Histological Examination of Finely Comminuted Sausages.

Torsten Nilsson

Department of Food Hygien, Royal Veterinary College, Stockholm.

Histological examination of meat products has long been used to demonstrate adulteration and particularly the addition of various organs to certain types of sausages. This check on quality still plays an important rŏle. In the case of sausage types in which the filling is finely ground in the machines now available, a histological examination is a necessary complement to certain chemical analyses. Usually one can do no more by histological methods than qualitatively determine the various tissues which are included in the sausage filling. The relationship between the amounts of collagen and meat protein, however, can be determined in histological specimens by using Kotter'(1) method.

Histological methods have also been utilized to study changes in various tissues, such as musculature, during the manufacturing process and to observe the distribution of fat in the sausage mixture and in the finished product. (Schönberg /2,3/, Hansen /4/).

The most satisfactory histological specimens are obtained by embedding in paraffin but this method is too time-consuming for routine work. Frozen sections must therefore be used in most cases. In order to maintain stability during sectioning some specimens must be embedded in gelatin or alginat before sections are made. Bolonga and wieners, and other firm sausage types can be sectioned directly with a

freezing microtome.

The most widely used stains are eosin and haemalun, van Gieson's, and Calleja's. 276

There are several methods used in histological and histopathological work to demonstrate fat. Of these, Scharlach Red combined with haemalun is quite suitable for frozen sections. During quality checks including histological study of bolonga (falukorv) and luncheon sausage (frukostkorv) delivered to some army detachments this method was applied. Specimens were also embedded in paraffin and then stained with eosin - haemalun and van Gieson's stain. Fixation was in 10 per cent formalin at 45°C. Both frozen and paraffin sections were cut at 5 micra.

When the frozen sections are passed through the various staining baths some of the larger fat drops are washed out and this will result in large unstained vacuoles in the stained histological section. Actually, the absence of the large fat drops makes it easier to examine the sections. This can be shown by comparing the usual type of section with a fat stained section cut from a gelatin embedded specimen and in which all the fat drops remain.

(Figs. 1 and 2)

Scharlach Red stains fat red and this makes it possible to detect even very small fat droplets. When the fat stain is combined with haemalun the other tissue components are stained in shades of blue. These other tissue components are relativily easily identified, partly because of their warying staining properities but mostly through the morphological characteristics of the particular cells. Dermal tissue is easily recognized. None of the various staining methods, when used alone, was greatly superior to any other.

If several different stains are required in a particular instance it is preferable to utilize paraffin - embedded material. 277

(Figs. 3 and 4)

Lean beef and pork are the main ingredients in wieners and bolonga and similar types of sausages. Through curing and intensive chopping in the cutters the muscle tissue becomes very finely comminuted. The muscle fibres are disintegrated and the final result is a suspension of muscle protein and collagen (Schönberg). The fat is also very finely divided. According to Hansen the batter is a true emulsion of fat particles in a continous protein phase. He has demonstrated that the saltsoluble proteins of the muscle, myosin and actomyosin, act as emulsifying agents and surround the fat particles with a protein film which has affinity for fat but which repels the film round other particles so that the fat particles are held separated. After smoking and specially after cooking the protein coagulates and in well-made sausages the fat is uniformly distributed throughout the filling.

In Sweden up to 4 per cent potato starch may be used as a binder in some type of sausages. Dried blood plasma and dried skim milk may also be added. In order to see how these substances affected the histological structure including the emulsification of fat, several experimental sausages were made. To a batter containing lean beef, pork, spices, glucose, and water made according to a standard recipe for bolonga we added either potato starch, dry blood plasma, or dry skim milk and then passed the various mixtures through a cutter for three minutes. Test sausages were made from each mixture, then smoked and finally cooked at 80°C for

35 and 45 minutes. After cooling samples from each sausage were taken for histological examination. Frozen sections stained with Scharlach Red and haemalun were prepared from each specimen. 278

R e s u l t s:

Test sausage made of batter without starch and cooked for 35 minutes:

Histologically the sausage is composed of a homogenous protein matrix in which there are innumerable fat drops. The size of the fat drops varies greatly but small ones predominate. A few muscle fibres persist, either alone or in small groups. Patches of connective tissue can also be seen. There is no distinct protein membrane surrounding the small or the large fat drops ; judging by its staining intensity the protein material has the same density adjacent to and remote from the fat drops. In the places where fat drops have fallen out during the histological process the resulting vacuoles seem to have a more pronounced border but this may be an artefact.

(Fig. 5)

Test sausage made of batter with potato starch added and cooked for 35 minutes:

This sausage differs from the first inasmuch as the protein material contains small vacuoles with amorphous and strongly basophilic contents which represent the starch. Occasionally the starch appears as small rings with a thick wall. The distribution of fat is much the same as in the previous sausage but the average size of the fat drops appears to be somewhat larger. Here again there were no signs

of a film surrounding the fat drops.

(Fig. 6) 279

Test sausage made of batter with potato starch added and cooked for 45 minutes:

This sausage differs from the former in that the starch is no longer amorphous but appears instead as intensely basophilic small grains arranged in groups resembling bunches of grapes or beads on a string.

(Fig. 7)

The addition of dried blood plasma or dried skim milk does not affect the histological appearance. The fat drops are perhaps somewhat larger than was the case for the sausage made with potato starch but the differences are insignificant. There are once again no signs that an emulsion is formed.

(Figs. 8 and 9)

These results show that the histological methods used can demonstrate the presence of potato starch added as a binder without the use of iodine staining. The addition of blood plasma and dried milk cannot be detected with this method. Neither potato starch nor the other substances added to the test sausages appear to influence fat emulsification to any demonstrable extent.

References:

1: Kotter, L.: Wien.Tierärztl. Mschr., 44: 706, 1957.

2: Schönberg, F.: Arch. f. Lebensmittelhygiene., 8: 121,1957.

3: - " - : - " - ,12: 106,1961.

4: Hansen, L.: Food Technol.: 14: 565, 1960.