RESEARCH PRIORITIES IN FERMENTED AND DRIED MEAT PRODUCTS

Kalman Incze

Hungarian Meat Research Institute, 1097 Budapest, Gubacsi út 6/b. Hungary

Abstract
Several fields of research interest are listed concerning fermented and dried meat products out of which some are discussed in detail. Extension of starter culture application is further on experienced partly in form of new strains, species and genera, partly by applying them in regions where no such technology has been practised before. This way traditional technology is shrinking, at least with sausages. Among sensory attributes results of texture, flavor and aroma research is discussed, advantages and disadvantages of speeding up the process are mentioned and some further research needs are given, too. Safety issue became more timely than ever for known reasons, special requirements in this respect with raw fermented commodities are evaluated and safety in case of manufacturing with less sophisticated equipments is also discussed. As a relatively new field of research development of probiotic fermented meat products is mentioned, where to find a sound compromise among safety, sensory value and efficiency as probiotic food is and will be a real challenge.

1. Introduction
Although fermentation and drying of meat products are probably the most ancient ways of preservation, and experience of several thousand years in practice as well as results of half a century intensive research are available, the complexity of this process influenced by physical, chemical, biological, biochemical and microbiological factors still offer ample opportunities for doing research. Here are some more interesting fields:

1.1. Fields of main interest in microbiology
New isolation of starter cultures, new mixed cultures, new countries and new fermented products, GMO, protective cultures, speeding up of the process (bacteriological enzymes, chemical acidulants), proteolysis and lipolysis, sensory characteristics, food safety, pathogenic microorganisms, spoilage microflora, antagonisms, bacteriocins, shelf stability, biogenic amines, probiotic microorganisms.

1.2. Fields of main interest in terms of nutritional value
Fat reduction, alteration of fatty acid composition (through feeding of animals and/or using PUFA as food additive), new additives for partial substitution of fat and/or meat, technological and sensory changes induced by substitution or supplementation, oxidative changes, antioxidation.

1.3. Fields of main interest in terms of sensory characteristics
Development of binding and structure, color formation and color stability, aroma formation influenced by tissue and by added enzyme activity or by microbial growth and metabolism, flavor and aroma affected by tenure of ripening and drying, detection and identification of aroma components.

1.4. Fields of interest concerning technological modifications
Improving drying conditions, improving control and automation, new technologies for dried meat products under less favourable conditions yet meeting food safety requirements.

Considering that this list (far from complete) can be quadrupled because the processes differ depending on whether the product in question is *sausage* or *ham* and whether the fermentation is *traditional* or using *starter culture*, it can easily be accepted that the research opportunities in the field of fermentation of meat products are inexhaustible.
In the following chapters evidently only a few research priorities will be discussed, among others extension of use of starter cultures, texture, flavor and aroma formation, food safety and requirements for making fermented meat products probiotic.

2. Globalization of starter culture application
Globalization, favourable for some and unfavourable for others in general, in case of starter culture application has brought advantages in most instances for everyone. After pioneering works of Niinivaara and Niven application of different starter cultures was initiated and this technology soon became popular in many states of Europe and North America. It has to be emphasized that this technology came into general use not only through replacing old technology of fermented meat manufacturing but production of fermented sausages was also started in many places where no such product was known earlier and the reason of introducing was the reliability, rapidity of the process and safety assured by the new technology. This "conquest" by starter culture technology has accordingly two main streams, one: replacing traditional technology, like in Italy, France, and the other: introducing fermented sausage production, practically unknown before. As for today very few factories apply the "old world style" fermentation technology, that, meeting the requirements of low temperature long ripening-drying time offers an even higher food safety level. A good example for this safe technology is as Hungarian traditional salami (Pick and Herz) is manufactured. The typical characteristics of these products in terms of food safety and sensory value will be discussed more detailed later.

A very definite demand of countries where starter culture technology is intended to be introduced is the selection of cultures from the home environment. This tendency is clear also from the planned research cooperation among countries applying for financial support (e.g. grants for SME-s). Although there is a surplus in the supply of starter cultures both in varieties (different mixes) and their capabilities, it is easy to understand that mostly in mediterranean countries they prefer the flavor and aroma direction they are accustomed to. (It is well-known that in northern part of Europe fermented sausages are more acidic than in Southern-Europe.) On the other hand finding the microbes which possess the favoured characteristics by *selection* rather than by *genetic manipulation* is and probably also will be preferred. As Lücke put it: "Genetic engineering of cultures may improve certain properties of the strains but benefits to consumers and industry are too small to make them acceptable by consumers and regulatory bodies in the near future" (Lücke, 1999). This statement seems to be valid, even if successful genetic modifications (cloning and expressing specific antistaphylococcal gene in lactobacilli) are known (Hammes and Hertel, 1998), because there are also technological measures available for the inhibition of staphylococcal growth in fermented sausages, and by these measures also other undesired microorganisms can be inhibited, i.e. this method is more advantageous because of its "broader spectrum".

4 Development of binding, structure and texture

In addition to color and taste structure and texture of meat products are also very important features perceived and judged by consumers and this perception plays a definite role in their buying-or-not-again decision. This observation evidently refers also to fermented and dried meat products. The situation is basically different on the one side in case of fermented and dried *intact muscle* type of products (ham, loin, butt, beef cuts etc.), where mostly tissue enzymes play the decisive role in texture and on the other in case of *chopped* (ground) meat products (sausages), where in addition to the probably less effective tissue and bacterial enzyme activity several other factors influence binding, structure and texture. Some of the more important factors:

- type of meat (pork, beef)
- ratio of meat and fat
- particle size of meat and fat
- temperature of sausage batter when stuffing
- pH-value and pattern of pH-changes
- salt level
- microflora (starter and/or spontaneous)
- drying rate
- extent of drying
- type and amount of non-meat additives and spices

Among the factors listed above pH-value and pH-changes have distinguished influence on binding and texture. Sausages with relatively high pH (traditional salami with pH-value above 5,6-5,8) need a much longer period of time, sometimes several months to become sliceable, than sausages with a pH-value below 5,3. Since in case of traditional sausages drying and reduction of water activity are the main factors in evolving gel structure and sliceability, the extent of drying has to be greater, consequently hardness is higher, elasticity is lower, than in short fermented sausages.

In sausages with lower pH-value the major structural changes take place during the first 48 hours (van't Hooft, 1999), or more generally: during the period of pH-drop to 5,3 or below. During this period and because of acidification the solubilised myofibrillar proteins after their salt induced diffusion from muscle cell forming meat exudate are gelified and this evolved matrix ensures the fixation of meat and fat particles affecting binding. Binding in this case means cohesion within and adhesion between particles. Myosin is the main gelating protein, but in adhesion also collagen fibrils take part. This rapid process of binding caused by acid-denaturation and enhanced by drying results in a sliceable structure within days, yet, for safety reasons drying has to be continued. Sodium chloride plays an important role in binding and it was shown by Gimeno et al. (1999), that texture of fermented sausage where NaCl is replaced by a mixture of NaCl, KCl, $MgCl_2$ and $CaCl_2$ (same ionic strength as with NaCl alone) was softer than that of with NaCl alone.

It is interesting to mention that van't Hooft (1999) quantified the amount of meat exudate necessary for good binding and he stated that exudate values below 15 % gave poor cohesion, adhesion and fat holding, especially if fat is smeared when its temperature was not low enough during chopping (> - 7,5°C). The author also found that salt chopping time of 60-80 sec is optimal, getting less exudate with shorter and greasiness with longer time. (Extractable protein content of the chopped meat increased by 40-80 % when chopping with salt.)

All this information is necessary and useful if consistent quality is aimed at by applying pertinent process control.

Influencing and possibly controlling the texture of products made of whole meat muscles (ham) is also a rather complicated process not only because raw material selection plays a decisive role much more than with sausages and controlling technological steps is even more critical (diffusion of bacterial growth inhibiting salt is slow), but also because the ham has to be of superior quality in terms of taste, aroma and tenderness usually without using seasonings and evidently without applying muscle tissue disintegration that is normal with sausages.

In addition to undesired microbial growth (causing spoilage, health risk and eventually also texture problems) the main problems of texture in dry-cured hams are the soft and pasty texture, the rather heterogenous texture between different muscles and the possibly occurring holes (Ordóñez et al., 1999). For this reason it is important to investigate the physico-chemical, compositional and structural characteristics of ham determined by the technological parameters (Parolari et al., 1994; Bañón, 1996). Spanish authors (Ordóñez et al., 1999) found significant correlation between moisture/NaCl, NPN and denaturated protein as expected. Not unexpectedly there was a correlation between hardness and moisture as well, NPN also correlated with hardness, especially with pastiness, and high moisture level was also responsible for pasty ham (for Parma ham 63,5 % moisture is given as upper limit for normal quality [Parolari et al., 1999.]).

It can be assumed, that as a consequence of high moisture content and raw material characteristics the high proteinase activity causes high level of NPN responsible for pastiness. For high moisture content temperature, relative humidity and air velocity, in other words drying-rate and extent of drying are responsible. (High pH-value could also slow down drying, but such hams are sorted out.) The reason for differences between muscles can be, in addition to structural differences, also the location of the muscle, this is why Díez and Pérez-Villareal (1999) found that after ripening of ham Semimembranosus muscle showed the highest hardness and the lowest chewiness value due to the greater extent of drying, since the muscle was not covered by fat.

Texture problems can be caused by spoilage microflora, too, as it was proven by Carrascosa et al. (2000). Using Serratia liquefaciens and Proteus vulgaris isolated from spoiled, swollen ham for inoculation experiments, it was possible to cause the same phenomena, soft texture, discoloration and sometimes gas formation. This kind of texture failure occurs nevertheless seldom, and can be prevented by high hygienic quality of raw material and by well controlled process.

4. Flavor and aroma

Flavor and aroma belong also to most important characteristics of meat products influencing consumers' perception significantly. This is especially true for fermented and dried meat products, where richness of aroma is more pronounced than with any other item, due to the fact that microbial metabolism and tissue enzymes are not inactivated by heat treatment, thus they can influence aroma formation significantly and very positively.

Several factors may contribute to the final aroma of fermented and dried meat products, some of which can be influenced, others not:

- raw material (animal species, age and type of animal)
- tissue enzymes
- microbial metabolism (original microflora, starter culture)
- smoking
- spices
- additives
- pH-value, pattern of pH changes, types of acids formed
- surface moulds
- redox potential
- fermentation time
- rate and extent of drying
- type and extent of break-down of lipids, proteins and peptides

Out of this - also not complete - list just a few will be discussed, since there are excellent reviews on the topic both in the proceedings of ICoMST meetings (Montel et al., 1998) and elsewhere (Dirinck and Opstaele, 1999; Flores et al., 1999), yet there are some topics, that are less dealt with.

Efforts have been made for long to reduce processing time of most technologies, that is especially true for curing and for fermentation and drying. The real break-through in reduction of drying time came when lactobacilli were introduced as starter culture, that reduced pH and speeded up drying significantly. (Reduction of drying time has been 3-6 fold compared with traditional products.) Although application of starter cultures is known also with fermented meats (ham, loin etc.) they are applied more generally in sausage manufacturing, where also drying time reduction is more detectable. Even if time reduction has been very successful, further reduction is also aimed at while sensory characteristics and safety should not be worsened. Main questions in this respect:

- How can we assure similar break-down of components in order to get similar aroma compounds in shorter period of time?
- If whole processing time will be shorter, how can we assure similar safety as with longer dried products?

Trying to solve the first problem different combinations of starter cultures with higher and broader metabolic activity (e.g. proteinase) and direct additions of enzymes are applied and tested (Bruna et al., 2000). This way ripening takes place in a shorter time, yet good aroma formation is claimed during this reduced time.

As for the second question, the answer and solution is far more remote. The trouble is that some pathogenic bacteria (e.g. listeriae, EHEC) are rather resistant to low pH and lowered water activity and sometimes even "normal" length of ripening time is insufficient for several log reduction. If GHP is followed and HACCP is operated properly the health risk can be kept low (fermented meat products have a good safety record anyway), but reduction of ripening-drying time has to be supported by challenge tests if we want to avoid health (and sometimes also spoilage) risk.

A much higher level of safety is offered, if ripening-drying time is extended, in this case namely water activity decreases significantly and has a long lasting effect causing several log reduction in EHEC count as it was reviewed (Incze, 1998). Longer ripening and drying time favours also aroma formation: richness is much more pronounced after longer ripening, since aroma compounds are more abundant.

5. Food safety

Fermented meat products have always had an excellent safety record, if ample time was given for drying, reducing this way water activity, one of the main factors assuring shelf stability and safety. If however water activity is relatively high (as with short fermented sausages, like "Mettwurst"), and lowered pH alone is expected to do its duty in inhibiting growth of undesired microorganisms, a higher risk has to be calculated with either in case of EHEC against which organism low a_w is more effective, or with staphylococcal growth, when lactic acid is metabolized by surface moulds and as a result pH raises.

Meeting safety requirements seems more controllable with sausages than with whole muscle meat products because of significant differences in salt diffusion time, yet this favourable situation with sausages must not be abused. Rapid salt diffusion alone is namely insufficient for assuring safety, since initial water activity is only slightly inhibitory and incubation temperature favours undesired microbes, too. The well-known factors assuring safe fermented and dried meat products are:

- GHP with raw material and processing technology
- rapid pH-drop in case of sausages with accelerated (starter culture) fermentation
- low temperature fermentation and drying until critical a_w-value is reached (traditional meat products)
- a_w-drop to the extent until product becomes safe either through low a_w-value alone (traditional products) or through combination with low pH.
- precisely controlled drying air parameters

5.1. Efforts to raise level of safety

Since fermented and dried meats are raw products where we have no opportunity to drastically reduce initial microbial contamination and since newly emerging pathogens (listeriae, EHEC) are rather salt and acid tolerant, it seems reasonable to look for methods to enhance safety. One of the most extensively studied possibilities for this purpose is the use of lactobacilli that produce bacteriocins, peptides or proteins broken down in gastro-intestinal tract of humans, consequently not considered problematic as antibiotics. After initial enthusiasm reality seems to offer limited application, for two reasons: antibacterial spectrum of bacteriocins is rather narrow (and resistant mutants occur quite frequently) and bacteriocin molecules are bound to food constituents becoming thus unable to inhibit efficiently target microbes. Application of bacteriocin producing lactobacilli should be considered only "as an *additional measure* to good manufacturing, processing and distribution practices" (Hugas, 1998), that may help mainly against listeriae and staphylococci (Aymerich et al., 2000).

Under conditions when it is difficult to meet GHP-requirements precisely, yet there is a demand for manufacturing dried meat product, there are basically two ways of making it safely:

- dry salting of relatively thin meat pieces with excessive amount of salt assuring rapid drying and a_w-drop
- heat treatment of the product.

Evidently fermentation in true sense occurs in neither case, and in first case meat usually has to be soaked before consumption, but shelf stability characteristics of heat treated dry product may resemble that of raw dried meats. Heat treatment can be applied either before or after drying, causing some differences in sensory characteristics. A well detectable difference between the two types of heat treatments was found in concentrations of fatty acids of C3-C18 (Tas; personal communication) and also in bacteriological status. Should the product be heated before drying, most vegetative bacteria are destroyed but enterococci, that can grow during drying, and their role is judged as controversial. Should the heat treatment be applied after drying, previous growth of staphylococci may occur with toxin production that is not inactivated by pasteurizing heat treatment. Although these possibilities are not only theoretical, heat treatment seems a fairly good solution for building in a safety factor in cases technical level makes it necessary. Training the staff in this case is of paramount importance.

6. Fermented meat products and probiotic microorganisms

Therapeutic use of lactic acid bacteria as an aid to cure some types of gastro-intestinal disorders has been suggested for almost a century (Hawley et al., 1959). Ever since keen interest in the topic can be noticed in general (Mitsuoka, 1996; Sanders, 1999) and in special with meat (Andersen, 1998; Arihara et al., 1998; Hammes and Haller, 1998; Hammes and Hertel, 1998; Incze, 1998).

Most well-known members of probiotic bacteria are different strains of lactobacilli and bifidobacteria, that are generally used in dairy products. Since investigations with probiotic bacteria has been going on several decades, many positive health effects has been found, yet not all of them can be supported scientifically. Just to give a short list of the beneficial effects, the positive gastro-intestinal effect seems to be most thoroughly investigated. This positive effect includes not only the promotion of normal gut microflora and inhibition of harmful microbes e.g. after broad spectrum antibiotic treatment, achlorhydria, or infection, but probiotic bacteria can act also as immune stimulators, can split food ingredients (lactose) the organism is not capable for, they are probably able to counteract mutagenic and genotoxic effects, and these bacteria or their metabolites decrease cancer cell proliferation. As further beneficial effects anti-allergic characteristics, antihypertensive effect can be mentioned, as well as the influence on Helicobacter pylori, healing this way chronic gastritis and peptic ulcer (Sanders, 1999).

Dairy products as probiotic food are well-known and available on the market. Development of probiotic meat products has only been started. The reason of this gap may be explained by the basic differences in characteristics of dairy and meat products.

The main differences between milk-based and meat-based probiotic foods are that

- there is no way of elimination or drastic reduction of the initial sausage microflora as is the case with milk (pasteurization)
- the a_w-value of final meat product (fermented sausage) is critically low for most probiotic microorganisms, that is not the case with dairy products.

Since probiotic food is effective only if relatively large number of probiotic microorganisms is ingested (min. 10^6/g of food, more often: 10^{10}-10^{12}/day is the proposed dosis), strains have to be found that can compete the sausage microflora, can survive in large number the a_w-drop during drying and of course the gastrointestinal environment, too.

7. Discussion

Although starter culture technology has a history of about 40-50 years in meat industry, extension of its application is still going on in many parts of the world. While in countries where starter cultures have been in use since several decades, extension arises in form of application of new strains, new species or new genera (e.g. yeasts became quite "fashionable"), in other parts of the world the well proved technology is introduced either to replace traditional fermentation and drying or to start the process completely new for the region.

Sensory characteristics directly influence consumers' buying decision and in addition to color, texture, flavor and aroma are most important attributes. Dry-cured ham is a favoured topic of research in both texture, flavor and aroma, yet less attention has been paid to texture research with sausages. Because of the nature of technological differences between ham and sausage processing, more emphasis has been put on microbial role in sausage aroma research while in case of dry-cured ham the emphasis has been put on the role of tissue enzymes concerning their contribution to aroma formation. Now-a-days traditional long ripening-drying is a technology, which is practised almost exclusively with dry-cured ham, and is applied regretfully less and less with sausage manufacturing. In order to speed up the ripening-drying process even more than with starter culture, enzymes and enzyme-combinations have been tested and similar sensory characteristics were found in shorter time than with starter culture sausages. It has to be pointed out, that as for today no exact knowledge is available, how aroma-richness of long fermented meats could be produced in a shorter time. As a consequence it is the long fermentation which results in a superior quality product (e.g. Parma ham, Iberian ham, Hungarian salami etc.), to put it more bluntly, quality needs its time.

By the help of modern analytical methods very detailed information becomes available on volatile and non-volatile aroma components qualitatively as well as quantitatively. This way we are able to make distinction between products that are smoked or not, that are ripened for short or long time, we can determine what spices are used as typical flavouring ingredients etc. Further research is nevertheless needed if we want to find out in what concentration the different volatile and non volatile components should be mixed and how many of them would be needed for producing similar sensory perception as with consuming the very meat product.

Because of numerous outbreaks caused by foodborne diseases (salmonellosis, listeriae, EHEC, dioxin-crisis etc.) even more attention has been paid to safety issues than before (e.g. White Paper on Food Safety). As for prevention of foodborne diseases caused by pathogenic microorganisms the easiest and safest way (heat treatment) evidently cannot be the method of choice with *raw* fermented products. Keeping this in mind those factors have been and also will be mainly investigated, that play decisive role in inactivating or inhibiting pathogenic and spoilage microbes. In case of raw fermented meat products good hygienic status of raw material, GMP and operation of HACCP system are more important than in any other types of products where high intensity treatment can inactivate undesired microorganisms. Low initial count of raw material, high initial count and viability of starter culture, optimal environment for growth and metabolism of starter culture in the critical period, inhibitory environment for undesired microbes through the whole period of processing either in form of lowered pH-value and lowered a_w-value combination or low temperature and low a_w-value combination (traditional, long ripened products) and of course hygiene during all technological steps are about the most effective measures to be taken if we want to avoid health and spoilage risk with these types of meat products. In cases when not all of these requirements can be met, yet safe dried meat product is to be manufactured, heat treatment can be applied either before or after drying, nevertheless sensory value of such sausages will not be equal to that of raw fermented products.

On top of the enjoyment when tasting this delicious food rich in aroma, further advantages could also be "built in", if raw fermented items are transformed to probiotic food. Well-known and generally practised in dairy industry, the technology is just starting in meat industry by the help of which known meat product can be transformed to probiotic food without loosing its favourable sensory characteristics. In making this several difficulties have to be overcome such as: the product (chiefly sausage) has to carry large number of *viable* probiotics, but at the same time it has to be safe, assured by low pH-and/or low a_w-value, the viable probiotics should survive the gastro-intestinal environment and they must not alter the good sensory value of known product, or at least should not interfere with the acceptable sensory characteristics of a newly developed commodity, and finally, reliable human intervention experiments have to prove the efficiency of the product as probiotic food. Considering all these requirements one can see ample opportunities for doing research also in this field, which seems worthwile if we consider that meat has much less to do with allergic reactions than dairy products (Tarrant, 1998).

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