

Starter Cultures in the Processing of Meat by Fermentation and Dehydration

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When Dr. Acton asked me last summer to give a lecture in this meeting, he made me the following suggestion: "Personally, I would like to hear about your life's research in the area of meat preservation by fermentation and dehydration, and about the importance of bacteria in this process." So, following his suggestion, let me tell you today about my research on starter cultures, an endeavour in which I have spent half of my life.

The History of Food Fermentation

The preservation of food by fermentation is as old a custom as is the history of man. It was, surely, learned by chance. The purpose of the fermentation is not only to preserve the food, but also to improve its flavor, consistency, texture and nutritive value. So, fermentation creates new and unique products from a given raw material: wine from grapes; cheese from milk; beer from malt; and salami or dried ham from meat. Dried, fermented meat products have a shelf-life of months and a delicious flavor, quite different than the flavor of meat.

Fermented sausages probably originated in the Mediterranean area during Roman times (Buckenhüskes, 1990). By studying the circumstances leading to a particular type of fermentation, people learned how to control the otherwise spontaneous process with remarkable success. Products such as Salami Milanese and Hungarian Salami have prevailed for centuries and are still being consumed.

Fermentations were carried out during centuries without any scientific knowledge about the nature of the processes involved. Up until recently, sausage-makers would transfer old curing brine to the newly-prepared one. Thus new brine would become inoculated with beneficial microorganisms, causing the desired changes in the cured meat during ripening. This traditional method was based on empirical observations, with almost no knowledge of bacteriology. Therefore, the results were not always satisfactory. Failure was not uncommon.

In the 19th century, Pasteur showed that fermentations are caused by specific types of organisms. The first defined bacterial starters, intended for the fermentation of milk, were introduced about 100 years ago. It was not until the 1950's, however, that a pure starter culture became available for the fermentation of meat (Lücke et al., 1989). Shortly thereafter, pure cultures were mixed for even better results. The scienti-

fic basis of the use of starter cultures in the meat industry was for the first time presented in my doctoral dissertation in 1955. This study will be described later.

People often ask me: How did I become interested in the use of bacteria for the production of dried fermented sausage?

As a student and later as an assistant of Finnish Nobel Laureate (Biochemistry, 1945) Professor Artturi I. Virtanen, I became quite well acquainted with the importance of the bacterial cultures used in the processing of dairy products: cheese, butter, sour milk products (yoghurt), among others. Professor Virtanen had spent a great part of his life in the field of Dairy Science, having made many important discoveries. The results of his research significantly improved the quality of dairy products by ensuring the success of the delicate biological processes utilized then.

In 1947, Professor Virtanen asked me to initiate and to organize a new field of endeavour: meat research in Finland. Consequently, I left my work in biochemistry and started a brand new career without having the slightest idea about this new discipline, unaware of the nature of the problems to be solved.

I found my experience in Dairy Science to be quite useful. The fermentative changes in cheese were quite well known at that time, thanks to Professor Virtanen's previous research work. The formation of the typical flavor, aroma and consistency depended on the activity of the appropriate microbial flora in the cheese. Cultured microbes were used in these processes.

The ripening of dry sausage, I supposed, might have been by a similar process, but the information on the kind or kinds of bacteria which played a role therein was non-existent. Some microbes could have been useful and desirable but also harmful if found to cause discoloration, unpleasant odor or putrefaction within the system.

Therefore, I concluded that it might be an interesting and economically important task to clarify the relationship between different microbes, the changes produced during the process and the quality of the final product.

Decisive for my future work was the discussions with Dr. Niven and Dr. Deibel in Chicago in 1953. Both of these scientists were interested in the role of microbes in meat fermentation, e.g., in the ripening of dry sausage. This was my first study trip to the U.S.A.

The First Successful Achievement with Pure Cultures for Meat Processing

In spite of the many unsuccessful experiments in the U.S.A., I decided to start the research in order to isolate bacteria and test the influence of these organisms in the

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manufacture of dry sausage. The main purpose of the studies was to isolate bacteria from good quality dry sausage and to determine their influence on the ripening process. The ultimate purpose of the study was to inoculate sausage batter with cultures of the isolated bacteria in order to improve the process by shortening the fermentation time, improving color and flavor and eliminating the risk of spoilage and discoloration inherent in the traditional process.

In addition to the organoleptic evaluation, microbiological, chemical and physical determinations were carried out. Variations in moisture content, drying loss, fat content, pH value, redox potential, photometric color determinations, and the content of glucose, lactic acid, nitrate, nitrite ammonia and hydroxylamine in the sausage, were carried out.

Microbiological analyses included differences in total bacterial count, aerobic and anaerobic microorganisms and micrococci were determined. The technological trials were carried out in the pilot plant of the Research Institute for the Meat Industry, in Hämeenlinna, Finland.

In the preliminary tests, the best results were achieved with a bacterial strain which we called "M-53." In accordance with Bergey's Manual, this organism was classified to be closest to *Micrococcus aurantiacus*. The "M-53" organism was then used as the starter organism in the original and subsequent research work. The results of this research work became the author's Doctoral Dissertation in 1955 (Niinivaara, 1955).

In my dissertation, the following important observations were made and published. Using starter cultures:

- Color formation was speeded up.
- The pH value of the system was lowered more rapidly.
- The desired consistency was achieved more rapidly.
- Total processing time could be shortened considerably, a great economical advantage.
- The process became fail-safe in view of the antagonistic nature of the starter culture which inhibited many spoilage or pathogenic organisms.

"It is a Long Way From Idea to Success."

There were many factors which led—through many difficulties—to the success of utilizing starter cultures industrially. The first of these, a very important one, was the industrial propagation of suitable strains. Fortunately, I was able to start a good cooperation with an enthusiastic person from Germany, Herr Rudolf Müller, who agreed to develop the cultivation of microbes, i.e., "starter cultures", on an industrial scale. At that time, there was no model to follow for this kind of product and, therefore, we had to overcome numerous difficulties. The greatest one of these was the preconceived opinion of many people in industry as well as in research institutes.

The bacterial culture methodology I had used at pilot plant scale was not directly applicable at industrial scale. A new method had to be developed. Lyophilizing technology was still in quite a primitive stage: it was not cost-effective, and it significantly decreased the activity of the microorganisms. If

that were not enough, later on we had problems with bacteriophage, which destroyed the cultures towards the final stages of cultivation. Therefore, we were forced to isolate new phage-resistant strains exhibiting the characteristics we had determined to be desirable in starter cultures for sausage: nitrate reduction capacity, acid production capacity and an antagonistic effect towards harmful/undesirable bacteria.

Finally, when after much toil lyophilized starter cultures became available, we met the skeptical attitude and preconceived opinion of the meat industry.

Fortunately, at that time I was working in the Research Institute for the Meat Industry in Hämeenlinna and therefore had the opportunity to organize experiments in order to demonstrate the advantageous influence of the starter cultures. Through Herr Müller, the positive, encouraging results obtained by the Finnish meat industry were transmitted to the German meat industry, where initially the attitude was very skeptical, as well. In fact, many leading European Meat Research Institutes would stubbornly not accept this innovative methodology and, therefore, discouraged further research on the subject.

Thus, Finnish meat industry was the first test laboratory in the development of the starter cultures for industrial use. The Finns (filled with "Suomalainen Sisu," a mixture of courage, determination, inspiration and stubbornness), went from pilot-plant scale to the industrial level of applications. But slowly, through continued experimental work in Finnish industrial plants, and later on in the German meat industry, the beneficial influence of starter cultures on the production, quality and economics of dry fermented sausage was finally recognized. The use of starter cultures was accepted and became a reality. This was a key accomplishment!

In 1972, at my initiative, the International Starter Culture Symposium was organized in Helsinki. This event strongly influenced the opinion and effectively removed the prejudice from many meat scientists. Afterwards, cooperation with many countries developed and collaborative studies were carried out and published (Proceedings, Starter Culture Symposium, Helsinki, 1972).

Mixed Starter Cultures

After having gathered a great deal of information about the importance and role of the micrococci in the ripening process of dry sausage, we started to clarify the role of lactobacilli in the process. These investigations led to Esko Nurmi's Doctoral Dissertation in 1966. As a result we were the first to develop the combined inoculation with mixed starters containing a pure culture of *Micrococci* and a pure culture of *Lactobacillus plantarum*. The *Micrococcus* ensured color formation whilst the *Lactobacilli* was responsible for the decrease in pH-value and for the formation of the desired texture and consistency. This was another key accomplishment!

Before Nurmi's findings, it was commonly believed that *Lactobacilli* were the main spoilage organism in European dry sausage (Coretti, 1958). These studies showed that lactobacilli are also useful and, in many cases, necessary organisms in the ripening process.

Some Aspects of the Properties of the Starter Cultures

Antagonistic Properties

Research carried out during recent years has proved that the antagonistic properties of starter cultures is a very important conservation factor. In addition to their role in fermentations, the suppression of spoilage and pathogenic bacteria offers new application for the use of starter cultures in food manufacturing. Just in the last years, the use of microorganisms as protective flora has expanded, for example, in the packages of cooked sausages.

In my first publication on starter cultures (Niinivaara, 1955), the inhibitory effect of starter cultures was already mentioned. This was shown in laboratory trials, but also in technological experiments at pilot plant scale. We were able to show that the microbial formation of hydroxylamine is possible only when nitrate was present in the growth milieu. Hydroxylamine had an inhibitory effect on the growth of spoilage bacteria.

In the work by Pohja (a dear, inspiring co-worker of mine) and Niinivaara (1957), we showed how the starter organisms inhibited the growth of many undesired organisms. Later, the antagonistic influence of starter cultures on the growth of *Salmonella senftenberg* in the dry sausage (Niinivaara et al., 1977) was shown. Pohja's doctoral dissertation pointed out the first selection system of useful Micrococci. Later on, his work has been used as the model for other microbes. Thus, the starter cultures improve the hygienic conditions during processing and minimize the potential health risk caused by the pathogenic organisms.

The Gram-Negative Bacteria in the Fermentation

There are many indications that gram-negative bacteria play an advantageous role in the fermentation process. For instance, the proteolytic activity of the bacteria in this group can degrade proteins to form the desired aroma. On the other hand, we usually try to avoid the growth of gram-negative bacteria because many pathogenic organisms belong in this group.

Esko Petäjä (1977) started an investigation to clarify the role of gram-negative bacteria in sausage ripening. He isolated a *Vibrio* strain (*Vibrio costicolus*) that he used in the drying and fermentation of dried ham with limited success. Although it had a positive influence on the flavor of ham, *Vibrio costicolus* was found to be very sensitive, difficult to cultivate and apt to lose its fermentative capacity after freezing. Therefore, it never came into commercial use.

Petäjä also isolated several strains of *Aeromonas*. Two strains of his collection, *Aeromonas X* and *Aeromonas 19*, had the most favorable effect on the quality of sausage. The best results he achieved when *Aeromonas* was inoculated together with *Lactobacilli*.

These strains are not available commercially despite the fact that 12 years ago we achieved very interesting results in production trials carried out under the direction of Dr. Abraham Saloma in Argal S.A., a commercial operation in the city of Lumbier (Navarra), in Spain. This factory has for several

years successfully used these starter cultures in the commercial production of Spanish dry sausages, Salchichon and Chorizo.

Starter Cultures and the Nitrite Problem

During the last decades, investigations have been carried out on the fate of nitrate/nitrite in cured meat products. Evidence exists that under certain circumstances the formation of carcinogenic nitrosamine is possible. This is one reason why many laboratories are trying to find out how to minimize the concentration of nitrite in meat products to a level where bacterial spoilage could be avoided without jeopardizing color formation.

My co-worker Eero Puolanne has worked on this problem. In 1977, he published his Doctoral Dissertation "The effect of lowered addition of nitrite and nitrate on the properties of dry sausage." He was able to show that by using starter cultures it is possible to lower the nitrite and nitrate addition by one-third from the normal level.

Investigation by Niven

By an astonishing coincidence, at the same time my work on the use of Micrococci was published, Charles Niven published his study on the use of *Pediococcus cerevisiae* (*P. acidilactici*) as starter culture in the American type of dry sausage known as "summer sausage" (Niven & Wilson, 1955). We had worked independently of each other. After the discussion in Chicago in 1953, we did not have any contacts with each other, but our studies were published exactly at the same time (April 1955). Niven's *Pediococci* also became commercially available and was sold under the name ACCEL (E. Merck, Rahway, N.J.).

Future Research

The future opens many new perspectives to create new starter cultures and utilization of microorganisms in new fields.

Leistner, et al. (1990) have mentioned the genetic possibilities of improving certain properties of bacteria. In the future we could, through gene technology, improve the production and activity of microbial protease, lipase, catalase, nitrate reductase, to name a few. In that way we could give new properties, or strengthen the desirable ones already existing in the microbe.

It seems also possible to transfer new genes into a given microorganism so that it may produce aroma components, vitamins, specific desirable metabolites, and so on. In addition to the research oriented towards the solution of old, traditional problems of meat fermentation, there are new elegant methods to create better cultures with stronger activity in those desirable reactions that favor a good fermentation process.

Much research has been carried out in the field of meat fermentations. Yet, we are far away from understanding the complete interrelations between the microbiology, the technology and external factors influencing the fermentation and ripening process (Buckenhüskes, 1990).

Financial Aid

A very significant step in starter culture research was the financial aid received from the United States Department of Agriculture. Two grants of considerable magnitude became available for this research in the years 1959-1963 and 1964-1969. This financial support made it possible to continue the research work on starter cultures in Finland. Thirty-eight scholarly papers were published on this subject, thanks to the generosity of the USDA.

International Cooperation

The year after my studies on starter cultures were published, we started the international cooperation with Germany. The objective was to create starter culture technology and a distribution organization to deliver the cultures to the meat processors. This cooperation continued for about 20 years.

Many other international contacts came into being later on, which proved to be very beneficial for the research and development work. In that fashion, we were able to exchange thoughts and ideas, bacterial strains and even research work personnel between countries. Mutual cooperation was carried out with Yugoslavia, Hungary, Bulgaria and later with Spain. In all these countries, fermented sausages have for centuries constituted a very important gastronomical tradition. In addition, they play a significant role in the meat industry.

I would especially like to allude again to a very interesting and sympathetic co-operation with a meat processing firm in Spain. This cooperation started in 1976 by the initiative of Dr. Abraham Saloma, who was then the technology director of this company, Argal, S.A., then a subsidiary of the Antonio Porta Labata Group. Under Dr. Saloma's direction, important improvements were achieved in the production of fermented salchichón and chorizo at commercial scale.

One significant accomplishment at Argal was the establishment of one of the most complete starter culture programs anywhere in the meat industry. More than 12 different strains of microbes (bacteria, yeasts and fungi) were daily propagated and incorporated into fermented products. Each particular sausage was inoculated with a tailored mixed starter culture that ensured distinctive characteristics and high quality in the final product. I would like to thank Dr. Saloma for these pleasant years of fruitful cooperation, friendship and international understanding.

The year 1955 was the birth year not only of starter cultures for meat, but also the birth year of the international cooperation called the European Meeting of Meat Research Workers (EMMRW), renamed the International Congress of Meat Science and Technology (ICoMST) in 1987. The birthplace of this Congress was the same small Finnish town, Hämeenlinna, where much of the starter culture work originated and was carried out.

This Congress has gathered annually, without interruption, during 35 years, having become an international forum for lectures concerning not only starter culture research but meat science and technology across many international borders. More than 30 countries participate each year in this congress. It has been a wonderful feeling that the idea of

scientific collaboration, originally expressed in a small circle amongst good friends and colleagues, was the seed for a great intercontinental cooperation.

Some Remarks About My Life's Other Activities

As I mentioned at the beginning of this lecture, I started meat research in Finland in 1947. There was no model in Europe to learn what meat science was all about and which problems had to be solved. Even in countries where meat research had been started, like in Germany, everything was destroyed by the war. Meat science laid in primitive stages in ruined Europe. The only way was to identify the problems and to produce solutions alone, and through research build the knowledge, to improve the quality of the products, to improve the economy of the production, to improve the utilization of the byproducts. We started our independent research work from zero in meat science and technology.

The most important question in the production of cooked sausages was the waterbinding capacity of meat. This was the theme with which we worked during the first years, since 1952 in co-operation with German meat scientists Grau and Hamm.

The next step was to concentrate on the problems of the fermented meat products. As I described at the beginning of this lecture, the role of the beneficial microbes should be cleared up. The final goal was to find and cultivate microbes, add them as the pure cultures in the meat mass and by that way speed and ensure safety in the processing. Both things mean better economy in the production. Of decisive importance for this work was the encouraging discussion in the American Meat Institute Foundation with Dr. Niven (in 1953) and later the financial aid by USDA in the form of two grants of considerable amount. In many scholarly papers, new information about the basic problems in meat science was published in the international scientific journals and congresses. This research activity created a new field of food science. This made it possible to establish a professorship at the University of Helsinki for Meat Science and Technology. Through this research work, meat technology gained "saloon competence," as people say in Europe. It became working knowledge.

Through financial aids by the meat producers, the chair and professorship of Meat Technology was founded. My duty was to start this academic activity in 1961 again from zero, like the industrial meat research 14 years earlier.

Besides the scientific research—partly continuing the old themes—the education of new generations of young students for leading technical positions in the meat industry was my duty with high challenge.

This professorship in Meat Technology was the first one in Europe. No models existed for the content of the instruction. No academic text books existed. The lectures, the exercise works had to be created from zero, again.

Now, more than 100 meat technologists are in leading positions in the Finnish meat industry. Eero Puolanne, one of my students, is continuing my academic career as professor of Meat Technology and Director of "my" Meat Research Institute.

Just before retiring and leaving my duties as university professor, I started a new activity in the field of meat.

It was generally known that consumers had lots of wrong information about the nutritive value of meat. Housewives, who in most homes are responsible for the preparation of meals, were confused when buying meat: which meat or which part of the carcass for which purpose. The strong propagation from the side of *Die Grünen*, the "greens," vegetarians and raw food eaters, made the housewives still more confused. They often ask: "Is meat healthy for my family or is it not?" To improve the knowledge of the consumers about the quality and nutritive value of meat, to facilitate the choice of meat, to give new ideas in food preparation, we founded a society that we called "Meat Information Center," of which I was the first chairman and charter member.

During the last years, we have been able to build a useful cooperation with many corresponding organizations abroad. The international cooperation helps us to get new ideas and to evaluate experiences about different activities.

Last, but not least, I should like to mention the constructive cooperation with students in Finland: The Society of Food Science ("Lipidi"). I have made numerous studying excursions with my excellent students in many countries in the world, sharing ideas, promoting knowledge of food science, creating strong bonds with fellow professionals and fostering good will amongst many people internationally. This

Society has selected a few honorary members, one of them being Abraham Saloma (since 1978). Other ("Lipidi") honorary members are Professor Robert S. Harris (U.S.A.), Doctor Peter Zeuthen (Denmark), Professor Lothar Leistner (Germany), Professor Ferenc Lorincz (Hungary), Professor Torsten Storgards (Sweden), Professor Rainer Hamm (Germany), Professor Velimir Oluski (Yugoslavia), Doctor Sandor Balogh (Hungary), and Professor Ralston Lawrie (U.K.).

During the 22 years I was active in the University of Helsinki, I performed the duties of the chairman of this Society for 20 years. Like my job as University Professor, my position in this society was inherited by my successor in the office, my dear student, Professor Eero Puolanne.

In Conclusion

I am very happy to be able to review today, before this wonderful audience, many of the magnificent developments which have taken place during the past 35 years. Time has quickly flown from the first experiments when we inoculated sausages experimentally to the present, when starter cultures are indeed an integral, indispensable part of fermented meat and other food products. The reason why I was invited to participate in this very interesting meeting is that I was there when this beautiful story began.

References

- Buckenhuskes. 1990. Lecture at the FIA-Conference 1990 at Singapore.
- Coretti. 1958. Die Bakterienflora Frankfurter Rohwurst. *Arch. Lebensmittelhygiene*. 9: 32-35.
- Krol, B. 1984. Progress and problems of fermented sausages. *Trends in Modern Meat Technology*—1. pp. 55-57. Pudoc Wageningen.
- Kunz, B. 1989. Aspects in the use of starter cultures in meat products.
- Kuusela, K.; Puolanne, E.; Petäjä, E.; Niinivaara, F.P. 1978. A rapid method to estimate the activity of lactobacilli used as starter cultures. 24th European Meeting of Meat Research Workers, Kulmbach.
- Leistner, L. 1987. Perspectives of fermented meats. International Congress of Meat Science and Technology. Helsinki. pp. 323-326.
- Leistner, L. and Scinunsefridze als Starterkulturen beider Rohwurstherst ellemg. Starterkultur Symposium, Helsinki, 1972.
- Lucke et al. 1989. Starter culture development. Proc of the COST 91 bis sub-programme "Food Technology of the EEC." Göteborg. October 3-5.
- Niinivaara, F.P. 1955. About influence of bacterial pure cultures on the ripening and color formation of the dry sausage (in German). *Acta Agralia Fennica*. 85.
- Niinivaara, F.P.; 1964. Bacterial pure cultures in the manufacture of fermented sausages. *Food Technology* 18, 2:25-31.
- Niinivaara, F.P.; Sederholm, H. 1963. Über die knotinuerliche Züchtung von Mikroorganismen. 9th European Meeting Meat Research Workers.
- Niinivaara, F.P.; Sirviö, P.; Nurmi, E.; Puolanne, E. 1977. Der Einfluss von Starterkulturen und verschiedenen Zusatzstoffen auf das Wachstum von *Salmonella senftenberg* in Rohwurst. *Fleischwirtschaft* 57, 5: 1007-1008, 1011, 1012.
- Niven, C.F.; Deibel, R.H.; Wilson, G.D. 1955. The use of pure culture starters in the manufacture of summer sausage. *Am. Meeting Amer. Meat Inst.* 5 p.
- Nurmi, E. 1966. Effect of bacterial inoculations on characteristics and microbial flora of dry sausage. *Acta Agralia Fennica*. Nr. 108. p. 7-73.
- Petäjä, E. 1977. The effect of gram-negative bacteria on the ripening and quality of dry sausage. Dissertation. J. Scientific Society of Finland. 49: 107-166.
- Pohja, M.S.; Niinivaara, F.P. 1957. Über die antagonistische Wirkung eines Mikrokokken-Stammes gegen die in Rohwurst vorkommenden Bakterienstämme. *Zeitschrift für Lebensmittel-Untersuchung und-forschung* 106, 4: 298-301.
- Pohja, M. 1960. Micrococci in fermented meat products. Dissertation. *Acta Agralia Fennica*, 96.
- Puolanne, E. 1977. The effect of lowered addition of nitrite and nitrate on the properties of dry sausage (in German). Dissertation. J. Scientific Agricultural Society of Finland. 49:1, 1-106.
- Roca, M.; Incze, K. 1989. Antagonistic effect of some starter cultures on Enterobacteriaceae (*E. coli*). *Meat Science* 25, 123-131.