement of sodium chloride on rancidity development in raw and cooked ground pork, *J. Food Prot*., 46, 578, 1983.

114. **Lücke, F. K.,** The microbiology of fermented meats, *J. Sci. Food Agric*., 36, 1342, 1985.

115. **Dierick, N., Vandekerckhove, P. V., and Demeyer, D.,** Changes in nonprotein compounds during dry sausage ripening, *J. Food Sci*., 39, 301, 1974.

116. **Martin, M.,** *Technologie du saucisson sec. Contribution a l'etude des phénoménes physico-chimiques*, Doctoral thesis, Universidad Paul Sabatier, Toulouse, France, 1975.

117. **Law, B. A.,** *Microorganisms and Nitrogen Sources*, Payne, J. W., Ed., John Wiley & Sons Ltd., Chichester, 1980.

118. **Khalid, N. M. and Marth, E. H.,** Lactobacilli — their enzymes and role in ripening and spoilage of cheese: a review, *J. Dairy Sci*., 73, 2669, 1990.

119. **Zourari, A., Accolas, J. P., and Desmazeaud, M. J.,** Metabolism and biochemical characteristics of yogurt bacteria. A review, *Le Lait*, 72, 1, 1992.

120. **Pritchard, G. G. and Coolbear, T.,** The physiology and biochemistry of the proteolitic system in lactic acid bacteria, *FEMS Microbiol. Rev*., 12, 179, 1993.

121. **Reuter, G.,** Laktobazillen und eng verwandte mikroorganismen in fleisch und fleischwaren. 6. Mitteilung: proteolytische enzymaktivitaten, *Fleischwirtsch*., 51, 945, 1971.

122. **Reuter, G.,** Classifications problems, ecology and some biochemical activities of lactobacilli of meat products, in *Lactic Acid Bacteria in Beverages and Foods*, Carr, J. G., Cutting, C. V., and Whiting, G. C., Eds., Academic Press, London, 1975, 221.

123. **Cantoni, C., d'Aubert, S., Bianchi, M. A., and Beretta, G.,** Alcuni aspetti del metabo-

lismo dei lattobacilli durante la maturazione degli insaccati stagionati (salami), *Ind. Aliment.*, 13, 88, 1975.

124. **Montel, M. C., Talon, R., Cantonnet, M., and Cayrol, J.,** Peptidasic activities of starter cultures, in *Proc. 38th Int. Congr. Meat Sci. Technol.*, Clermont-Ferrand, 1992, 811.

125. **Vignolo, G. M., de Ruíz Holgado, A. P., and Oliver, G.,** Acid production and proteolitic activity of *Lactobacillus* strains isolated from dry sausages, *J. Food Prot.*, 51, 481, 1988.

126. **Law, B. A. and Kolstad, J.,** Proteolytic systems in lactic acid bacteria, *Antoine van Leeuwenhoek*, 49, 225, 1983.

127. **Lücke, F. K. and Hechelmann, H.,** Cultivos starter para embutido seco y jamón curado. Composición y efecto, *Fleischwirtsch. español*, 1, 38, 1988.

128. **Bermell, S., Molina, I., Miralles, C., and Flores, J.,** Study of the microbial flora in dry-cured ham. VI. Proteolytic activity, *Fleischwirtsch.*, 72, 1684, 1992.

129. **Robertson, P. S. and Perry, K. D. S.,** Enhancement of flavor of Cheddar cheese by adding a strain of *Micrococcus* to the milk, *J. Dairy Res.*, 28, 245, 1961.

130. **Desmazeaud, M. and Hermier, J.,** Isolement, purification et propiétés d'une protéase exocellulaire de *Micrococcus caseolyticus*, *Ann. Biol. Anim. Biochem. Biophys.*, 8, 565, 1968.

131. **Bhowmik, T. and Marth, E. H.,** Esterolytic activities of *Pediococcus* spices, *J. Dairy Sci.*, 72, 2869, 1989.

132. **Sajber, C., Karakas, R., and Mitic, P.,** Influence of some starter cultures upon the change in protein of "stajer" sausages during fermentation, in *Proc. 17th Eur. Meet. Meat Res. Work.*, Bristol, 1971.

133. **Bacus, J. N.,** Fermented meat and poultry products, in *Advances in Meat and Poultry Microbiology*, Pearson, A. M. and Dutson, T. R., Eds., Macmillan, Loudres, 1986, 123.

134. **Chen, M. T. and Guo, S. L.,** Studies on the microbial flora of Chinese-style sausage. II. Action of selected organisms isolated from Chinese-style sausage on porcine muscle proteins, *Fleischwirtsch.*, 72, 1126, 1992.

135. **Creamer, L. K. and Olson, N. F.,** Rheological evaluation of maturing Cheddar cheese, *J. Food Sci.*, 47, 631, 1982.

136. **Trieu-Cuot, P. and Gripon, J. C.,** A study of proteolysis during Camembert cheese ripening using isoelectric focusing and two-dimensional electrophoresis, *J. Dairy Res.*, 49, 501, 1982.

137. **Lenoir, J. and Auberger, B.,** Activity of acid and neutral protease of *Penicillium caseicolum* in Camembert cheese ripening, in *Proc. XXI Int. Dairy Congr.*, Moscow, Vol. 1, Mir Publ. Moscow, 1982, 336.

138. **Leistner, L.,** Alimentos madurados con hongos, *Fleischwirtsch. español*, 1, 38, 1987.

139. **Grazia, L., Romano, P., Bagni, A., Roggiani, D., and Guglielmi, G.,** The role of molds in the ripening process of salami, *Food Microbiol.*, 3, 19, 1986.

140. **Trigueros, G., García, M. L., Casas, C., Ordóñez, J. A., and Selgas, M. D.,** Proteolytic and lipolytic activities of mold strains isolated from Spanish dry-fermented sausage, *Z. Lebensm. Unters. Forch.*, 201, 298, 1995.

141. **Toledo, V. M., Selgas, M. D., Casas, M. C., Ordóñez, J. A., and García, M. L.,** Effect of selected mold strains on the proteolysis in Dry-fermented sausages, *Z. Lebensm. Unters. Forsch.*, 204, 385, 1997.

142. **Verplaetse, A., Demeyer, D., Gerard, S., and Buys, E.,** Endogenous and bacterial proteolysis in dry sausage fermentation, in *Proc. 38th Int. Congr. Meat Sci. Technol.*, Clermont-Ferrand, 1992, 851.

143. **Demeyer, D., Claeys, E., Ötles, S., Caron, L., and Verplaetse, A.,** Effect of meat species on proteolysis during dry sausage fermentation, in *Proc. 38th Int. Congr. Meat Sci. Technol.*, Clermont-Ferrand, 1992, 775.

144. **Garriga, M., Calsina, M. D., and Monfort, J. M.,** Estudio de la proteolisis en la maduración de salchichones manufacturados con carne de porcino de buena calidad, *Invest. Agric.: Prod. Sanid. Anim.*, 3, 17, 1988.

145. **Hierro, E. M.,** *Origen y agentes responsables de las sustancias componentes del sabor y aroma de los embutidos*, Doctoral thesis, Facultad de Veterinaria, Universidad Complutense de Madrid, Spain, 1997.

146. **Díaz, O., Fernández, M., García de Fernando, G. D., de la Hoz, L., and Ordóñez, J. A.,** Effect of the addition of pronase E on the proteolysis in dry-fermented sausages, *Meat Sci.*, 34, 205, 1993.

147. **Díaz, O., Fernández, M., García de Fernando, G. D., de la Hoz, L., and Ordóñez, J. A.,** Effect of the addition of papain on the dry-fermented sausage proteolysis, *J. Sci. Food Agric.*, 71, 13, 1996.

148. **Toldrá, F., Rico, E., and Flores, J.,** Cathepsin B, D, H, and L activities in the processing of dry-cured ham, *J. Sci. Food Agric.*, 62, 157, 1993.

149 **Sárraga, C., Gil, M., and García-Regueiro, J. A.,** Comparation of calpain and cathepsin (B, L, and D) activities during dry-cured ham processing from heavy and light large white pigs, *J. Sci. Food Agric.*, 62, 71, 1993.

150. **Koohmaraie, M., Siedeman, S. C., Schollmeyer, J. E., Dutson, T. R., and Crouse, J. D.,** Effect of postmortem storage on Ca^{++}-dependent proteases, their inhibitor and myofibril fragmentation, *Meat Sci.*, 19, 187, 1987.

151. **Dransfield, E., Etherington, D. J., and Taylor, M. A. J.,** Modelling post-mortem tenderisation II: Enzyme changes during storage of electrically stimulated and non-stimulated beef, *Meat Sci.*, 31, 75, 1992.

152. **Sárraga, C., Gil, M., Arnau, J., Monfort, J. M., and Cusso, R.,** Effect of curing salt and phosphate on the activity of porcine muscle proteinases, *Meat Sci.*, 25, 241, 1989.

153. **Shahidi, F.,** Flavor of meat and meat products — an overview, in *Flavor of Meat and Meat Products*, Shahidi, F., Ed., Blackie Academic and Professional, London, 1994, 1.

154. **Crocker, E. C.,** The flavor of meat, *Food Res.*, 13, 179, 1948.

155. **Bender, A. E. and Ballance, P. E.,** A preliminary examination of the flavor of meat extract, *J. Sci. Food Agric.*, 12, 683, 1961.

156. **MacLeod, G. and Seyyedain-Ardebili, M.,** Natural and simulated meat flavors (with particular reference to beef), *CRC Crit. Rev. Food Sci. Nutr.*, 14, 309, 1981.

157. **Bailey, M. E.,** The Maillard reaction of meat flavor, in *The Maillard Reaction in Foods and Nutrition*, Waller, G. R. and Feather, M. S., Eds., American Chemical Society, ACS Symposium Series, 215, 1983, 169.

158. **Toldrá, F.,** Desarrollo del aroma y sabor de la carne y productos cárnicos. II. Reacciones químicas en la carne, *AICE*, 46, 8, 1994.

159. **Shahidi, F., Rubin, L. J., and D'Souza, L. A.,** Meat flavor volatiles: a review of the composition, techniques of analysis and sensory evaluation, *CRC Crit. Rev. Food Sci. Nutr.*, 24, 141, 1986.

160. **Shahidi, F.,** Flavor of cooked meats, in *Flavor Chemistry: Trends and Developments*, Teranishi, R., Buttery, R. E., and Shahidi, F., Eds., ACS Symposium Series, 388, 1989, 188.

161. **Buttery, R. G., Ling, L. C., Teranishi, R., and Mon, T. R.,** Roasted lamb fat: Basic volatile components, *J. Agric. Food Chem.*, 25, 1227, 1977.

162. **MacLeod, G.,** The flavor of beef, in *Flavor of Meat and Meat Products*, Shahidi, F., Ed., Blackie Academic and Professional, London, 1994, 4.

163. **Dwivedi, B. K.,** Meat flavor, *CRC Crit. Rev. Food Sci. Nutr.*, 5, 487, 1975.

164. **Maga, J. A.,** Flavor potentiators, *CRC Crit. Rev. Food Sci. Nutr.*, 18, 231, 1983.

165. **Moody, W. G.,** Beef flavor — a review, *Food Technol.*, 37, 227, 1983.

166. **Langner, H. J.,** Aroma substances in dry sausage, *Fleischwirtsch.*, 52, 1299, 1972.

167. **Hammer, G. F.,** The stabilising effect of various natural spices in dry sausage during storage, *Fleischwirtsch.*, 57, 1957, 1977.

168. **Mendiolea, R., Ledward, D., and Lawrie, R. A.,** Antioxidative activity of spices in intermediate moisture meats, in *Proc. 36th Int. Congr. Meat Sci. Technol.*, La Habana, 1990, 896.

169. **Berdagué, J. L., Monteil, P., Montel, M. C., and Talon, R.,** Effects of starter cultures on the formation of flavor compounds in dry sausage, *Meat Sci.*, 35, 275, 1993.

170. **Burgos, J.,** Factores tecnológicos que controlan la calidad de los embutidos crudos y maduros, *Filón*, diciembre, 16, 1981.

171. **Hinrichsen, L. L. and Pedersen, S. B.,** Relationship among flavor, volatile compounds,

chemical changes, and microflora in Italian-type dry-cured ham during processing, *J. Agric. Food Chem.*, 43, 2932, 1995.

172. **Croizet, F., Denoyer, C., Tran, N., and Berdagué, J. L.,** Les composès volatils du saucisson sec. Evolution au cours de la maturation, *Viandes Prod. Carnés*, 13, 167, 1992.

173. **Ockerman, H. W., Blumer, T. N., and Craig, H. B.,** Volatile chemical compounds in dry-cured hams, *J. Food Sci.*, 29, 123, 1964.

174. **Ramaswamy, H. S. and Richards, J. F.,** Flavor of poultry meat — a review, *Can. Inst. Food Sci. Technol. J.*, 15, 7, 1982.

175. **Hamilton, R. J.,** The chemistry of rancidity in foods, in *Rancidity in Foods*, Allen, J. C. and Hamilton, R. J., Eds., Elsevier Applied Science, Elsevier Science Publishers LTD, London, 1989, 1.

176. **Langner, H. J., Heckel, U., and Malek, E.,** Aroma substances in ripening dry sausage, *Fleischwirtsch.*, 50, 1193, 1970.

177. **Halvarson, H.,** Formation of lactic acid, volatile fatty acids and neutral, volatile monocarbonyl compounds in Swedish fermented sausage, *J. Food Sci.*, 38, 310, 1973.

178. **Frankel, E. N.,** Chemistry of autoxidation. Mechanism, products and flavor significance, in *Flavor Chemistry of Fats and Oils*, Min, D. B. and Smouse, T. H., Eds., American Oil Chemists' Society, Champaign, 1985, 1.

179. **Kinsella, S. E. and Hwang, D.,** Biosynthesis of flavors by *Penicillium roqueforti*, *Biotechnol. Bioeng.*, 18, 927, 1976.

180. **Weber, H.,** Elaboración de embutidos secos. La importancia de los denominados cultivos protectores y sus productos metabólicos, *Fleischwirtsch. español*, 2, 34, 1994.

181. **Hirai, C., Herk, K. O., Pokorny, J., and Chang, S. S.,** Isolation and identification of volatile flavor compounds in boiled beef, *J. Food Sci.*, 38, 397, 1973.

182. **Belitz, H. D. and Grosch, W.,** *Química de los Alimentos*, Acribia, Zaragoza, 1997.

183. **García, C., Berdagué, J. L., Antequera, T., López-Bote, C., Córdoba, J. J., and Ventanas, J.,** Volatile components of dry cured Iberian ham, *Food Chem.*, 41, 23, 1991.

184. **MacLeod, P. and Morgan, M. E.,** Differences in the ability of lactic streptococci to form aldehydes from certain aminoacids, *J. Dairy Sci.*, 41, 908, 1958.

185. **Berdagué, J. L., Denoyer, C., Le Quere, J. L., and Semon, E.,** Volatile components of dry-cured ham, *J. Agric. Food Chem.*, 39, 1257, 1991.

186. **Van Straten, S.,** *Volatile Compounds in Food*, 4th ed., CIVO-TNO Publisher, Zeist, The Netherlands, 1977.

187. **Rembold, H., Wallner, P., Nite, S., Kollmannsberger, H., and Drawert, F.,** Volatile components of chickpea (*Cicer arietinum* L.) seed, *J. Agric. Food Chem.*, 37, 659, 1989.

188. **Barbieri, G., Bolzoni, L., Parolari, G., Virgili, R., Buttini, R., Careri, M., and Mangia, A.,** Flavor compounds of dry-cured ham, *J. Agric. Food Chem.*, 40, 2389, 1992.

189. **Berdagué, J. L., Bonnaud, N., Rousset, S., and Touraille, C.,** Volatile compounds of dry cured ham: identification and sensory characterisation by sniffing, in *Proc. 37th Int. Congr. Meat Sci. Technol.*, Kulmbach, 1991, 1135.

190. **Viallon, C., Berdagué, J. L., Denoyer, C., Tran, N., Bonneau, M., and Le Denmat, M.,** Analysis of volatile components from backfat of pig and relations with androstenone content, in *Proc. 38th Int. Congr. Meat Sci. Technol.*, Clermont-Ferrand, 1992, 987.

191. **Buscailhon, S., Berdagué, J. L., and Monin, G.,** Time-related changes in volatile compounds of lean tissue during processing of French dry-cured ham, *J. Sci. Food Agric.*, 63, 69, 1993.

192. **Wittkowski, R.,** Analysis of liquid smoke and smoked meat volatiles by headspace gas chromatography, in *Agriculture, Food Chemistry and the Consumer*, in *Proc. 5th Eur. Conf. Food Chem.*, INRA, Versailles, Vol. 2, 1989, 639.

193. **Min, D. B., Ina, K., Peterson, R. J., and Chang, S. S.,** Preliminary identification of volatile flavor compounds in the neutral fraction of roast beef, *J. Food Sci.*, 44, 639, 1979.

194. **Kato, H., Rhue, M. R., and Nishimura, T.,** Role of free amino acids and peptides in food

taste, in *Flavor Chemistry. Trends and Developments*, Teranishi, R., Buttery, R. G., and Shahidi, F., Eds., ACS Symposium Series, 388, 1989, 158.

195. **MacLeod, A. J.,** Instrumental methods of meat flavor analysis, in *Flavor of Meat and Meat Products*, Shahidi, F., Ed., Blackie Academic and Professional, London, 1994, 231.

196. **Yamaguchi, S.,** The umami taste, in *Food Taste Chemistry*, Boudreau, J. C., Ed., ACS Symposium Series, 115, 1979, 33.

197. **Fuke, S. and Shimizu, T.,** Sensory and preference aspects of umami, *Trends Food Sci. Technol*., 4, 246, 1993.

198. **Flores, J.,** Potenciadores del sabor en la industria cárnica, *AICE*, 44, 5, 1994.

199. **Maga, J. A.,** Umami flavor of meat, in *Flavor of Meat and Meat Products*, Shahidi, F., Ed., Blackie Academic and Professional, London, 1994, 98.

200. **Kirimura, J., Shimizu, A., Kimizuka, A., Ninomiya, T., and Katsuya, N.,** The contribution of peptides and amino acids to the taste of foodstuffs, *J. Agric. Food Chem*., 17, 689, 1969.

201. **Lemieux, L. and Simard, R. E.,** Bitter flavor in dairy products. II. A review of bitter peptides from caseins: their formation, isolation and identification, structure masking and inhibition. *Le Lait*, 72, 335–382, 1992.

202. **Nishimura, T. and Kato, H.,** Taste of free amino acids and peptides, *Food Rev. Int.*, 4, 175, 1988.

203. **Pereira-Lima, C. I.,** *Identificación de las sustancias responsables del sabor de los caldos de carne de vacuno*, Doctoral thesis, Facultad de Veterinaria, Universidad Complutense de Madrid, Spain, 1996.

204. **Rice, S. and Koehler, P. E.,** Tyrosine and histidine decarboxylases activities of *Pediococcus cerevisiae* and *Lactobacillus* especies and the production of tyramine in fermented sausages, *J. Milk Food Technol*., 39, 166, 1976.

205. **Rice, S. L., Eitenmiller, R. R., and Koehler, P. E.,** Biologically active amines in food: a review, *J. Milk Food Technol*., 39, 353, 1976.

206. **Ten Brink, B., Damink, C., Joosten, H. M. L. J., and Huis In't Veld, J. H. J.,** Occurence and formation of biologically active amines in foods, *Int. J. Food Microbiol*., 3, 73, 1990.

207. **Bardúcz, S.,** Polyamines in food and their consequences for food quality and human health, *Trends Food Sci. Technol.*, 6, 341, 1995.

208. **Koehler, P. E. and Eitenmiller, R. R.,** High pressure liquid chromatographic analysis of tyramine, phenylethylamine and tryptamine in sausage, cheese and chocolate, *J. Food Sci*., 43, 1245, 1978.

209. **Tabor, H., Rosenthal, S. M., and Tabor, C. W.,** Biosynthesis of spermidine and spermine from putrescine and methionine, *J. Biol. Chem*., 233, 907, 1958.

210. **Spinelli, A. M., Lakritz, L., and Wasserman, A. E.,** Effects of processing on the amine content of pork bellies, *J. Agric. Food Chem*., 22, 1026, 1974.

211. **Blackwell, B. and Mabbit, L. A.,** Tyramine in cheese related to hypertensive crises after MAO inhibition, *Lancet*, 1, 938, 1965.

212. **Sandler, M., Youdin, M. B. H., and Hanington, E.,** A phenylethylamine oxidizing defect in migraine, *Nature* (London) 250, 335, 1974.

213. **Hanington, E., Horn, M., and Wilkinson, M.,** *Migraine Symp. 3rd*, London, Heinemann Medical Books, London, 1970.

214. **Bjeldanes, L. F., Schutz, D. E., and Morris, M. M.,** On the aetiology of scombroid poisoning: cadaverina potentiation of histamine toxicity in the guinea-pig, *Food Cosmet. Toxicol*., 16, 157, 1978.

215. **Hotchkiss, J. H., Scanlan, R. A., and Libbey, L. M.,** Formation of bis (hidroxyalkyl)-*N*-nitrosamines as products of the nitrosation of spermidine, *J. Agric. Food Chem*., 25, 1183, 1977.

216. **Halász, A., Barath, A., Simon-Sarkadi, L., and Holzapfel, W.,** Biogenic amines and their production by microorganisms in food, *Trends Food Sci. Technol*., 5, 42, 1994.

217. **Pfannhauser, W. and Pechanek, U.,** Biogenic amines in foods: formation, ocurrence, analysis and toxicological evaluation, *Z. Ges. Hyg*., 30, 66, 1984.

218. **Askar, A. and Treptow, H.,** *Biogene Amine in Lebensmitteln. Vorkommen, Bedeutung und Bestimmung*, Eugen Ulmer GmbH & Co., 1986, Stuttgart.

219. **Teuber, M.,** *Progress in Food Fermentation*, Benedito de Barber, C., Cellar, C., Martínez-Anaya, M. A., and Morell, J., Eds., IATA CSIC Valencia, vol. 1, 1993, 16.

220. **Voigt, M. N. and Eitenmiller, R. R.,** Production of tyrosine and histidine decarboxylase by dairy-related bacteria, *J. Food Prot*., 40, 241, 1977.

221. **Dainty, R. H., Edwards, R. A., Hibbard, C. M., and Ramantanis, S. V.,** Bacterial sources of putrescine and cadaverine in chill stored vacuum-packaged beef, *J. Appl. Bacteriol*., 61, 117, 1986.

222. **Sumner, S. S. and Taylor, S. L.,** Detection method for histamine-producing, dairy-related bacteria using diamine oxidase and leucocrystal violet, *J. Food Prot*., 52, 105, 1989.

223. **Zee, J. A., Simard, R. E., Vaillancourt, R., and Boudreau, A.,** Effect of *Lactobacillus brevis, Saccharomyces uvarum* and grist composition on amine formations in beers, *Can. Inst. Food Sci. Technol. J.*, 14, 321, 1981.

224. **Lagerborg, V. A. and Clapper, W. E.,** Amino acid decarboxylases of lactic acid bacteria, *J. Bacteriol*., 63, 393, 1952.

225. **Edwards, R. A., Dainty, R. H., Hibbard, C. M., and Ramantanis, S. V.,** Amines in fresh beef of normal pH and the role of bacteria in changes in concentration observed during storage in vacuum packs at chill temperatures, *J. Appl. Bacteriol*., 63, 427, 1987.

226. **Rice, S. L., Eitenmiller, R. R., and Koehler, P. E.,** Histamine and tyramine content of meat products, *J. Milk Food Technol*., 38, 256, 1975.

227. **Santos-Buelga, C., Peña-Egido, M. J., and Rivas-Gonzalo, J. C.,** Changes in tyramine during chorizo-sausage ripening, *J. Food Sci*., 51, 518, 1986.

228. **Maijala, R., Nurmi, E., and Fischer, A.,** Influence of processing temperature on the formation of biogenic amines in dry sausages, *Meat Sci*., 39, 9, 1995.

229. **Tschabrun, R., Sick, K., Bauer, F., and Kranner, P.,** Formation of histamine in sliceable raw sausages, *Fleischwirtsch*., 70, 448, 1990.

230. **Hudson-Arnold, S. and Duane-Brown, W.,** Histamine toxicity from fish products, in *Advances in Food Research*, C.O. Chichester, Mrak, E. M. and Schweigert, B. S., Eds., Academic Press Inc., Orlando, vol. 24, 1978, 113.

231. **Straub, B. W., Tichaczek, P., Kicherer, M., and Hammes, W. P.,** Formation of tyramine by *Lactobacillus curvatus* LTH972, *Tagung der Deutschen Gesellschaft für Hygiene und Mikrobiologie*, München 43, 317, 1991.

232. **Maier, H. G.,** Volatile flavoring substances in foodstuffs, *Angew. Chem*., 9, 917, 1970.

233. **Hartmann, T.,** Biosynthesis of volatile amines in apples by aldehyde amination, *Z. Pflanzenphysiol*., 57, 368, 1967.

234. **Moat, A. G. and Foster, J. W.,** *Microbial Physiology*, a Wiley-Interscience Publication, John Wiley & Sons, Inc. New York, 1988.

235. **Andersen, H. J. and Hinrichsen, L. L.,** Changes in curing agents, microbial counts and volatile compounds during processing of green bacon using two different production technologies, *J. Sci. Food Agric*., 68, 477, 1995.

236. **Stahnke, L. and Zeuthen, P.,** Identification of volatiles from Italian dried salami, in *Proc. 38th Int. Congr. Meat Sci. Technol*., Clermont-Ferrand, 1992, 835.

237. **Hinrichsen, L. L. and Andersen, H. J.,** Volatile compounds and chemical changes in cured pork: role of three halotolerant bacteria, *J. Agric. Food Chem*., 42, 1537, 1994.

238. **MacLeod, P. and Morgan, M. E.,** Leucine metabolism of *Streptococcus lactis* var. *maltigenes*. I. Conversion of alpha-ketoisocaproic acid to leucine and 3 methylbutanal, *J. Dairy Sci.*, 38, 1208, 1955.

239. **Ventanas, J., Córdoba, J. J., Antequera, T., García, C., López-Bote, C., and Asensio, M. A.,** Hydrolysis and Maillard reactions during ripening of Iberian Ham, *J. Food Sci*., 57, 813, 1992.

240. **Edwards, R. A., Dainty, R. H., and Ordóñez, J. A.,** Volatile compounds of microbial origin in dry, fermented Spanish sausage, *Poster, 2nd Int. Symp. on the Interface between Analytical Chemistry and Microbiology-Chromatography and Mass Spectrometry in Microbiology*, Lund, 1991.

241. **Dainty, R. and Blom, H.,** Flavor chemistry of fermented sausages, in *Fermented Meats*, Campbell-Platt, G. and Cook, P. E., Eds., Blackie Academic and Professional, Suffolk, 1995, 176.

242. **Stahnke, L. H.,** Aroma components from dried sausages fermented with *Staphylococcus xylosus*, *Meat Sci*., 38, 39, 1994.

243. **Bills, D. D., Morgan, M. E., Libbey, L. M., and Day, E. A.,** Identification of compounds responsible for fruity flavor defect of experimental Cheddar cheeses, *J. Dairy Sci*., 48, 1168, 1965.

244. **Hosono, A., Elliot, J. A., and McGugan, W. A.,** Production of ethyl esters by some lactic acid and psychrotrophic bacteria, *J. Dairy Sci*., 57, 535, 1974.

245. **McGugan, W. A., Blais, J. A., Boulet, M., Giroux, R. N., Elliott, J. A., and Emmons, D. B.,** Ethanol, ethyl esters, and volatile fatty acids in fruity Cheddar cheese, *Can. Inst. Food Sci. Technol. J*., 8, 196, 1975.

246. **Bodrero, K. O., Pearson, A. M., and Magee, W. T.,** Evaluation of the contribution of flavor volatiles to the aroma of beef by surface response methodology, *J. Food Sci*., 46, 26, 1981.

247. **Cheftel, J. C. and Cheftel, H.,** *Introducción a la Bioquímica y Tecnología de los Alimentos*, Volumen I, Acribia, Zaragoza, 1982.

248. **Berger, R. G., Macku, C., German, J. B., and Shibamoto, T.,** Isolation and identification of dry salami volatiles, *J. Food Sci*., 55, 1239, 1990.

249. **Salzer, U. J.,** The analysis of essential oils and extracts (oleoresins) from seasonigs. -A critical review, *CRC Crit. Rev. Food Sci. Nutr*., 9, 345, 1977.

250. **Russell, G. F. and Else, J.,** Volatile compositional differences between cultivars of black pepper (*Piper nigrum*), *J. Assoc. Off. Anal. Chem*., 56, 344, 1973.

251. **Govindarajan, V. S.,** Pepper. Chemistry, technology and quality evaluation, *CRC Crit. Rev. Food Sci. Nutr*., 9, 115, 1977.

252. **Richard, H. M. J.,** Spices and condiments I, in *Volatile Compounds in Foods and Beverages*, Maarse, H., Ed., Marcel Dekker Inc., New York, 1991, 411.

253 **Lawrence, B. M.,** Major tropical spices: pepper (*Piper nigrum* L.), in *Essential Oils* 1979-1980, Allured Publ. Corp., Wheaton, MD., 1981, 140.

254. **Richard, H.,** Varietal studies on some volatile constituents of black pepper nigra, Doctoral Thesis, University of California, Davis, 1970.

255. **Analytical Methods Committee,** Application of gas-liquid chromatography to the analysis of essential oils. XI. Monographs for seven essential oils, *Analyst*., 109, 1343, 1984.

256. **Debrauwere, J. and Verzele, M.,** Constituents of peppers. IV. The hydrocarbons of pepper essential oil, *J. Chromatogr. Sci*., 14, 296, 1976.

257. **Debrauwere, J. and Verzele, M.,** New constituents of the oxygenated fraction of pepper essential oil, *J. Sci. Food Agric.*, 26, 1887, 1976.

258. **Whitfield, F. B. and Last, J. H.,** Vegetables, in *Volatile Compounds in Food and Beverages*, Maarse, H., Ed., Marcel Dekker Inc., New York, 1991, 209.

259. **Stoll, A. and Seebeck, E.,** ‹ber alliin, die genuine muttersubstanz des knoblauchˆls, *Helv. Chim. Acta*, 31, 189, 1948.

260. **Huffman, V. L., Schadle, E. R., Villalon, B., and Burns, E. E.,** Volatile components and pungency in fresh and processed Jalapeno peppers, *J. Food Sci*., 43, 1809, 1978.

261. **Buttery, R. G., Seifert, R. M., Guadagni, D. G., and Ling, L. C.,** Characterization of some volatile constituents of bell peppers, *J. Agric. Food Chem*., 17, 1322, 1969.

262. **Buttery, R. G.,** Vegetable and fruit flavors, in *Flavor Research*, Teranishi, R., Flath, R. A., and Sugisawa, H., Eds., Marcel Dekker, New York, 1981, 175.

263. **Varga, L., Fekete, M., and Kozma, L.,** Quantitative determination by computerized spectrum analysis of the pigment components in ground paprika, *Acta Alimentaria*, 13, 295, 1984.

264. **Fisher, C. and Kocis, J. A.,** Separation of paprika pigments by HPLC, *J. Agric. Food Chem*., 35, 55, 1987.

265. **Mateo, J. and Zumalacárregui, J. M.,** Volatile compounds in chorizo and their changes during ripening, *Meat Sci.*, 44, 255, 1996.

266. **El-Soda, M.,** Accelerated maturation of cheese, *Int. Dairy J*., 3, 513.

267. **Zalacain, I., Zapelena, M. J., Astiasarán, I., and Bello, J.,** Dry-fermented sausages elaborated with lipase from *Candida cylindracea.* Comparison with traditional formulations, *Meat Sci*., 40, 55-61, 1995.

268. **Zalacain, I., Zapelena, M. J., Astiasarán, I., and Bello, J.,** Addition of lipase from *Candida cylindracea* to a traditional formulation of a dry-fermented sausage, *Meat Sci.*, 42, 155–163, 1996.

269. **Zalacain, I., Zapelena, M. J., Paz de Peña, M., Astiasarán, I., and Bello, J.,** Use of lipase from *Rhizomucor miehei* in dry-fermented sausages elaboration: microbial, chemical and sensory analisis, *Meat Sci.*, 45, 99–105, 1997.

270. **Zalacain, I., Zapelena, M. J., Paz de Peña, M., Astiasarán, I., and Bello, J.,** Application of Lipozyme 10,000 L (from *Rhizomucor miehei*) in dry-fermented sausage technology: study in a pilot plant and at the industrial level, *J. Agric. Food Chem*., 45, 1972-1976, 1997.

271. **Fernández, M., Hoz, L., Díaz, O., Cambero, I., and Ordoñez, J. A.**, Effect of the addition of pancreatic lipase on the ripening of dry-fermented sausages. Part I. Microbial, physicochemical and lipolitic changes, *Meat Sci*., 40, 159–170, 1995.

272. **Fernández, M., Hoz, L., Díaz, O., Cambero, I., and Ordoñez, J. A.**, Effect of the addition of pancreatic lipase on the ripening of dry-fermented sausages. Part II. Free fatty acids, short chain fatty acids, carbonyls and sensorial quality, *Meat Sci.*, 40, 351–362, 1995.

273. **Naes, H., Holck, A. L., Axelsson, L., Andersen, H. J., and Blom, H.,** Accelerated ripening of dry-fermented sausage by addition of a *Lactobacillus* proteinase. *Int. J. Food Sci. Technol.*, 29, 651–659, 1995.

274. **Blom, H., Hagen, B. F., Pedersen, B. O., Holck, A. L., Axelsson, L., and Naes, H.,** Accelerated production of dry-fermented sausage, *Meat Sci*., 43, S229-S242, 1996.

275. **Zapelena, M. J., Zalacain, I., Paz de Peña, M., Astiasarán, I., and Bello, J.,** 1997. Addition of a neutral proteinase from *Bacillus subtilis* (Neutrase) together with a starter to a dry-fermented sausage elaboration and its effect on the amino acid profiles and the flavor development, *J. Agric. Food Chem*., 45, 472–475, 1997.

276. **Melendo, J. A., Beltrán, J. A., Jaime, I., Sancho, R., and Roncalés, P.,** Limited proteolysis of myofibrilar proteins by bromelain decreases toughness of coarse dry sausage, *Food Chem.*, 57, 429–433, 1996.