4 - 65

MICROSTRUCTURAL ANALYSIS IN MEAT AND MEAT PRODUCTS STUDIES

P. D. VELINOV[1] A. A. BELOUSOV[2]
[1][2]INSTITUTE OF MEAT INDUSTRY, SOFIA, BULGARIA
[2]ALL-UNION RESEARCH INSTITUTE OF MEAT INDUSTRY, MOSCOW, USSR

Introduction

The traditional methods of investigations - physico-chemical and biochemical proved insufficient in solving many essential questions, regarding the preparation and the quality of meat products. The application and the development of modern microsrtuctural methods in studying the meat and meat products brought about the appearance of a new direction in meat science.This new direction became popular as meat microstructure and in the last decade, separated from normal histology and pathohistology. Cohen et al.(I98I) underlined that the solution of present day problems in food industry requires increasingly sophisticated tools and approaches. Such modern approaches include microscopic examination of foods and ingredients and efforts to understand the factors which lead to the microstructures observed.
Lewis (I96I)pointed out that water and fat binding in meat and meat products is a complex subject and there are many mechanisms which may operate. Mostly the mechanisms are structural than chemical and the key to their understanding involves the use of all types of microscopy. A quite close liaison exists between microscopists and meat technologists and it has been possible to turn the mechanistic theory to practical use in producing stable meat products.

Cassens et al.(I984) stated that in morphological study of the effect of various processing procedures on meat two areas deserve coment. The first is compartmentalization. Structural studies reveal where components (such as fat) are concentrated and located.The second area of interest is interfaces in which polar and non-polar groups are aligned.It is essential to be known that the authors pointed out in conclusion that the era of description without utilization of the information is drawing to a close. The most likely opportunities lie in regulating biological processes to produce custom-made meat, in devising morphological control procedures for manufacturing processes and in utilizing the now available immunological procedures.

Shmidt (I984) in conclusion of a review paper on processing effect on meat product microstructure stated that species of meat, product produced, degree of comminution, application of mechanical energy, composition desired, and thermal treatment of the product affect basic properties of raw and finished material.Well designed factorial experiments should be utilized to determine basic effect as well as interaction. Basic research tools should be used to determine which treatment effects are compatible with industrial production practices.Basic research on the components of processed meat products is useful to produce background material for application in product testing.Integration of modern research tools to determine which factors affect meat product properties shoud be a continuing effort of researchers.

Davis and Gordon (I982) underlined that studies of food microstructrue, regardless of the type of system being studied, are not entities unto themselves but are portions of information needed in an integration of data leading to a fuller understanding of the total system.

The objective of this paper is to analyse the possibilities of microstructural analysis as a practical tool in the assessment and directing of the technological processes, in the improvement of existing and the elaboration of new meat products and equipment and in the veterinary-sanitary and technological control of meat and meat products. The review of literature is selective to prevent excessive length.Recommendations are presented for future work.

Importance, place and role of microstructural analysis in studying meat and meat products

In studying meat and meat products, physico-chemical, biochemical, microbiological, and sensorial methods of investigations have found wide application.But all of them are not sufficient for the thorough study of the changes in meat resulting from technological processing.Microstructural analysis is invaluable in the improvement of meat processing. Chemical methods are used very often to determine the ultimate, and less often the intermediate products of the conversion of individual meat components.These methods are based on investigations of homogenized samples which have been destructed by their preparation. It should be mentioned that the destruction of the samples results in significant changes in the studied material. By microbiological methods of investigations we can only establish the counts of the microorganisms and their species, but not the changes resulting under the action of their enzymes. Sensorial methods allow us to get only an overall evaluation of meat and meat products quality without discovering the mechanisms which are determining these properties.In thi s respect the application of microstructural analysis in meat and meat products studies plays very important role. The microscopical methods give opportunities to make the investigations on an undestructed substrate, when necessary, from the surface to the inside. Moreover even the most insignificant changes arising in meat are detected considerably earlier with microscopic analysis than with physico-chemical analysis.For example, the microstructural changes occurring under the action of putrefactive microoraganisms (pycnosis and decreasing the staining ability of the nucleus) are detected 3-4 days earlier with histological methods than with physico-chemical methods of investigations (Skalinski and Belousov,I978). In studying meat and meat products the main thing should be taken into account: that they are not simple combinations of different substances- fat, ptotein, water,but in every separate case a complex structure. The latter determines the properties and the quality of the meat products which differ from each other according to their parameters and their evaluation is unthinkable without conducting microstructural

analysis.Microstructural analysis is nowadays the only available method for determining the components of sausage meat and their location and relationship. Very valuable information can be obtained in the assessment of the quality of water-protein-fat emulsions which are used in the production of sausages and paste s.
Recognizing the peculiarities of the studied object - meat during different technological treatments, sausages, hams,paste s etc., the specificity of the selection and investigation of the materials, it should be mentioned that a new direction in morphological investigations was formed. It is specific and differs greatly from normal histology and pathohistology. That is why it can be said convincingly that in the last decade a new science in meat investigations has appeared. This science indicated as meat microstructure is getting every year greater and greater recognition all over the world.
It should be pointed out that some microstructural methods are used succssefully in the practical work of the production laboratories in meat plants. In big meat plants in the USSR, there are histological laboratories created where microstructural analysis plays a great role in the veterinary-sanitary and technological control of meat and meat products.

Potential of microstructural analysis in development of meat science

Microstructural analysis allows to:
- study the quality of raw material as the basis of the formation of the initial properties of meat (feeding, managing, transportation, lairage of animals, composition and structure of the individual muscles etc.);
- study the microstructural changes of meat upon slaughtering, refrigeration and processing.

On the basis of the knowledge revealed by microstructural analysis microstructural criteria were developed, which characterize technological processes. These microstructural criteria allow to effect:
a) veterinary -sanitary control of the quality of raw materials (meat) and processed meat and meat products (dystrophia;condition of animals in the moment of slaughter healthy,undergoing forced or casualty slaughter; assessment of the freshness of meat,hams and sausages).
b) technological control:(quality of fresh meat PSE,DFD; influence of the method of stunning-mechanical,electrical; electrical stimulation; extent of meat ageing; quality of refrigeration-cold shortening of muscle; influence of refrigeration and the storage of refrigerated meat; influence of curing; degree of the mechanical treatment of meat; degree of the comminution of meat; composition of the raw material for sausages; characteristics of heat treatment; state and stability of water protein-fat emulsions in sausage products).
c) control of arbitration: (forced slaughter animal or killed animal; dystrophic or normal meat; degree of meat freshness; composition of sausage meat and its adulteration; utilization of inadmissible raw materials or admixtures in sausages).

Increasing interest towards the application of microstructural investigations on meat and meat products

In this respect we would like to point out some important facts which could be a good illustration of the progress of the microstructural investigations of food, meat and meat products included. First of all it deserves to be mentoined that begining in 1979, programs on food microstructure are held at annual Scanning Electron Microscopy (SEM) meetings (Cohen et al.1981).Food Microstructure 1985 program held in Las Vegas,USA consists of five special programs including Meat Microstructure(7). The annual meeting in Las Vegas this year has been very successful. A great number of food scientists have presented their most recent findings as well as reviews on a variety of subjects.
It is interesting to mention that thirty-six papers based on 1979,1980 and 1981 programs were collected to form a book "Studies of Food Microstructure" which was published in 1981 by Scanning Electron Microscopy,Inc.In this book leading scientist have demonstrated that microstructural studies of food stuffs are invaluable. Reading the book carefully we join in the nice comments made on it.

In 1982 the international journal "Food Microstructure" was established. It is specialized in papers dealing with the microstructure and microanalysis of foods, feeds and their ingredients. The science reported in Food Microstructure contributes to the development of new manufacturing processes, better understanding of food products and improved quality of food supply.
It merits to mention the underlined interests towrds conducting well designed factorial experiments. Very recently Schmidt (1984) stated that well designed factorial experiments should be utilized to determine basic effects as well as interactions and basic research tools should be used to determine which treatment effects are compatible with industrial production practices. Cassens et al.(1984) directed our attention to devising morphological control procedures for manufacturing processes.
It is characteristic of the development of microstructural studies in the USSR and in Bulgaria that, already with their appearance they have an applied-science direction. As a result of that, in big meat packiging plants in the USSR, histological laboratories have been organized and standatrs are elaborated for the assessment of technological processes using microstructural indices.
Another very important fact which we would like to underline is the increasing interest of technologists towards studying the technological prcesses through microstructural investigations. In the USSR, there is no research work in the field of the technology of meat and meat products without microstructural investigations.

Consistency of the microstructural investiagtions

The microstructural analysis of meat is conducted by different methods of morphological investigations for studying the changes of muscle tissue which result from the technological treatment of meat. The choice of the method depends on the characteristics of muscle tissue structure and the objective of investigation. In every case we should start with macrostructural analysis, then, if it is necessary to continue with microstructural analysis, light microscopy for studying the changes of the tissues in meat processing and electron microscopy / TEM and SEM / for studying the changes of cell structure. If we need to study the structure of the protein molecule and its changes during meat processing we have to resort to roentgenostructural analysis. For studying the location, distribution and penetration of ions in meat during meat processing x-rays microanalysis opens a new field of investigations of meat and meat products. In short, the investigations should be conducted from the simple to the complex with the purpose to elucidate changes in meat and meat products. The consistency of the microstructural analysis is well demonstrated by Skalinski and Belousov (1978).

Principles of selection and investigation of studied materials

Regardless of the objective of investigation, the selection of material and its sampling should take into consideration the structural and functional characteristics of muscle tissue.
The data, obtained in model experiments on individual muscle fibres or pieces of meat cannot entirely reflect all the changes in the muscle which is a component of the whole carcass, side or cut in the course of the process under industrial conditions.
When studying the structural changes in meat during storage and technological processing, developing new technological regimes, evaluating new production equipment and in veterinary-sanitary and technological control over finished product quality, it is necessary that sampling takes into consideration the time, conditions and peculiarities of the technological process.
Skalinski and Belousov (1978) indicated the basic factors, which influence the results of microstructural investigations. We would like to point out some of them.
<u>High reactivity of muscle tissue.</u> The sampling of muscle tissue in the first hours after the slaughter of animals leads to changes of muscle structure due to the contraction of muscle fibres. On the cut surfaces and in individual muscle fibres contraction nodes are formed.
<u>Muscle integrity.</u> Muscles, separated from the carcass do not preserve meat quality so well and get spoilt faster. For this reason the results of investigations, obtained from pieces of meat, separated from the carcass cannot be valid for muscles,

lying on the carcass.

Attachment of the muscles to the carcass. Separating the muscles from the carcass immediately after the slaughter of animals leads to their significant shortening in the process of rigor mortis development.

Contact of the muscles with environment. Muscles on the surface of the carcass are exposed to faster cooling, drying or moistening, microbial contamination, etc. In this connection, it is recommended to carry out microstructural investigations from the surface to the depth.

Sterility of deep lying muscles from healthy animals. It should be taken into consideration, that the spoilage of the meat, produced from healthy animals develops mainly from the surface to the depth of the tissues.

Autolytic character of the development of postmortem structural changes in muscle tissue. The postmortem structural changes in muscle tissue are determined to a great extent by the condition of meat storage: temperature, humidity and the duration of storage.

The samples for the investigations of meat and meat products should be taken from 3 cm below the surface.

In studying the microstructure of meat batter the samples are taken from the superficial, middle and deep layers.

The samples which are taken by meat technologists have to be accompanied by a description of the raw meat or meat products, from where they have been taken, the time of sampling and the characteristics of the technological treatment with all of its parameters.

Assessment of the obtained results and their correlation with data from other methods of investigations

As we have already mentioned, meat and meat products are not simple combination of different substances-fat, protein, water, etc. but in every separate case a complex structure. The physico-chemical and biochemical changes which occur in meat processing are in a close correlation with microstructural ones. A good illustration in this respect is given by Belousv et al.(1980). In assessing the extent of mechanical treatment of meat by microstructural characteristics, they found that, upon superficial tenderization, water holding capacity (WHC), is enhanced by 5-7%, upon moderate tenderization, WHC is increased by 8-12 %, in optimum tenderization, by 14-18 %, and in overtenderization, WHC decreases by 5-7 % compared to optimum tenderization. The knowledge of the correlation with data from other methods of investigations allows to assess the quality of meat and meat products and to control manufacturing processes only by microstructural characteristics.

In describing the microstructural changes of meat and meat products as a result of processing it is essential to use terms which reflect as accurately as possible the observed structures. In this respect we feel that there are some problems and we would like to comment some of them. It is well known that the mechanical treatment, massaging or tumbling, causes a disruption of muscle fibres and a destruction of myofibrils. Under the action of the salt solution some part of the myofibrillar proteins dissolve. On the surface of meat chunks a sticky material is formed. It contains salt soluble myofibrillar proteins, in particular myosin, muscle fibres and myofibrils at a different stage of destruction, fats as fat cells or fat globules, collagenous fibres etc. Weiss (1974) / cited by Theno et al.1978 / called it protein suspension. Theno et al.(1978a,b,c), Siegel et al.(1978a,b), Cassidy et al.(1978), Motycka and Bechtel (1983), Voyle et al.(1984) described this material as exudate. Studying the microstructure of binding junction in selected and formed ham Theno et al.(1978c) found in the binding matrix, emulsion-like areas which possess histological characteristics similar to those of true emulsion meats such as frankfurters or bologna. In our investigations we have also observed the same areas. As it can be seen the most popular term of the above mentioned sticky material is exudate. But the term exudate originates from the Latin word exsudatum and means a liquid, rich in protein, which flows out from the walls of small blood vessels (capillaries, etc.) during inflamation (Arnaudov,1975). That is why in our investigations we describe the sticky material on the surface of meat chunks formed during massaging or tumbling, as a finely granulated protein mass. Discussing the term exudate we are far from any criticism. We strongly believe that all meat microscopists would like to hold meat microstructure on a very high level and they will do

their best to improve terminology.

Future Research

Some aspects of further microstructural investigations of meat and meat products are very well defined by Cassens et al;(I984), Shmidt (I984) and Voyle (I98I). Cassens et al.(I984) pointed out that the rapidly emerging immunological methods offer great potential for specific identification and enzyme-linked-immunosorbent-assay (ELISA) procedures now offer precision and automation. When antibodies are coupled to markers, they can be used microscopically to relate presence to specific location. The now available immunological procedures should be utilized.
Shmidt (I984) stated that research in the area of the thermal alteration of the structure of myofibrillar, stromal and sarcoplasmic proteins is needed. Additional research is needed to understand the microstructural changes that take place during dried sausage production. Research also is needed to develop methods to accurately identify the components of processed meat products.
Voyle (I98I) believes that energy dispersive x-ray analysis through the scanning electron microscope would give useful information about the location of curing salts in processed meats.
We think that X-ray microanalysis of frozenhydrated sections and fractured bulk specimens is now giving significant data concerning the distribution and concentration of electrolytes in a wide variety of plant and animal tissues and its use in studying the microstructure of meat and meat products is an interesting and promising direction of future research.
The modern instruments for quantitative analysis offer an interesting area in meat and meat products studies. It could be very useful in obtaining the needed information for the improvement of the existing and the development of new technological processes and equipment.

Conclusion

A large amount of details about muscle structure are already known. Microstructural analysis of meat and maet products gains not only theoretical importance, but it plays a greater and greater role for the practce in studying, directing and improving technological processes.
The science of meat microstructure has been making great progress and it sets great requirements to meat microscopists. Their efforts should be directed to the use of increasingly sophisticated tools and approaches for microscopic analysis with the purpuse of detecting new details and knowledge on meat structure and on its changes during technological process. In this respect quantitative microscopy and X-ray microanalysis open wide opprtunities in studying the localization of protein, lipid, water and ions within meat products and would be very useful in determining the functionality of these components and in the improvement of existing and newly developed technological processes and equipment. At the same time, work should be done on the elaboration of morphological characteristics and procedures for the control of the manufacturing processes and for their implantation in practce.

References

Arnaudov GD.(I975). Terminologia medica polyglota.p.I63.Medizina i phiscultura. Sofia,Bulgaria.

Belousov AA, Oreshkin EF, Rostupkin VI,Bobricova EG, Bolshakov AS, Zabashta AG. (I980). Ispolsuvanie physicheskih metodov dlia obrabotki mjasa i mjasoproductov. pp. 56-6I. Sbornik nauchnih trudov. VNIIMP,Moscow.

Cassens RG,Carpenter CE,Eddinger TJ.(I984). An analysis of microstructural factors which influence the use of muscle as a food.Food Microstructure. 3, I-7.

Cassidy RD,Ockerman HM,Krol B, Van Roon PS, Plimpton RF, Cahill VR.(1978). Effect of tumbling method, phosphate level and final cook temperature on histological characteristics of tumbled porcine muscle tissue. J.Food Sci. 43, 1514-1518.

Cohen SH,Davis EA,Holcomb DN,Kalab M.(1981). Studies of FOOD MICROSTRUCTURE, p.iii.Scanning Electron Microscopy,Inc.Printed in USA.

Davis EA,Gordon J.(1982).Food Microstructure: An integrative approach.Food Microstructure.I, 1-7.

Food Microstructure.(1984).3, iii.

Lewis DF.(1981). The use of microscopy to explain the behaviour of food stuffs. Studies of FOOD MICROSTRUCTURE.pp 25-38.

Motycka RR,Bechtel PJ.(1983).Influence of Pre-Rigor Processing,Mechanical Tenderization,Tumbling Method and Processing Time on the Quality and Yield of Ham. J.Food Sci.48,1532-1536.

Schmidt GR.(1984). Processing effect on meat product microstructure. Food Microstructure. 3, 33-39.

Siegel DG,Theno DM,Schmidt GR.(1978a).Meat massaging: the effects of salt,phosphate and massaging on the presence of specific skeletal muscle proteins in the exudate of a sectioned and formed ham.J.Food Sci. 43, 327-330.

Siegel DG,Theno DM,Schmidt GR,Norton HW. (1978b). Meat massaging: the effects of salt,phosphate and massaging on cooking loss, binding length and exudate composition in sectioned and formed ham.J.Food Sci. 43, 331-333.

Skalinskii EI,Belousov AA. (1978). Microstructura mjasa.Moscow,USSR.

Theno DM,Siegel DG,Schmidt GR.(1978a).Meat massaging:effects of salt and polyphosphate on the microstructural composition of the muscle exudate.J.Food Sci. 43, 483-487.

Theno DM,Siegel DG, Schmidt GR.(1978b).Meat massaging:effect of salt and phosphate on the ultrastructure of cured porcine muscle. J. Food Sci. 43, 488-492.

Theno DM, Siegel DG, Schmidt GR.(1978c). Meat massaging: effects of salt and phosphate on the microstructure of binding junctions in selectioned and formed ham. J. Food Sci. 43, 493-496.

Voyle CA, Jolley PD, Offer GW.(1984). The effect of salt and pyrophosphate on the structure of meat. Food Microstructure. 3, 113-126.