Chemical and Microbiological Changes during Sausage Fermentation and Ripening

S. A. PALUMBO and J. L. SMITH

Eastern Regional Research Center, Agricultural Research Service,
U.S. Department of Agriculture, Philadelphia, Pa. 19118

Introduction

The production of sausage began as one of man's earliest attempts at food preservation, possibly as far back as 1500 B.C., when people learned that meat would not spoil if it were finely chopped, mixed with salt and spices, and allowed to dry in rolls. Salami is thought to be named after the city of Salamis on the east coast of Cyprus (1), an early producer of such products. With the passage of time, each individual region developed its characteristic sausage as evidenced in Italy by the well-known Genoa and the lesser known Milano, Sorrento, Lombardi (2), and Siciliano salamis (1). Sausage production involves three of the oldest forms of food preservation: salting, drying, and smoking.

The red or pink color typical of cured meat products, was first noted in Roman times (3). This color was subsequently found to be due to nitrate, occurring as an impurity in the salt, which is reduced by bacteria to nitrite; the nitrite then reacts with the meat pigment myoglobin to give the pink color, nitrosylmyoglobin. The nitrosylmyoglobin is subsequently denatured to give the stable form, nitrosohemochrome.

It was only in the early part of the 20th century that various bacteria (with their enzymes) were discovered to be the agents responsible for two changes that occur during production of dry fermented sausage: lactic acid production and nitrate reduction. Traditionally, the addition of the two bacterial types, i.e., lactic acid bacteria and micrococci (nitrate reducing), was left to chance. Much of the natural flora was contributed by the processor when leftover material from a previous batch was added ("back-slopping") or by reuse of equipment after limited cleaning. The meat was handled in some

[1]Agricultural Research Service, U.S. Department of Agriculture.

fashion to permit the development of its own starter culture. This often involved low temperature holding of meat, plus salt and cure in the form of nitrate, in shallow pans to permit the growth of nitrate reducing micrococci with subsequent formation of nitrite to effect curing. After the pan cure, sugars and spices were mixed in and the meat mixture was ground and stuffed into casings. The stuffed sausages were then held in the "green room" where lactic acid bacteria fermented the sugars and some drying occurred. The sausages were then moved into the dry room where further dehydration took place. Much of the chance has been eliminated in modern sausage manufacture by two processing advances: the use of nitrite alone in cures, and the addition of lactic acid starter cultures containing lactobacilli and/or pediococci.

The original development of starter cultures for use in dry fermented sausages followed separate routes in the U.S. and Europe (4). American sausage makers utilized nitrite cures and thus needed to add only lactic acid bacteria. Originally the culture, Pediococcus cerevisiae, was lyophilized (5); now it is available as a frozen concentrate (6). European workers favored cultures that would reduce nitrate such as a strain of Micrococcus, M 53, available as "Baktofermente" (Rudolf Müller Co.[2], Giessen, Germany). The Europeans changed their views when they observed that the addition of lactobacilli along with the micrococci gave better color development and a somewhat more acid product (7). This mixed culture is available as "Duplofermente," also from R. Müller.

The processing of dry fermented sausages occurs in three principal steps: formulation, fermentation, and drying or ripening. Palumbo et al. (8) described a process for the preparation of pepperoni as a fully dry sausage. During formulation of pepperoni, salt, glucose, spices, and cure (nitrate or nitrite) are mixed with the meats and stuffed into casings. An aging period (before addition of other ingredients) of salted meat is included to encourage the development of micrococci and lactobacilli (8, 9). The fermentation period at 35°C and 90% RH is one to three days, depending on the desired pH decrease. After fermentation, the sausages are dried at 12°C and 55-60% RH for six weeks. Major microbiological activity occurs during the second and third steps. In the second step, micrococci reduce nitrate to nitrite within the first 24 hr. of fermentation (9, 10,) with concomitant formation of nitrosylmyoglobin; lactobacilli convert glucose to lactic acid somewhat more slowly than nitrate is reduced, often requiring three days to lower the pH to 4.6-4.7 from a starting value of 5.6 (8).

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[2]Reference to brand or firm name does not constitute endorsement by the U.S. Department of Agriculture over others of a similar nature not mentioned.

During the third step, drying (or ripening as it is known in the European literature), the lipids and proteins of the sausage are attacked by various bacteria and their enzymes. The sausages lose 20-40% of their starting (green) weight (11). In the older production of sausage, fermentation usually occurred in the green room, generally held at a somewhat higher temperature than the dry room, and some dehydration occurred there. The sausage was then moved to the dry room where further drying occurred, along with desired flavor and textural changes. In the United States, drying room times and temperatures for sausages of a given diameter are specified by the requirements for trichinae inactivation (12).

Although there are almost as many sausage types as there are sausage makers and geographic areas, some classification of their types and characteristics is possible. Fermented sausages are usually described either as semi-dry (loss of 8-15% of starting weight) and fully dry, or simply dry, (loss of 25 to 40% of starting weight). Examples of semi-dry sausages include thuringer, cervelat, Lebanon bologna, and pork roll; examples of dry sausage include summer sausage, pepperoni, and various salamis (11). Some varieties, however, such as pepperoni, can be prepared as both dry and semi-dry forms. As a group, the Italian sausages are quite spicy, not smoked, and have moisture contents of 35-40%, while the northern European varieties are smoked and have somewhat higher moisture contents (13). Dry sausages can also be described by the maximum moisture/protein ratio (M/P) expected for individual varieties (14): salami, 1.9/1; pepperoni, 1.6/1; and Genoa, 2.3/1. (A M/P < 1.6/1 generally indicates a shelf stable product.) Today, the identity of many dry fermented sausage varieties is blurred and processors are marketing products with two or three varietal names. A sausage labelled "thuringer-cervalat-summer sausage" was observed recently in a local market.

Most of the enzymes that produce the changes during fermentation and ripening of sausages are considered to be associated with bacterial cells, located either within the cells, as in the case of glycolytic enzymes, or outside the cells, as in the case of lipases and proteases. Because of the nature of meat, it is not possible to "pasteurize" or "sterilize" it before sausage manufacture. Thus, it is not possible to do for sausage ripening what Reiter *et al*. (15) did for cheddar cheese ripening; they established that, because the milk was pasteurized, the added bacterial starter culture, with its enzymes, was responsible for the observed changes such as proteolysis, lipolysis, and flavor development that occur during ripening of the cheese.

Alteration of Sausage Components by Bacteria and Their Enzymes

Fermentation. There are two major changes produced in sausages by fermentation: sugars are converted into lactic acid and nitrate is reduced to nitrite.

Lactic acid production. The major activity of the lactic acid bacteria is the conversion of sugars, usually glucose, to lactic acid by the homolactic Embdem-Myerhoff pathway. In their investigation of the stoichiometry of carbohydrate fermentation in Belgian salami, DeKetelaere *et al*. (16) found that lactic acid was the major acid formed, with minor amounts of acetic, generally in the molar ratio of 10:1 lactic to acetic. They also detected trace amounts of butyric and propionic. Our data for pH decrease and acid production during fermentation of pork-beef pepperoni are shown in Fig. 1. (There is only a very slight pH change in pepperoni during the drying step (8).) Similar data were obtained for Lebanon bologna during fermentation.

The extent to which producers of commercial dry fermented sausage achieve a low pH (acid) product is illustrated in Table I. Some varieties such as Lebanon bologna and summer sausage have low pH values, while others such as pepperoni and Isterband have both higher pH values and a wide range of pH values. Often European dry sausages have pH values indicating virtually no acid production. Their formulations usually have little or no glucose added. For example, Niinivaara *et al*. (4) added only three grams glucose to 100 kg meat and obtained a pH of 5.3, while Mihalyi and Kormendy (17) added no glucose to their formulation of Hungarian dry sausage and observed a pH of their finished sausage of 6.28.

Table I

pH Values of Some Commercial Fermented/Dry Sausages

Sausage/Type	pH Range	Processors	Reference
Belgian salami	4.48 - 5.10	10	16
Pepperoni	4.7 - 6.1	9	8
Isterband	4.6 - 5.7	10	32
Lebanon bologna	4.6 - 4.9	6	9
Summer sausage	4.6 - 5.0	12	33
Salami	5.1 - 5.5	8	10
Thuringer	4.9 - 5.1	4	10

Nitrate reduction. Many studies have been performed with micrococci, the bacteria that carry out the reduction. Micrococci developed during cold (5°C) aging of salted pork and beef for pepperoni (8) (Fig. 2) and beef for Lebanon bologna (18) and declined during pepperoni fermentation (Fig. 3). A further decline of micrococci occurred during the drying of pepperoni. About midway through the drying period, when the titratable acidity reached 1%, viable micrococci were no longer detected (8). The acid content of the pepperoni was concentrated as the sausages dehydrated in the dry room, thus destroying the acid sensitive micrococci. However, micrococci are usually isolated from less acid pepperoni (8). DeKetelaere *et al*. (16) stated that micrococci were present through the ripening period of Belgian salami, even though its acid content was above 1%. They utilized a salt-containing medium (S 110 agar), as we did in our study of pepperoni during drying. However, we also employed the gram stain to examine representative colonies on the medium and thus observed that while the count on the medium remained constant, micrococci colonies were replaced by bacilli colonies. It is important for investigators to verify the colony types on the selective agars and not rely solely on the supposed specificity of a given medium.

The reduction of nitrate occurs within the first 24 hr of the fermentation period, usually during the first 2 to 16 hr (10). Zaika *et al*. (19) observed that formation of most of the cured meat pigment (nitrosylmyoglobin) occurred within the first 24 hr also. They indicated that the micrococci of the natural flora are capable of reducing various levels of nitrate with satisfactory nitrosylmyoglobin formation (Fig. 4). With the lowest level of nitrate tested, 100 ppm, pigment conversion was somewhat slow; levels of 200 up to 1600 ppm nitrate gave equally satisfactory pigment conversion.

The need for nitrate reducing flora in fermented sausage production has been superseded by the use of nitrite. Cures containing only nitrite are adequate for the normal processing of most sausages; however, many sausage makers feel that dry fermented sausages must be cured with nitrate. The occasional lack of natural flora of micrococci that possess desired levels of nitrate reductase was overcome by Niinivaara and coworkers (4) who isolated strains of micrococci having intense nitrate reductase activity, along with other traits desirable for the production of dry fermented sausages.

Bacteria or, more specifically, the end products of their metabolism, can produce two color defects in fermented sausages: "nitrite burn" and "greening." Reliance on microbial reduction of nitrate to yield the nitrite necessary for the curing reactions can lead to instances in which various micrococci and non-pathogenic staphylococci reduce excessive amounts of nitrate (20). The resulting excess nitrite reacts with the meat pigments to give a greenish discoloration. This defect usually occurs on

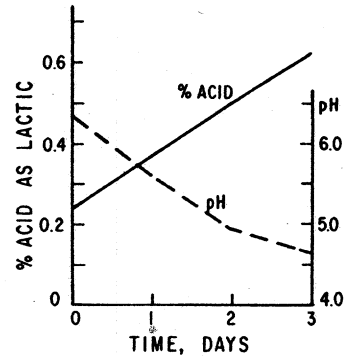

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Figure 1. Changes in pH and % acid (as lactic) during the fermentation of a pork-beef pepperoni at 35°C (8)

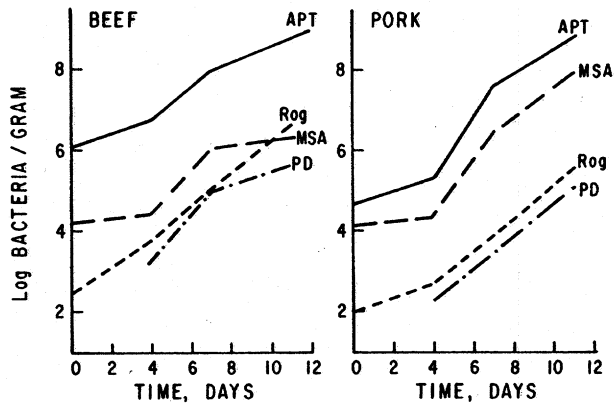

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Figure 2. Changes in microflora plated on various media during aging of salted (3% NaCl) beef and pork at 5°C. Media designations: APT *(for total count);* Rog *(Rogosa SL agar for lactic acid bacteria);* MSA *(Mannitol salt agar for micrococci);* PD *(Potato Dextrose agar for yeast) (8)*

the product surface where the aerobic bacteria proliferate. Another surface color defect which also yields a green pigment is caused by Lactobacillus viridescens (21) growing on the surface of cured meat products, especially emulsion and low acid sausages. The organism produces excessive amounts of H_2O_2 (22) which reacts with nitrosylhemochrome to give a green pigment.

Drying (Ripening). Deibel (13) stated that "Essentially, the microbiology associated with the (fermented sausage) product is restricted to the green room" (the fermentation). Palumbo et al. (8) reported that the total count and the number of lactobacilli remained essentially constant during the drying period of pepperoni, while micrococci declined to undetectable levels. DeKetelaere et al. (16) reported that the number of lactobacilli and micrococci remained constant during ripening of Belgian salami. Palumbo et al. (8), Smith and Palumbo (18), and Deibel et al. (10) reported that various commercial dry fermented sausages contained large numbers of viable bacteria. Thus, dry fermented sausage do contain, after processing, large numbers of viable bacteria. Because of the acidity and low water activity of these products, the bacteria cannot multiply.

However, microbial caused changes do occur during the drying or ripening stage. Recent studies on dry fermented sausages have indicated that bacteria and their enzymes can attack various sausage components during the ripening period, so that while the different bacterial populations appear to be stable, important changes are occurring in the lipid and protein components of the sausage. The end products of microbial action on lipids and proteins include compounds that give each individual sausage type its unique flavor and textural characteristics. Recent studies, especially by various European researchers, have yielded some interesting findings on the fate of the lipid and protein components of dry fermented sausages.

Lipolysis. Of the two components present in dry sausages, fat is higher in concentration than protein. In products such as pepperoni fat can comprise more than 50% of the final product weight (8). The fat in many sausage formulations is pork fat.

As indicated above, micrococci are important in reducing nitrate in dry fermented sausages. Work at our laboratory (23) and Cantoni's (24) indicated that micrococci can degrade fresh lard. Smith and Alford (23) found that, of the various microbial cultures surveyed, Micrococcus freudenreichii was the most active in attacking components of fresh lard. This organism substantially increased the peroxide value, and the 2-enals and the 2,4-dienals of fresh lard; further, though not as active as other cultures tested, M. freudenrichii also increased the alkanal content of fresh lard. Thus, M. freudenrichii markedly increased the carbonyl content of the fresh lard, probably by cleaving the unsaturated fatty acids at the site of the double bonds.

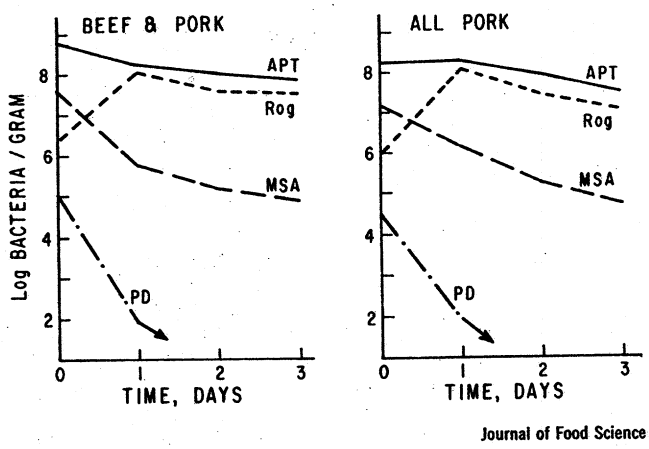

Figure 3. Changes in microflora plated on various media during the fermentation of a pork-beef and an all-pork pepperoni at 35°C (see Figure 2 for media designations) (8)

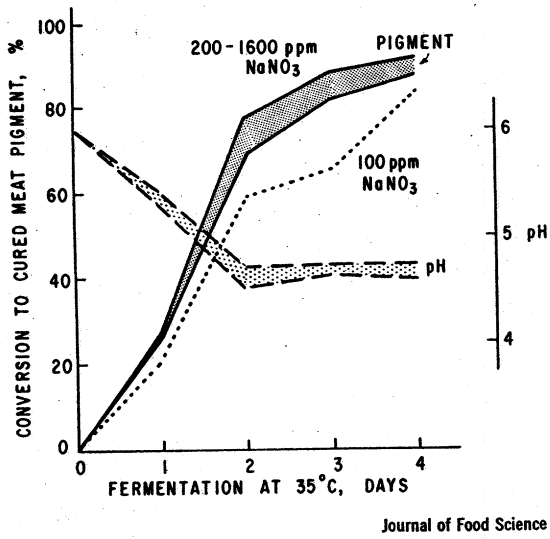

Figure 4. Formation of cured-meat pigment and changes in pH in Lebanon bolognas prepared with 100, 200, 400, 800, and 1600 ppm $NaNO_3$ during fermentation with natural flora (19)

Cantoni et al. (24), studying the action of micrococci on lard (Table II), indicated that micrococci are very active in degrading lard. The organisms produced lipases which cleave both long and short chain fatty acids from the triglycerides. Micrococci can degrade long chain unsaturated fatty acids as indicated by the formation of carbonyls.

The presence of micrococci in finished sausages such as pepperoni (8), as well as others (16, 18), and the work of Smith and Alford (23) and Cantoni et al. (24) on the action of pure cultures of micrococci on lard suggest that the lipids of various sausages should be actively attacked during ripening. Data on various European sausage type support this contention. The most complete study appears to be the work of Demeyer et al. (25) on Belgium salami; other workers have also studied this attack (4, 17, 26).

Table II

Action of Micrococci on Pork Fat (from Reference 24)

	Culture	
	D10	C13
C_4 - C_{20}, g FA/100 g fat[a]	23.1	45.2
(FA most easily released: oleic, myristic, palmitoleic, linoleic)		
Volatile FA, mg/100 g fat[b]	87	50
(Principal VFA: propionic, acetic)		
Carbonyls, μM/1 of culture[c]	660	667
(Prinicpal carbonyls: propionaldehyde, isovaleraldehyde)		

[a]After 28 days of culture; FA - fatty acid.

[b]After 28 days of culture; VFA - volatile fatty acid.

[c]After 24 days of culture.

Demeyer et al. (25) observed increases in the free fatty acid (FFA) and carbonyls of Belgian salami during ripening. Triglycerides were partially degraded to FFA (Fig. 5) and the unsaturated FFA's were degraded into carbonyls. The triglyceride content decreased with a corresponding increase of FFA's and diglycerides (DG); though based on limited hydrolysis, these data suggest that the enzymes of the microflora removed only one fatty acid from the triglyceride molecule. In a rate study of limited duration, they quantitated and identified the FFA's released (Fig. 6). Though the actual changes were relatively small, the rate of lipolysis appeared to decease in the order: linoleic (18:2) > oleic (18:1) > stearic (18:0) > palmitic (16:0). The FFA distribution (Fig. 6) suggests that the micrococcal lipase has specificity for triglycerides containing linoleic acid.

Demeyer et al. (25) then went on to quantitate the total carbonyl content of fat of the sausage during ripening. Using two methods (benzidine derivatives and D.N.P. hydrozones), they observed a several-fold increase in total carbonyls during ripening. They did not record changes in individual carbonyls, as did Halvorson (26) in his study of Isterband, a Swedish fermented sausage. Halvorson observed significant changes in n-hexanals, n-octanals, and some of the 2-alkenals during ripening; these compounds can contribute to the aroma of the finished sausages. He also detected increases in the level of several short chain branched carbonyls which could arise from amino acids by the Strecker degradation.

Though most lipase activity is associated with micrococci, it has also been reported in lactobacilli (Fryer et al. (27); Oterholm et al. (28)). These investigators used strains of lactobacilli of dairy origin and observed very active degradation of tributyrin, with relatively little activity against other triglycerides. However, the activity of meat lactobacilli against pork fat (lard) and other fats present in dry sausages needs further study.

Proteolysis. After the lipids, the most plentiful component of dry fermented sausages is protein. Less work has been done on the changes in the nitrogenous compounds released during sausage ripening, although progress has been made. Mihalyi and Kormendy (17) reported a 7% decrease in total protein and a 36% increase in non-protein nitrogen (NPN) occurring from the 10th to the 100th day of ripening of Hungarian dry sausage. It was not possible to determine whether the proteolytic enzymes were of meat or microbial origin. They also reported changes in the sacroplasmic and myofibrillar fractions, but since the methods utilized were developed for fresh (not cured or dried) meat, the significance of these changes cannot be properly accessed.

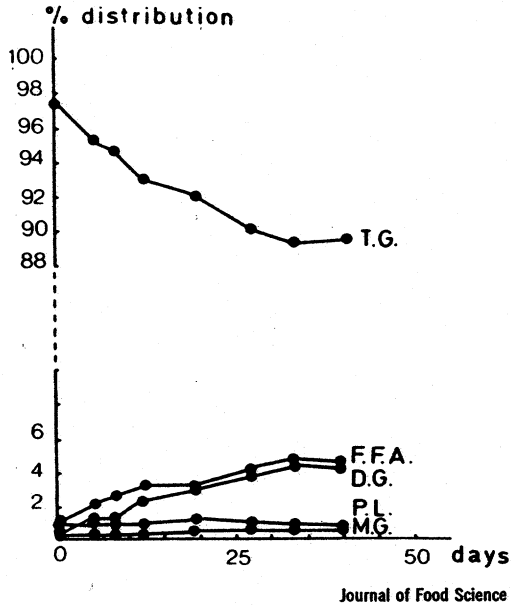

Figure 5. Changes in fatty acid distribution over lipid classes (%) during ripening of Belgian salami: M.G. *(monoglycerides),* D.G. *(diglycerides),* T.G. *(triglycerides),* F.F.A. *(free fatty acids), and* P.L. *(polar lipids)* (25)

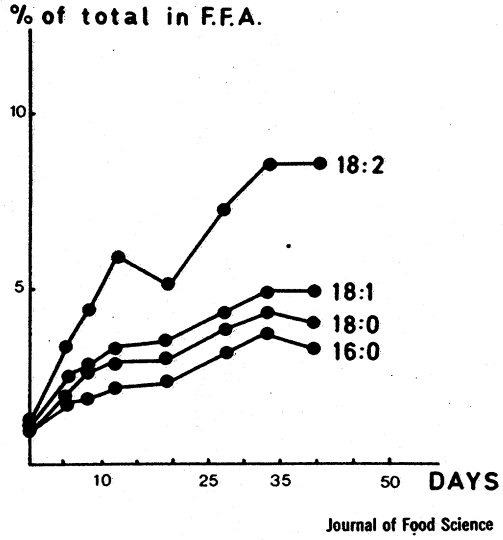

Figure 6. Percent of total palmitic (16:0), stearic (18:0), oleic (18:1), and linoleic (18:2) acid present in F.F.A. (25)

Dierick et al. (29) studied the changes in the NPN fractions during the ripening of Belgian salami (Table III) and found an increase of free ammonia from deamination of amino acids and an increase of free α-amino nitrogen (amino acids) from the breakdown of proteins. Peptide-bound nitrogen decreased slightly, with a decrease in nucleotide nitrogen and an increase in nucleoside nitrogen.

Relative changes in the concentration of free amino acids (Table IV), for the sake of convenience, are presented in three groups: those amino acids showing a large increase during ripening, those acids showing a small increase, and those showing a decrease. Of unusual interest is the decrease of glutamic acid that was added to the sausage mix in the form of MSG (monosodium glutamate). Even though it was added for flavor, a considerable part of the glutamate was degraded by the time the sausage was fully ripened and ready for consumption.

Table III

Concentration of Nonprotein Nitrogen Compounds during Ripening of Belgian Salami (from Reference 29)

	Days of Ripening		
	0	15	36
NH_3	24*	58	76
Free α-NH_2-N	141	234	255
Peptide bound α-NH_2-N	161	152	145
Nucleot.-N	34	13	12
Nucleos.-N	33	78	83

*mg N/100 g of dry matter.

Table IV

Changes in Free Amino Acids During 36 Days of Ripening of Belgian Salami (from Reference 29)

Large Increase, >12X	Small Increase, <12X	Decrease
Thre	Asp, Ala, Ileu	Glu
Pro	Ser, Val, Phen	His
Leu	Gly, Met, Lys	Tyr
γ-NH_2-BA		Orn

Dierick *et al*. (29) observed a decrease in histidine (his), tyrosine (tyr), and ornithine (orn) concentrations and corresponding increases in the concentrations of histamine, tyramine, and putrescine, the decarboxylation products of his, tyr, and orn. Rice and Koehler (30) attempted to identify the organism(s) that possess tyr and his decarboxylase activities. They investigated the lactic acid starter culture organisms, including strains of lactobacilli, streptococci, and *Pediococcus cerevisiae*. Only the streptococci showed tyr decarboxylase activity; however, streptococci are not usually starter cultures for fermented sausages and the significance of this finding cannot be adequately evaluated. They did not test other organisms such as micrococci that are present in many fermented sausages in large numbers and could conceivably carry out this metabolic activity.

Despite their inability to locate the source of decarboxylase activity, Rice *et al*. (31) surveyed various dry fermented sausages for their histamine and tyramine content (Table V). The histamine content of most sausages was low. Emulsion-type products such as bologna weiners were low, while braunschweiger, because of its liver content, was slightly higher than ground beef. In contrast, tyramine was found in larger quantities in a variety of dry fermented sausages. Those sausages containing the higher concentrations could provide sufficient tyramine in moderate servings to produce pressor responses in tyramine susceptible individuals.

Table V

Amine Content of Various Sausages

(from Reference 31)

Histamine	Avg.	Tyramine	Avg.
Dry sausage	2.87*	Hard salami	210*
Semidry sausage	3.59	Pepperoni	39
Bologna	1.89	Summer sausage	184
Weiners	1.75	Farmers sausage	314
Braunschweiger	3.60	Genoa salami	534
Ground beef	2.70	Smoked land-Jaeger	396
		Lebanon bologna	224

*μg/g of sausage.

Virtually all the changes during sausage fermentation and ripening are enzyme-catalyzed and most of the enzymes are of bacterial origin. The various types of changes reported here have not all been studied in a single sausage type; however, where overlapping studies exist, their results are similar. In summary, bacteria and their enzymes: ferment sugars to lactic acid; reduce nitrate to nitrite; degrade triglycerides to fatty acids and cleave unsaturated fatty acids and amino acids to form various carbonyls which can contribute to flavor. Proteins are degraded to various simpler nitrogen-containing compounds such as amino acids, ammonia, and amines by both meat and microbial enzymes.

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