**THE CHEMISTRY OF DRY SAUSAGES**

J. C. Acton*

**INTRODUCTION**

The characteristics of semi-dried and dried sausages are numerous because of the many types available, the varieties within any type, and the raw materials used in their preparation. As denoted from the word "dried," a considerable difference in the moisture content is present in this class of sausage products. The moisture content generally falls between 50% to 45% for the semi-dried types and 35% or lower for the dried-types.

Dried and semi-dried sausages may be made from pork, mixtures of pork and beef, or from beef, depending on the particular product category desired. Two major added ingredients are recognized as being necessary for proper preparation: NaCl and carbohydrates, usually glucose and/or sucrose.

The major function of the salt is to set up a "screen" against most of the normal raw meat microflora, thus allowing lactic acid bacteria to become the predominant bacterial group. In the finished product it also serves with flavor contribution and preservative effects, particularly in the presence of a low moisture content of dried sausages.

The major function of added carbohydrate is to provide a substrate for the biological acidulation by lactic acid bacteria. The acidity produced in fermentation results in a characteristic "tangy" flavor and development of proper texture, sometimes described as chewy. Furthermore, the metabolites of carbohydrate breakdown play an important role in aroma formation. In the finished product, lactic acid, in combination with salt, exerts preservative effects to dry sausages.

Preparation methods for semi-dry and dry sausages have been noted by many researchers to be either traditional processes or starter culture processes (Table 1). Traditional processes in the pre-1940 period were based on natural fermentation by the indigenous lactic acid bacteria present in the meat microflora or those introduced from equipment. A form of inoculation in traditional processes occurred when a portion of a recently fermented meat batch was added to a freshly prepared batch, a procedure frequently referred to as "back-slopping." Following the successful development and use of starter cultures in cheese fermentations, attempts were made to develop starter cultures for meat fermentations. Jensen and Paddock (1940) first introduced starter cultures for sausage fermentations, but the majority of the bacteria were dairy-type starters and did not grow well in the meat environment.

The primary genera of bacteria which have been used for cultures are *Micrococcus* (Niinivaara, 1955; Nurmi, 1966), *Lactobacillus* (Nurmi, 1966; Everson et al., 1970), and *Pediococcus* (Deibel and Niven, 1957). *Micrococcus* are often added due to their nitrate reducing activity while the lactobacilli and pediococci are responsible for the acidification of sausage. Currently single bacteria cultures and mixed cultures of these are available in lyophilized form and in a frozen concentrate.

The fermentation phase in the traditional process, whether occurring by chance contaminants and the natural flora or starter culture induced, is longer. Generally, the fermentation is a gradual process lasting over a 3 to 7 day period (Table 1) and controlled by temperature. Such a fermentation constitutes the "ripening" phase in traditional processes. However, use of starter cultures do permit a more rapid fermentation phase of from 32-40 hr using lyophilized cultures and 18-24 hr using the frozen concentrate cultures. Rapid fermentations are conducted at higher temperatures (generally 30°C to 37°C) than in the "ripening" for the traditional process (generally 15°C to 22°C). A typical comparison of the rate of acidity development in both processes is shown in Figure 1.

Starter cultures have been promoted on the basis of control of the fermentation progression to prevent development of off-flavors, gassiness, proteolysis, slime formation, lack of acid production, and incidences of "greening." More desirable and uniform product characteristics are attained from batch to batch.

Following "ripening" in the traditional senses, or rapid fermentation with starters, sausages are then either subjected to some further heating, in the case of semi-dry sausages, or placed in an air-conditioned drying room, in the case of fully dried sausages. Some

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TABLE 1

PREPARATION METHODS OF FERMENTED AND DRIED SAUSAGES

<table>
<thead>
<tr>
<th>Method Category</th>
<th>Fermentation Microflora</th>
<th>&quot;Active&quot; Fermentation Period</th>
</tr>
</thead>
<tbody>
<tr>
<td>Traditional or Natural Process</td>
<td>indigenous lactobacilli (fermenters) micrococci (nitrate reducers)</td>
<td>3 to 7 days</td>
</tr>
<tr>
<td>Starter Culture Process</td>
<td>added lactobacilli (fermenters) pediococci (fermenters) micrococci (nitrate reducers)</td>
<td>32 to 48 hours (freeze-dried) 15 to 20 hours (frozen concentrates)</td>
</tr>
</tbody>
</table>

varieties of heated sausages may also be further dried.

CHEMICAL CHARACTERISTICS

General Composition

As previously indicated, the moisture content of various sausage types covers a wide range. A recent survey of the composition of dried sausages retailed in our area of South Carolina is presented in Table 2. Quantities of each chemical constituent reflect the end result of the degree of drying practiced for each type product listed. In many studies, the increase of protein, fat, ash, and salt content have been significantly (P<0.05) correlated to the decrease in moisture content during sausage dehydration (Acton and Keller, 1974; Keller et al., 1974; Lu and Townsend, 1973; Wardlaw et al., 1973). The compositional data of Table 2 agrees with other published values (Palumbo et al., 1973; Palumbo, et al., 1976; Rice, 1971).

Moisture

The initial moisture content in sausage preparations will vary due to the type and quantity of meat tissues and trimmings used. Although added moisture may or may not be a part of the formulation, the orderly removal of moisture after drying is of primary concern to the processor.

Kramlich (1971) stated that the pH of sausages after fermentation should be near 5.1 to ensure satisfactory removal of moisture from the sausages on drying. In most studies, weight loss of sausages on drying has been used as the measurement of the rate or amount of moisture lost. The results of Townsend et al. (1975) using dry salami products with pH chemically-adjusted to 6.6 and 5.5 and a control sausage of pH 5.9 showed no practical differences in weight loss over a 24 day period. Similarly, the results of Palumbo et al. (1976) with pepperoni of pH values ranging from 6.1 to 4.7, showed no direct dependence of weight loss with pH. However, Acton and Keller (1974) found that summer sausages subjected to drying with a pH in the range of 5.9 - 5.5 lost weight significantly slower than sausages in a pH range of 4.8 - 4.6. It was demonstrated (Figure 2) by measurements of the water holding capacity of a sausage mix undergoing fermentation, that the water holding capacity decreased to a minimum at pH 5.2. In view of these reports, it is possible that the pH effect in controlling moisture loss is not critical as long as pH range difference of approximately 0.4 - 0.6 units is under scrutiny, but, the effect is still open to question. The higher water holding capacity of sausage mixes at a pH lower than 5.2 (Figure 2) suggests that some protein does remain functional to bind moisture and was not completely denatured, in this case, by a 39°C fermentation temperature over a 24 hr period.

In a study of other variables affecting moisture loss on drying of pepperoni (Palumbo et al., 1976), the meat particle size obtained by grinding through a
TABLE 2

AVERAGE COMPOSITION AND CHARACTERISTICS OF SOME COMMERCIAL DRY SAUSAGES

<table>
<thead>
<tr>
<th>Product Typeb</th>
<th>Moisture (%)</th>
<th>Fat (%)</th>
<th>Ash (%)</th>
<th>Salt (%)</th>
<th>Protein N (%)</th>
<th>NPN (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lebanon bologna (5)</td>
<td>58.92 ± 2.84</td>
<td>15.12 ± 0.72</td>
<td>4.30 ± 0.57</td>
<td>3.53 ± 0.65</td>
<td>2.55 ± 0.23</td>
<td>0.47 ± 0.04</td>
</tr>
<tr>
<td>Thuringer (13)</td>
<td>48.14 ± 1.93</td>
<td>29.77 ± 2.80</td>
<td>3.58 ± 0.43</td>
<td>2.74 ± 0.32</td>
<td>2.26 ± 0.16</td>
<td>0.32 ± 0.04</td>
</tr>
<tr>
<td>Semi dry salami (8)</td>
<td>46.62 ± 4.67</td>
<td>30.24 ± 3.79</td>
<td>4.36 ± 0.40</td>
<td>4.09 ± 0.55</td>
<td>2.40 ± 0.12</td>
<td>0.35 ± 0.10</td>
</tr>
<tr>
<td>Summer sausage (19)</td>
<td>46.34 ± 5.56</td>
<td>31.06 ± 5.61</td>
<td>3.74 ± 0.52</td>
<td>3.15 ± 0.51</td>
<td>2.27 ± 0.15</td>
<td>0.35 ± 0.07</td>
</tr>
<tr>
<td>Genoa salami (8)</td>
<td>36.21 ± 2.57</td>
<td>33.67 ± 1.77</td>
<td>5.84 ± 0.62</td>
<td>4.54 ± 0.54</td>
<td>3.04 ± 0.24</td>
<td>0.58 ± 0.08</td>
</tr>
<tr>
<td>Dry salami (11)</td>
<td>35.49 ± 3.02</td>
<td>34.04 ± 3.50</td>
<td>5.43 ± 0.68</td>
<td>4.67 ± 0.53</td>
<td>3.02 ± 0.19</td>
<td>0.54 ± 0.05</td>
</tr>
<tr>
<td>Pepperoni (14)</td>
<td>28.50 ± 3.83</td>
<td>42.96 ± 3.73</td>
<td>5.14 ± 0.49</td>
<td>4.43 ± 0.74</td>
<td>2.91 ± 0.25</td>
<td>0.43 ± 0.04</td>
</tr>
<tr>
<td>S.F. dry salami (4)</td>
<td>26.36 ± 3.39</td>
<td>38.08 ± 2.26</td>
<td>5.94 ± 0.17</td>
<td>4.70 ± 0.52</td>
<td>3.83 ± 0.47</td>
<td>0.42 ± 0.04</td>
</tr>
</tbody>
</table>

aData from Acton and Dick (1976)
bNumber of samples analyzed given in parentheses
cShipped from California.

The casing diameter or sausage size has a significant effect on the rate of drying for most products (Keller et al., 1974; Palumbo et al., 1976), with larger sizes drying more slowly (Figure 3). Length of the sausage or sausage weight does not influence weight loss.

The percent fat has a significant effect on weight loss (Table 3). Palumbo et al. (1976) reported that with pepperoni, as the fat content of initial mixes increase, sausages will lose less moisture, because there is less moisture in the initial mix. Thus the yield results are higher.

Small effects on yield of pepperoni were reported (Palumbo et al., 1976) for variation in meat type (pork, beef, and blends), cure type (none, nitrate, nitrite, and combination), and post-fermentation heating (no heating, 49°C, 60°C). They are probably insignificant in their effect on moisture loss, however other sausage characteristics such as flavor and texture are probably affected by these variables. Interestingly, salt levels from 0-4% had no effect on yields, but did affect sausage diameter when 0% salt (49.5 ± 0.5 mm) was compared to 1% through 4% salt levels (42 ± 2 mm). Kramlich (1971) has stated that salt addition should occur toward the end of sausage mixing or blending to ensure proper bind but not to the extent that large amounts of proteins are extracted. He stated that..."This procedure helps assure the orderly removal of moisture from the sau-
AMERICAN MEAT SCIENCE ASSOCIATION

Comparison of percent moisture of the inner and outer portions of fermented sausages of varying casing diameters during the drying phase (Keller et al., 1974).

FIGURE 3

TABLE 3

EFFECT OF MEAT PARTICLE SIZE (GRINDER PLATE) AND INITIAL FAT CONTENT ON SAUSAGE YIELD FOR A PORK BEEF PEPPERONI DRIED 42 DAYS.

<table>
<thead>
<tr>
<th>Variable No.</th>
<th>Plate size (inch)</th>
<th>Percent fat</th>
<th>Sausage Yield, %</th>
<th>Mean ± S.E.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3/16c</td>
<td>13.3</td>
<td>48.0±</td>
<td>0.10</td>
</tr>
<tr>
<td>2</td>
<td>3/16c</td>
<td>17.4</td>
<td>53.0±</td>
<td>0.30</td>
</tr>
<tr>
<td>3</td>
<td>3/16c</td>
<td>25.1</td>
<td>58.25±</td>
<td>1.05</td>
</tr>
</tbody>
</table>

*aAdapted from Polumbo et al. (1976).

*bMeans within a column having one of the same letters are not significantly different at the 95% confidence level. S.E. = standard error of mean.

cSimilar data were obtained for sausages prepared from meat fat mixtures ground through 3/8 and 5/8 in. plates.

sage interior during drying.* No studies have been conducted to ascertain whether this be true or not.

A point here should be noted about weight losses and the relationship to moisture-to-protein ratios. Once a specified moisture-to-protein ratio is attained during drying, there should be no reason to continue drying a product. A proper adjustment of the moisture and fat content of an initial meat selection, key to standard drying room criteria of airflow, humidity, and temperature, can determine the minimum time requirements for moisture removal. This can have economic meaning in terms of product yield being at its maximum in the minimum of time as long as the moisture-to-protein ratio is attained. Moisture-to-protein ratios are the most useful criteria to judge the amount of moisture removal necessary for the various types of dried product categories. For example, a moisture-to-protein ratio of 1.9 to 1.0, the maximum permitted for Italian dry salami, and generally a moisture-to-protein ratio of no greater than 1.9 to 1.0 and a pH of 5.0 or lower is necessary for a product, by USDA permission, to be marketed in a nonrefrigerated state.

FATS AND THE ROLE OF FATS IN FLAVOR DEVELOPMENT

The type of fat used in the preparation of dry sausage will influence flavor characteristics, particularly as the source of fat shifts from being all beef origin to all pork origin. However, species-specific flavors in cured products may not be as important as they are in uncured products. The distinctive flavors of dry sausages are due in part to the hydrolytic and oxidative changes that occur in the lipid fraction during ripening. Other product flavor characteristics are definitely associated with spicing combinations, salting, acidity developed for "tanginess," residual sugars, and the amount of smoking practiced (Deibel, 1974).

Hydrolytic Changes

The hydrolytic changes in fats are due mainly to the action of bacterial lipases, resulting in variable quantities of free fatty acids and glycerol. In the case of non-heated dry sausages, it is possible that some hydrolysis may occur through muscle and adipose tissue lipases (Wallach, 1968). The flavor developed by lipase activity depends on the composition of the fat. Hydrolysis of pork and beef lipids generally results in the production of long-chain free fatty acids which do not contribute substantially to off-flavors unless fatty acid oxidation also occurs (Bennion, 1972).

Micrococci are generally accepted as being the predominant group of micro-organisms responsible for hydrolysis of fats in dry sausage (Cantoni et al., 1967), but recent studies show that some species of lactobacilli produce very active lipases at 20°C and 37°C (Stoychev et al., 1972a, 1972b; Coretto, 1965). Lipases preferentially hydrolyze the outer fatty acids of a triglyceride molecule (Alford et al., 1971).

There is extensive literature that demonstrates that the quantity of free fatty acids increases during ripening. Mihalyi and Kormendy (1967) reported an in-
crease in free fatty acid values in both the inner and outer zones of a Hungarian dry salami (of freshly chopped pork and bacon) aged for 100 days (Table 4). The outer zone showed a higher level of fatty acids than the inner zone and this was attributed to the possible lipolytic activity of surface mold covering their product. It is important to note that their product never exceeded a temperature of 15°C during preparation, smoking and subsequent drying. Lu and Townsend (1973) prepared a dry salami of coarse ground pork which was inoculated with *Pediococcus acidilactici* and fermented at 38°C for 18 hr, and then held at 13°C for 35 days for drying. Their sausage also showed an increase in free fatty acid values during the drying period and had parallel increases in peroxide values (Table 5).

Demeyer et al. (1974) recently reported that of the fatty acids distributed in the triglycerides of a dry sausage containing predominantly pork fat, linoleic acid was liberated at a faster rate than all of the other acids (Figure 4). Brockerhoff (1966) has shown that in pig fat triglycerides, most of the stearic acid (approx. 60%) is located at position 1, palmitic acid (approx. 60-80%) at position 2, and octadecenoic acids (approx. 50-60%) are located at position 3 on the triglyceride molecule. Demeyer et al. (1974) found that the rate of hydrolysis to *free fatty acids* decreased in the following order based on their content in the total free fatty acid fraction: linoleic > oleic > stearic > palmitic. This clearly indicated specificity of hydrolysis at position 3 of the triglyceride molecule.

The difference observed for linoleic and oleic acids was attributed to positional and/or structural specificity, a characteristic known to exist for microbial lipases (Alford et al., 1971).

During ripening of an Italian "pure pork" salami, Cerise et al. (1973) reported that oleic acid was the principal free fatty acid found in the lipid fraction. Dohbertin et al. (1975) examined fresh Mettwurst for lipolysis evidence during various periods of storage at several temperatures. They found yeasts, pseudomonads, and enterococci which exhibited a high lipase activity while the lactobacilli present showed no evidence of lipase activity. They further concluded

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**TABLE 4**

<table>
<thead>
<tr>
<th>Days of Ripening</th>
<th>Sausage Portion</th>
<th>Inner zone</th>
<th>Outer zone</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td></td>
<td>6.07</td>
<td>3.88</td>
</tr>
<tr>
<td>40</td>
<td></td>
<td>11.28</td>
<td>12.40</td>
</tr>
<tr>
<td>70</td>
<td></td>
<td>14.81</td>
<td>18.29</td>
</tr>
<tr>
<td>100</td>
<td></td>
<td>17.68</td>
<td>20.62</td>
</tr>
</tbody>
</table>

*Adapted from Mihalyi and Kormendy (1967).*

**TABLE 5**

<table>
<thead>
<tr>
<th>Days of Ripening</th>
<th>Free Fatty Acid Value</th>
<th>Peroxide Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>3.08</td>
<td>10.3</td>
</tr>
<tr>
<td>14</td>
<td>6.39</td>
<td>17.4</td>
</tr>
<tr>
<td>21</td>
<td>6.90</td>
<td>12.9</td>
</tr>
<tr>
<td>28</td>
<td>6.56</td>
<td>16.9</td>
</tr>
<tr>
<td>35</td>
<td>11.66</td>
<td>20.6</td>
</tr>
</tbody>
</table>

*Calculated from data for control sausages in study of Lu and Townsend (1973).*

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Percent of total palmitic (16:0), stearic (18:0), oleic (18:1) and linoleic (18:2) acid present in the free fatty acid fraction (Demeyer et al., 1974).
that the degree of lipase activity is independent of the total bacterial count.

Cerise et al. (1973) proposed that the ratio of free oleic acid at time “t” of drying to the free oleic acid content at time “t = 0” could be used as an indicator of lipolytic activity. This may serve some value, particularly if the sausage is not subjected to mild heating after fermentation since Coretti (1965) found that some lactobacilli appear to form lipase activity adaptively in later generations.

At the current time it appears that the quantity of free fatty acids generated is not directly related to the length of a drying period. Rather it appears that the quantity is primarily determined by the nature of the sausage microflora, either developed or controlled via inoculation, in the early fermentation phase and drying period.

Oxidative results

In the report of Cerise et al. (1973), using a traditional ripening process, there were two distinct phases of lipid changes. In the early ripening phase of fermentation, lipolysis occurred to yield free fatty acids, which in turn were oxidized by peroxides to carbonyl compounds in the drying phase. As the carbonyls were formed, there was a reduction in the amount of some free fatty acids as well as peroxides.

A generalized scheme of this sequence of events can be summarized as shown in Figure 5. Here we note that as peroxides begin to accumulate, and in the presence of free fatty acid generation from lipolysis, we have a good possibility for oxidation of the unsaturated fatty acids. As oxidation occurs, generally there is a decrease in peroxide values. Carbonyl content increases as a result of the oxidation of fatty acids.

The fact that peroxide values decrease rapidly after the fermentation phase was also shown (Figure 6) by Cerise et al. (1972). Peroxide values increased dramatically between the second and fourth days (fermentation of 3 days at 21°C) and remained high through 8 days before decreasing to below initial levels at 15 days of ripening. At day 4, the sausage had begun the drying phase at 12°C. The peroxide values were correlated with the formation of free fatty acids.

The source of peroxide in sausage preparations is from bacterial metabolism (Lamanna and Mallette, 1965) and more frequently occurs in aerobic growth conditions. Nurmi (1966) reported nearly equivalent amounts of peroxide formation at 3 days of sausage ripening with micrococci and/or lactobacilli. However, after the 3 day period of “active” fermentation, sausages containing micrococci decreased in peroxide content while sausages containing lactobacilli continued to show peroxide presence at higher levels than initially observed. Both micrococci and lactobacilli can be strong producers of peroxide. However, while micrococci are catalase-positive (denoting ability to convert H₂O₂ to H₂O), lactobacilli are generally catalase-negative although catalase-positive strains are known. Nurmi (1966) pointed out that faulty product flavor and color may occur when catalase-negative lactobacilli are used as starters for sausage ripening and further suggested that catalase-positive lactic bacteria should be isolated for fermented sausage use.

There are three factors that might be associated in
dry sausages to contribute to fatty acid oxidation following lipolysis:

1. The increase in peroxide content as a result of bacterial metabolism as previously noted;
2. The increase in NaCl (prooxidant) content during drying;
3. The change in pH value from near 6.0 to pH 5.0 to 4.8 (as evidenced from fresh pork sausage rancidity study of Watts and Peng (1947)).

The influence of pH as related to peroxide accumulation and fatty acid oxidation in dry sausage has not been investigated.

Hydrogen peroxide, as formed by bacterial metabolism, is an available reactant in the oxidation of free fatty acids generated by lipolysis. In addition, fatty acid oxidation may also result from autooxidation as a result of oxygen uptake to form unstable lipid hydroperoxides. Fatty acid oxidation leads to the formation of carbonyl compounds, which, as a group, are extremely important flavor contributors in dry sausage. Carbonyl compounds accumulate during both the “active” fermentation phase of ripening and the extended drying period. The evolution of total carbonyl compounds (isolated as the 3,4-dinitrophenylhydrazones) for a dry sausage of predominantly pork fat (as lipid constituent) is shown in Figure 7 (Demeyer et al., 1974). The carbonyl content increased during the first week of ripening which is probably due to those formed in carbohydrate fermentation, while the increase in the last stages of ripening or drying are probably the result of fatty acid oxidation. This explanation was suggested from the results of Cerise et al. (1973) and Demeyer et al. (1974). Lower levels of volatile monocarbonyl compounds were found in smoked-dried Swedish fermented sausage as compared to similar nonsmoked-dried sausages by Halvarson (1973). This was thought to be due to the presence of phenolic compounds in the smoked sausage which acted as antioxidants, thus providing some protection against fatty acid oxidations.

Some 22 volatile monocarbonyl compounds were qualitatively identified by Halvarson (1973) in their study of Swedish fermented sausage. Of these, 18 were quantified and the dominating substances were ethanal, propanal, propanone, and 2-methyl and 3-methyl butanal in concentrations of 0.6 to 3.6 mg per kg of sausage. All the straight chain alkanals up to octanal, with the exception of butanal were detected, as well as several methylketones, 2-alkenals, and 2,4-alkadienals. Langner et al. (1970) had earlier reported qualitative identification of twenty-nine carbonyl compounds during ripening of a dry salami. Formaldehyde and acetaldehyde made up the greater portion of the carbonyls. Langner et al. (1970) and Halvarson (1973) consider the lower molecular weight carbonyls (probably from carbohydrate fermentation) to possess minimal value for characteristic sausage aroma and both state that the main aroma producers are the higher molecular weight carbonyls (probably from fatty acid oxidation). In particular, unsaturated carbonyls, such as the 2-alkenals and the 2,4-alkadienals, are potent flavor compounds typically present in oxidized pork fat, and present to a limited extent in oxidized beef fat (Hornstein and Crowe, 1960; 1963). The actual content of total carbonyls reported for dry sausage ranges from approximately 16.7 to 143 mg/100 g of dry sausage or roughly 0.02% to 0.14% (Langner, 1972).

**PROTEINS OF DRY SAUSAGE**

Meat proteins in dry sausage preparation are not extracted to develop the same functional properties uniquely desired and associated with finely comminuted or emulsified products. Solubilization of the salt-soluble myofibrillar proteins is necessary but not to the extent found in comminution. The meat particles of dry sausage are larger, as the amount of cutting action is either restricted or coarse grinding is practiced.

Following meat selection and sequence addition of lean meats, then fat meats, the cure and seasoning in-
Ingredients are "cut-in" or blended into the meat mix. Salt addition is generally toward the end of blending. If a starter culture is used, salt will be added prior to batch inoculation.

**Protein Solubility in the Fermentation Phase**

Several recent studies by Klement *et al.* (1973; 1974; 1975) and Wardlaw *et al.* (1973) have characterized the solubility changes of the various protein fractions during the fermentation and drying phases. Three conditions that exist during fermentation or fermentation with mild heat processing must be noted with respect to protein changes:

1. The meat mix contains salt, 2.5 to 3.0% on a total weight basis, but since the meat mix is generally about 60% water, the actual salt concentration in the aqueous, brine phase is about 4 to 5%.

2. The meat mix is progressively enriched in hydrogen ions as lactic acid is produced from carbohydrate fermentation;

3. The meat mix is subjected to a continued and cumulative thermal energy input over the time course of fermentation.

As a result of these existing conditions, Wardlaw *et al.* (1973) reported a 36% increase in the total insoluble protein nitrogen fraction of a fermenting sausage mix. The increase could be explained by the denaturation of the myofibrillar and sarcoplasmic protein nitrogen fractions. Klement *et al.* (1973) reported decreases in the solubility of myofibrillar proteins of fermenting sausage ranging from 50-60%, and of sarcoplasmic proteins ranging from 21 to 47% under conditions of declining pH (from pH 5.3 to pH 4.9 or 4.6) and near constant temperature (about 37°C). The decrease in solubility of sarcoplasmic protein fractions due to pH was not as extensive nor as rapid when compared to the loss of solubility by the myofibrillar fraction.

Further study of the effect of pH and salt on extracted protein fractions was conducted by Klement *et al.* (1974) in a model system with bacterial fermentation occurring in the protein extracts (Figure 8). A 35% decrease (P<0.01) was observed for myofibrillar proteins as the pH decreased from 6.2 to 5.5. A further reduction (P<0.01) in solubility of about 25% occurred when the pH decreased from 5.5 to 4.6. Sarcoplasmic proteins, in the absence of salt, showed an approximately linear decrease in solubility amounting to 17% as pH decreased from 5.5 to 4.6. However, in the presence of salt, 0.67 M, a 28% solubility reduction occurred in the same pH range. At pH 4.6, both salted and unsalted fractions of sarcoplasmic proteins had retained greater solubility (P<0.1) when compared to the myofibrillar fraction.

In a third study by Klement *et al.* (1975) using the same model system but with direct acidification by lactic acid, the myofibrillar fraction was found to be more susceptible to denaturation by heat as compared to denaturation by pH reduction alone. As was previously noted, both conditions are interrelated during fermentation. Sarcoplasmic proteins also exhibited loss of solubility due to the acid and heat effects, but the reductions were not as great as the solubility losses found in the myofibrillar fraction.

In ripened sausages where the temperature does not exceed 22°C, similar losses of solubility in both protein fractions occur during the first 10 days (Mihalyi and Kormendy, 1967; DeMetelaere *et al.*, 1974).

The effects of heat, pH and salt concentration on each protein fraction agree well with the earlier, more basic studies of Paul *et al.* (1966); Trautman (1964), Scopes (1964); Sayre and Briskey (1963) and others. Thus the findings in fermented sausages do not vary from the expected or established solubility properties of the muscle proteins under similar conditions.

**Firmness Development in the Fermentation Phase**

In applying the results to explain the development of firmness of sausage during fermentation, the three
studies of Klement et al. (1973; 1974; 1975) suggest that salt, while aiding in solubilization of myofibrilar proteins (to the extent needed), also aids in the insolubilization of the sarcoplasmic proteins. Additionally, the progressive decrease in pH of fermentation results in a continual decline in the solubility of the myofibrillar proteins. When the salt effect and pH effect are further interacted with the cumulative heat effect, the solubilities of both fractions decrease. The major effect is on the myofibrillar fractions.

Shear analysis of sausage cores, conducted during fermentation, has shown that firmness development is related more to the effects of pH and heat on the myofibrillar proteins than on the sarcoplasmic proteins (Klement et al., 1973). Salt also aids in enhancing denaturation of sarcoplasmic proteins under fermenting conditions, but its primary role is probably greater in determining textural aspects related to binding the meat mass together, as was suggested by Palumbo et al. (1976). In fact, in nonfermented control sausages subjected to the same conditions as fermenting sausages in the study of Klement et al. (1973), no firmness development was evident until heat was applied at the end of fermentation. Even then, shear values were lower for the control sausages than for the fermented sausages.

**Firmness Development and Protein Changes in the Drying Phase**

In the drying phase there is a continual development of the textural characteristics of firmness and cohesiveness of meat particles. In comparing shear values between the various studies, it is difficult to ascertain what a specific value means. However, shear values during drying in each study do show a correlation to drying time (Acton and Keller, 1974; Keller et al., 1974; Wardlaw et al., 1973), sausage moisture content (Wardlaw et al., 1973), sausage diameter (Keller et al., 1974), and in some cases, dependence on the initial grinding procedure (Keller et al., 1974).

In sausages which have been heated to between 55°C and 63°C (Klement et al., 1973 and Wardlaw et al., 1973, respectively), very little myofibrillar or sarcoplasmic proteins remain soluble, most having been denatured under the conditions of low pH and heat application. However, in ripened sausages not subjected to heating beyond the mild conditions of fermentation, some protein of both fractions remain soluble (Mihalyi and Kormendy, 1967; DeKetelaere et al., 1974) in the early ripening period. Sokolov and Techkovskaya (1971) reported aggregation of myofibrillar proteins during dry sausage ripening, accompanied by the formation of disulfide bonds. There was also the appearance of electrostatic and hydrogen bonding among the proteins.

However, Sandholm et al. (1972) reported an increase in the content of sulfhydryl groups in dry sausage from approximately 47 μM - SH/g sample initially to approximately 554 μM - SH/g sample at the end of 20 days of ripening. They suggested that sulfhydryl groups increased due mainly to the reduction of disulfide groups of the meat proteins rather than to the unfolding of the protein molecules. These findings are not without question because between day-20 and the next and final analysis period at day-29, the concentration of sulfhydryl groups dramatically decreased to below the initial level found at day-0 of ripening.

**Nonprotein Nitrogen Fraction Changes**

Several studies have demonstrated that an increase in nonprotein nitrogen content occurs in dry sausage processing, primarily during mild heating and/or during ripening. Wardlaw et al. (1973) reported an approximate 60% increase in NPN content after heating fermented summer sausages to 63°C. Klement et al. (1973) reported only a slight increase for fermented samples as compared to a larger increase in control sausages heated to 55°C. Results in a model fermentation system (Klement et al., 1974) showed a marked increase in NPN content in myofibrillar protein preparations as compared to a slight increase in sarcoplasmic protein preparations (Figure 9). Irrespective of the origin of the NPN compounds, increases have also been noted during drying by Wardlaw et al. (1973) and during ripening by Mihalyi and Kormendy (1967), Reuter and Langner (1963), and Dierick et al. (1974).

Dierick et al. (1974) recently reported on concentration changes for ammonia, total and free amino acids, total peptides, nucleotides, nucleosides and some amines during ripening of dry sausage with and without starter cultures. The rate of production of free amino acids increased as peptides decreased when starters were used. Over a 36 day period the concentrations of all free amino acids, with the exception of glutamic, histidine, tyrosine, and ornithine, increased. The major free amino acids which increased in concentration were alanine, leucine, valine, serine, glycine and proline (Table 6). Decarboxylation products of the amino acids histidine, tyrosine, ornithine, and lysine were reported, with an approximate 10-fold increase in histamine, tyramine, and putrescine occurring in the 36 day period (Figure 10). Rice and Koëbler (1976) recently reported
that *Pediococcus acidilactici* and *Lactobacillus plantarum* from commercial starter cultures do not exhibit appreciable tyrosine or histidine decarboxylase activity. Extremely low levels of histamine and tyramine were found in their fermented sausages when compared to the levels found in the traditionally-ripened sausages of Dierick et al. (1974).

In the literature review of Dierick et al. (1974) studies are cited which indicate that the free amino acid production in sausages is partly due to bacterial protease activity. Since the major increases in free amino acid concentration were found in the first 15 days of ripening, the findings of Dierick et al. (1974) support the observations of Reuter et al. (1968) who found that the microflora associated with free amino acid production was usually predominated by lactobacilli. In addition, the report of Pezacki and Urbanjak (1965) indicated that the number of bacteria possessing proteolytic activity does not decrease during the drying phase of ripening.

The composition and concentration of the free amino acids, peptides, nucleotides, nucleosides, and amines contribute to a large extent, to the aroma and flavor developed in traditionally ripened dry salami (Dahl, 1970). No literature source is available currently to assess the extent of production of these compounds in sausage prepared with starter cultures under higher heat and/or mild cook processes.

**CARBOHYDRATES**

The range in carbohydrate content for dried sausage products is generally 1.2 to 1.7% in the finished state (Rice, 1971). The residual carbohydrate content following the active fermentation phase could be much lower but the residual is concentrated during the dehydration phase. As previously stated, the sole purpose for adding simple sugars to dry sausage

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**TABLE 6**

<table>
<thead>
<tr>
<th>Free Amino Acids</th>
<th>Days of Ripening</th>
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<tbody>
<tr>
<td>Alanine</td>
<td>0</td>
<td>10.20</td>
<td>20.90</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>1.06</td>
<td>11.50</td>
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<tr>
<td></td>
<td>36</td>
<td>1.44</td>
<td>6.35</td>
</tr>
<tr>
<td>Leucine</td>
<td>0</td>
<td>1.73</td>
<td>5.50</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>3.00</td>
<td>6.15</td>
</tr>
<tr>
<td>Valine</td>
<td>0</td>
<td>0</td>
<td>3.40</td>
</tr>
</tbody>
</table>

*Adapted from data of Experiment 2 in study of Dierick et al. (1974).*

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**FIGURE 9**

Change in nonprotein nitrogen (NPN) versus pH during fermentation of sarcoplasmic and myofibrillar proteins at 37°C (Klement et al., 1974).

**FIGURE 10**

Concentration of some amines at various stages of ripening in dry sausage (mg/100g dry matter) (Dierick et al., 1974).
formulations is to provide the fermentation substrates for the production of lactic acid. Tandler (1963) stated that the quantity of sugar needed depends on the type of sugar added, the curing substances used, and the ripening process followed. Andersen and Ten Cate (1985) reported that approximately 1% sugar is needed to yield a reduction of approximately 1 pH unit during fermentation. In methods using starter cultures, recommendations (Anon, 1969, 1972) are for the use of a minimum 0.75% sugar as glucose for proper starter activity. Carbohydrates other than glucose, primarily sucrose and corn syrups or corn syrup solids, are suggested ingredients in some formulations where starter cultures may or may not be included (Komárik et al., 1974; Kramlich et al., 1973).

The conversion of either sucrose or glucose to lactic acid is due primarily to the homofermentative bacteria (Deibel, 1974). Selective growth of this group of bacteria is favored by the anaerobic nature of the meat mixture and the presence of salt. However, in a normal mixed bacterial flora, part of the carbohydrate present may be metabolized by non-homofermentative bacteria via heterofermentative pathways (Pezacki and Szostak, 1962; Andersen and Ten Cate, 1965).

An additional source of carbohydrate disappearance may occur during the drying phase of manufacture. This occurrence appears to involve oxidative dissimilation of residual sugar by part of the surviving active flora, yielding measurable quantities of carbon dioxide and water (Pezacki and Jaroszewski, 1963; Pezacki and Fiszer, 1970).

Types of Carbohydrates in Fermentations

There are studies which demonstrate that carbohydrates other than the "simpler" mono- and disaccharides may serve as suitable fermentable substrates for bacterial production of acid in sausage (Tandler, 1963; Coretti and Tandler, 1965; Pyrcz and Pezacki, 1974, 1975; Urbaniak and Pezacki, 1975; and Acton et al., 1977). Tandler (1966) stated that acid production was more rapid in fermentations with monosaccharides as compared to disaccharides. When fermented, a dry starch syrup (undefined), dextrose, or sucrose, were found to yield equal amounts of acid in traditionally ripened dry sausage (Coretti and Tandler, 1965), whereas lactose yielded inferior product results.

In testing the fermentative ability of 129 bacterial isolates from ripened sausage, Urbaniak and Pezacki (1975) reported that all isolates fermented glucose, many fermented sucrose, and a few fermented maltose. Acton et al. (1977) found that for sausages containing 1% of carbohydrate, fermentations (24 hr at 38°C) with the starter Pedicoccus acidilactici showed equivalent pH reduction and lactic acid yields for sucrose or glucose. Maltose yielded 78% of the acidity found for glucose while lactose and dextrin yielded slight, insignificant levels of acid. When corn syrups were tested in the same manner, Acton et al. (1977) found that the amount of acid produced was dependent on the quantity of simpler carbohydrates, glucose and maltose, initially available in the corn syrup preparations. The yields of lactic acid were related to the available "simpler" substrates. Pyrcz and Pezacki (1974) found that the peak period of lactic acid production in ripening sausages was correlated with the molecular weight of the carbohydrate. As the molecular weight of the carbohydrate substrate increased, a longer period was required to attain adequate fermentation end products. Pyrcz and Pezacki (1974) also stated that the tendency toward homofermentation by the lactic acid bacteria in dry sausage increased as the molecular weight of the carbohydrate increased.

From a practical viewpoint, these studies do confirm the fact that at least 0.75 to 1.0% utilizable substrate would yield a satisfactory fermentation. However, adjustments of two types may be necessary:

1. The time period and temperature of fermentation should be suited to the type or source of carbohydrate substrate furnished;

2. The quantity of carbohydrate added should be based on initial mix pH. At pH values above 6.0, a minimum of 1% would be suggested; at pH values of 5.8 or below, 0.75% would be sufficient.

Lactic Acid and Other Fermentation End Products

As previously noted, additional components besides lactic acid are formed in the early "active" ripening phase associated with fermentation. DeKetelaere et al. (1974) related the stoichiometry of carbohydrate disappearance in terms of lactate and acetate production. Theoretically, for each mole of hexose fermented, two moles of lactate and/or acetate should be formed. In practice, results agree with the theoretical calculations, in most instances, averaging 95% of expected lactate and/or acetate production. Of the two acids, acetate generally is present at about 7% of the total of the two. When these results are experienced, it is indicative of an anaerobic conversion which is essentially homofermentative.
In cases where final yields of lactate and acetate average 65 to 85% (DeKetelaere et al., 1974; Fiszer, 1970), the deviation from theoretical yields may be related to stoichiometric imbalances found in the first two to three days of ripening and near the end of the drying phase.

Initially, the number of micrococci present in the sausage mix are fairly comparable to the number of lactobacilli present (Reuter et al., 1968). In addition, when sausages are stuffed without vacuuming the mix, a higher oxygen concentration will be present. With these above two factors in mind, coupled with the fact that the growth of micrococi, but not the lactobacilli, is stimulated by oxygen, the micrococi may contribute to a more complete oxidation of part of the total available carbohydrate. The result is the production of some carbon dioxide and water in the first two to three days of ripening, and thus a stoichiometric imbalance occurs.

Using 14C-labelled-glucose, Fiszer (1970) found production of labelled lactate, carbon dioxide, and small quantities of labelled aldehydes, ketones, and ethanol in the early ripening period for dry sausage. Pezacki and Jaroszewski (1963), Pezacki and Fiszer (1966), and Fiszer (1970) reported that during the latter stages of the drying phase, all fermentation processes ceased and the remaining glucose (and even some lactic acid) was oxidized. The oxidation was accompanied by an equivalent oxygen uptake from the air surrounding the sausages.

Two points need mentioning in this respect. First, oxidative dissimilation of available carbohydrate does not appear to occur if mixes are vacuumized (De-Ketelaere et al., 1974). Second, in the more rapid fermentation processes practiced with starter cultures, no data exists to indicate that heterofermentative activity or that oxidative processes are involved. The fact that many researchers, processors, and consumers detect or believe that the "acid flavor" of rapidly fermented sausages by starters is different than the "acid flavor" of traditionally slow ripened sausages suggests that this flavor difference may be due to differences in how added carbohydrates are metabolized by the various groups of bacteria that predominate in the "fast" and "slow" methods for sausage fermentation.

The presence of volatile fatty acids have also been determined in dry sausage (De-Ketelaere et al., 1974; Halvarson, 1973). In analyzing nine brands of dry salami, DeKetelaere et al. (1974) found small quantities of acetate, propionate, and butyrate (Table 7). In Swedish fermented sausages, Halvarson (1973) also reported the presence of formic acid in addition to acetate, propionate and butyrate (Table 7). Propionate concentration in the smoked sausages of Halvarson’s (1973) study was approximately 10-fold higher than the level in nonsmoked sausages. In the nonsmoked products, the presence of volatile fatty acids was suggested by Halvarson (1973) to be due to the activity of heterofermentative lactobacilli strains. However, as previously noted, the presence of low molecular weight volatile fatty acids and volatile monocarboxyl compounds have been suggested as contributing little to the characteristic aroma and flavor of traditionally-ripened dry sausage (Langner et al., 1973; Halvarson, 1973).

### Table 7

<table>
<thead>
<tr>
<th>Acid</th>
<th>DeKetelaere et al. (1974)</th>
<th>Halvarson (1973)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Nonsmoked</td>
<td>Smoked</td>
</tr>
<tr>
<td>Formate</td>
<td>-</td>
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<tr>
<td>Acetate</td>
<td>2.4 mM/100 g D.M.</td>
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<tr>
<td>Propionate</td>
<td>11.7 μM/100 g D.M.</td>
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<tr>
<td>n-Butyrate</td>
<td>14.5 μM/100 g D.M.</td>
<td>0.004</td>
</tr>
</tbody>
</table>

### References


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