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Dry Fermented Sausages

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ABSTRACT

Two major aspects of fermented sausages are discussed in the article. After a brief history of enterohemorrhagic Escherichia coli *with special regard to fermented meat products the basic experimental designs and results are tackled on the basis of which safety issue in this respect is thoroughly discussed. The results show a rather limited opportunity for meeting food safety requirements with short or medium time ripened raw fermented sausages while traditionally, long ripened sausages are in a much better position. Fermented meat products as probiotics are also discussed. After evolving the EHEC outbreaks caused by fermented sausages the possibilities for finding reliable methods and products have been narrowed and more research is needed to find optimum solution.* © *1998 Elsevier Science Ltd. All rights reserved*

INTRODUCTION

Fermentation and drying of meat products are probably the most ancient ways of preservation under normal climatic conditions and in some cases under hot climate, too.

After gathering experience of some thousand years and after several decades of intensive research in the field of fermentation and drying of meat products, manufacturing of fermented meat products still 'involves a lot of science, a little art and a certain amount of mistique' (Hand, 1997). From the 1950s on a real breakthrough in manufacturing of fermented meat products can be observed: thanks to the research of Niinivaara and Niven use of pure bacterial cultures in fermented meat products has been launched. This process was a 'triumphal march' characterized by mutual utilization of knowledge of experts in practice and in research. In some decades the new technology with application of starter cultures gained an overwhelming victory: manufacturing of traditional dry sausages has been changed not only 'inside' by addition of acidifying and aroma forming, nitrate reducing starter cultures but also 'outside' using mould starters. The new technology pushed gradually into the background those manufacturing practices where no starters have been applied, claiming rightly the higher level of safety in case of starter application, at least with sausages of short ripening time.

This almost idealistic situation lasted until such micro-organisms emerged or 'developed' that were less sensitive to the inhibiting factors produced by starter cultures and by drying. While growth of salmonellae is inhibited by lower pH, lower temperature and lower water activity and staphylococci are retarded in their growth by lower pH and lower temperature, listeriae grow almost undisturbed at lower temperatures and lower

pH-values and EHEC (enterohemorrhagic *E. coli*) strains survive for a long time these inhibiting factors that destroy many other micro-organisms.

Out of the almost innumerable interesting topics related to dry fermented sausages only some will be dealt with in this review, such as safety issues with special regard to EHEC strains and fermented sausages as probiotics.

ESCHERICHIA COLI IN DRY FERMENTED SAUSAGES

Escherichia coli, just as other Gram negative members of Enterobacteriaceae, has not exhibited earlier a real problem with dry fermented sausages, unless serious mistakes of hygienic and/or of technological nature have been made.

These microbes, being common contaminants of food raw material, usually disappear from the fermented sausages as a result of the combined effect of low pH, low temperature and low water activity. This effect is enhanced to some extent by the presence of nitrite and also by other metabolites in addition to lactic acid produced by starter cultures. As a consequence no *E. coli* could be found in a sausage fermented and dried according to good manufacturing practice and there was no risk of recontamination and growth of *E. coli* on dry sausages because of the inhibiting factors listed above. These factors have been effective against other undesired bacteria as well: a sound basis for the firm belief that fermented dry sausages have an excellent safety record. This was the situation until 1994 when an *E. coli* O157:H7 outbreak was linked to presliced dry fermented salami in the United States (Anon, 1995*a*; Tilden *et al.*, 1996) which was followed by an outbreak in Australia linked to mettwurst, an uncooked semi-dry fermented sausage (Anon, 1995*b*). Even though dry sausage as vehicle of enterohemorrhagic *E. coli* is far less frequent than either hamburger or other foods of animal and plant origin that are not or not sufficiently heated, the excitement and perhaps too extreme reaction of health authorities seem justified for three reasons:

- the increasing number of *E. coli* O157:H7 outbreaks and the severe consequences of even low bacterial numbers,
- to learn that a product of excellent safety record can be a time bomb threatening consumers with serious illness or even with death is a shocking new experience,
- the easy solution of the problem with other foods ('they have to be cooked') is not really a solution with *raw* sausage.

BRIEF HISTORY

Some strains of *E. coli*, a species used earlier as a non-pathogenic indicator of enteric pathogens, changed probably in the early 80s to virulent organisms represented by several strains. These enterohemorrhagic *E. coli* (EHEC) strains acquired their Shiga or Shiga-like toxin from Shigella. (*E. coli* strains acquired not only Shiga-like toxins but also other virulence factors causing pathogenicity to humans or animals. In addition to EHEC also enterotoxigenic /ETEC/, enteroinvasive (EIEC) enteroaggregative /EaggEC/, enteropathogenic /EPEC/ and diffusely adherent /DAEC/ strains are known. In this article nevertheless only enterohemorrhagic *E. coli* will be discussed.) In 1982, two hemorrhagic colitis (bloody diarrhea) outbreaks were observed where *E. coli* O157:H7 was indentified as causative agent and somewhat later the same micro-organism was found in feces of children with hemolytic uremic syndrome (HUS) (Karmali *et al.*, 1983; Riley *et al.*, 1983). HUS and other life-threatening complications like central nervous system deterioration,

stroke, renal impairment and end-stage renal failure, etc., may occur in patients suffering from hemorrhagic colitis (Buchanan and Doyle, 1997; Desmarchelier, 1997) the risk being the highest with young children and with elderly people.

As a result of the dry sausage outbreaks in the United States (California and Washington) the US Department of Agriculture and the Food Safety and Inspection Service developed guidelines (Reed, 1995) that required fermented sausage manufacturers to demonstrate a 5-log unit reduction in the enterohemorrhagic *E. coli* count during processing (Hinkens *et al.*, 1996). Since it was already known from earlier works (Buchanan and Klawitter, 1992; Glass *et al.*, 1992; Pozzi *et al.*, 1996) that EHEC strains are rather resistant to extrinsic and intrinsic factors, thus an easy solution for meeting the requirements of USDA Food Safety and Inspection Service was not foreseen.

First at University of Wisconsin a research project was launched sponsored by the National Cattlemen's Beef Association where raw meat batter was inoculated with a five-strain *E. coli* O157:H7 cocktail at levels of at least 10^7 CFU g^{-1}, the experiments were run in triplicate. Acid and heat sensitivity of the strains were tested, (Freeman, 1996) and since sausages were dried to different moisture:protein ratios also the effect of a_w was considered indirectly. On the basis of the results five options have been worked out and suggested, respectively.

Option 1: heat process, 63°C for 4 min
Option 2: companies develop and validate their inactivation treatment
Option 3: hold-and-test program for finished product (sub-samples from 15–30 individual chubs)
Option 4: combined treatments
Option 5: HACCP system including raw batter testing and a 2-log inactivation in fermentation and drying

Food Safety and Inspection Service insists options 1–4 result in a five-log reduction of *E. coli* O157:H7.

Following the publication of these options research work on the fate of enterohemorrhagic *E. coli* influenced by factors common during fermentation and drying of sausages, as well as on possible elimination of EHEC strains has been intensified.

It was already known that EHEC strains can be more tolerant to environmental factors, they have a high acid tolerance, surviving 2–7hr exposures to pH 2·5 and 37°C (Benjamin and Datta, 1995; Buchanan and Edelson, 1996) and their salt tolerance can also be remarkable compared to generic *E. coli* strains. There exist of course acid-tolerant non-enterohemorrhagic and acid-intolerant enterohemorrhagic *E. coli* strains, too (Buchanan and Doyle, 1997), but for safety reasons, chances of survival of more resistant strains have to be considered and challenged in 'five-log reduction' studies.

A real limiting factor in finding EHEC-inactivating methods of technology is the fact that intensifying almost any inhibitory effect of common dry sausage fermentation practice will alter palatability adversely, not to mention cooking that seems almost the only effective means of 5-log reduction, yet very atypical method with a group of traditionally *raw* products.

EFFECT OF FERMENTATION ON EHEC SURVIVAL IN MEAT ENVIRONMENT

Experimental designs are usually similar in using a 'cocktail' of EHEC strains isolated from outbreaks for inoculation studies but inoculation has not always been done in form

of mixing in the batter (Hinkens *et al.*, 1996; Yu and Chou, 1996; Calicioglu *et al.*, 1997; Gareis, 1997), injection of stuffed sausage was also used (Nissen *et al.*, 1997) or inoculation of slices of ready dry sausage was applied, too (Zeuthen *et al.*, 1997).

In two similar experiments (Hinkens *et al.*, 1996; Calicioglu *et al.*, 1997) pepperoni and all-beef summer sausage batter resp. were inoculated with a commercial Pediococcus starter culture and a five-strain mixture of *E. coli* O157:H7 ($\geq 10^7$ CFU g^{-1}). The sausages were fermented to pH 4·6 or 5·0 and then heated to different temperatures for different periods. The results can be summed up as follows. Regardless of the final pH fermentation alone resulted in only a 1·2–1·39-log CFU g^{-1} decrease in EHEC number and no further change could be observed after drying to a moisture: protein ratio of $\leq$1·6:1 either. Heating on the other hand resulted in a definite lethal effect the extent of which was influenced by final pH. Pathogen number was decreased by $\geq$7 log units in chubs of pH 4·6 after heating to an internal temperature of 54°C instantaneous but this decrease reached only a 3·2-log unit with the same heating if final pH was 5·0. When holding these sausages at 54°C for 30 or 60 min, pathogen numbers decreased by 5–7 log units, respectively. In pepperoni heating to internal temperatures of 53°C for 60 min resulted in a 5–6 log unit reduction while heating to 63°C instantaneously ended up with a $\geq$7 log unit decrease in number of pathogens.

Yu and Chou (1996) inoculated Chinese-style sausage batter with four strains of *E. coli* O157:H7 and dried in 50°C air-blast drier for 6 hr, taking samples every 2 hr. They found 2-log unit reduction in pathogen number by the end of drying, when internal temperature reached 48°C if sausage contained curing agents (a mixture of 13 additives including sucrose, salt, nitrite, sorbate and ascorbate). Without curing mixture no reduction occurred. In tryptic soy broth with curing agents 6-log reduction in 4 hr and without curing agents about 2-log unit reduction in 6 hr occurred with the same heating.

Nissen *et al.* (1997) inoculated freshly stuffed sausage (with Lactobacillus starter) with low (10^{3-4}) and high (10^{5-7}) numbers of *E. coli* O157:H7, Salmonella kentucky and Listeria monocytogenes. The sausages were fermented and dried according to common Norwegian practice and stored afterwards until 5·5 months at 4 and 20°C. In the low inoculum samples *E. coli* number increased in the first few days of fermentation but then decreased and no *E. coli* could be detected at the end of the storage period of 5·5 months regardless of the storage temperature. In case of high inoculum samples *E. coli* number decreased throughout the fermentation and drying period, yet they were undetectable at the end of storage of 5·5 months only if the chubs were kept at 20°C. In the sausages stored at 4°C about 5×10^2 CFU *E. coli* per sample were found at the end of storage. Water activity values were 0·91 (4°C) and 0·89 (20°C), respectively, pH values 4·8 and 5·1.

Zeuthen *et al.* (1997) sliced Danish fermented sausage ready for sale and inoculated with *E. coli* O157:H7 in two batches, with 4 and with 400 cells g^{-1} sausage. The pH value was 4·54 at the time of inoculation. Inoculated slices were vacuumpackaged and stored at ambient temperature. After 7 days storage no survival of pathogens was detected in an environment of 4·5 NaCl, 25·8% moisture and pH 4·5 with predominant growth of lactobacilli.

Duffy *et al.* (1996) investigated the effect of pH, sodium nitrite and NaCl as well as heat treatments of different intensity on the survival of *E. coli* O157:H7 in fermented sausage. Their findings were as follows: under extreme circumstances (pH 4·4; 4·8% salt; 300 mg kg^{-1} $NaNO_2$) a 4-log unit decrease in *E. coli* number could be detected, but the growth of starter culture was inhibited, too, consequently no fermentation in pepperoni occurred. With lowered salt concentration a reduction in *E. coli* number of approx. 2-log unit was found, an increase of about 0·5-log unit compared with the standard process. A further reduction in the number of *E. coli*, meeting the 5-log reduction requirement, could be achieved only by a mild heating at 55°C for 1 hr.

FERMENTED SAUSAGES AS PROBIOTICS

In the intestines of humans about one third of the faecal mass is made up by live bacteria known as intestinal flora. Its beneficial and harmful fractions are well balanced in healthy humans and the beneficial bacteria dominate. In gastro-intestinal disorders harmful bacteria outgrow beneficial ones, but if harmful bacteria dominate the detrimental effect is not necessarily immediate, serious consequences may occur delayed (Mitsuoka, 1996).

Therapeutic use of lactic acid bacteria as an aid to cure some types of gastro-intestinal disorders has been supported for almost a century (Cohendy, 1906; Hawley *et al.*, 1959). Ever since gets this topic intensive publicity, owing to other beneficial effect of lactic acid bacteria, yet pros and cons are equally represented.

The early works started with soured milk: yoghurt, koumis and kefir were used for medical purposes in Europe and in Asia long before the microbes responsible for the favourable effect were recognized and identified (Hawley *et al.*, 1959). Metchnikoff (1910) attributed senility and arterio-sclerosis to toxins produced in the intestines because of putrefaction, that can be cured by ingestion of large amounts of milk soured with Bacillus bulgaricus.

Rettger *et al.* (1935) summarized more than 60 years ago the conditions on which the success of lactobacillus therapy depends. They showed that it is necessary to use an intestinal strain (*L. acidophilus*) in form of large number of viable cells. These criteria are still valid and have been completed with some others since then. Whether administration of lactic acid bacteria, even in large number, has really an unequivocally beneficial effect on the health status is judged still contradictory. In the scientific literature one can find optimistic and guarded opinions equally (Sanders, 1993). Latter claim that the mechanism is still insufficiently known, and more research has to be done concerning antimicrobial, anticholesteremic and anticarcinogenic effect of bifidobacteria (Driessen and de Boer, 1989; Tannock, 1990; Kurmann and Rasic, 1991). Sanders considers a further problem in most cases the lack of direct evidence for a positive effect of lactic cultures on human health, since many papers deal with experiments on animals, *in vitro* or on humans with few subjects.

The questions whether the application of probiotic bacteria will bring the expected health benefit and what factors are necessary to be considered are manifold:

- apathogenicity
- viable number and viability of the micro-organism
- natural habitat of the organism
- adherence of the probiotic culture
- acid and bile tolerance of the micro-organism
- extent of pathogen inhibition
- action as a potential immunogenic factor
- fermenting ability
- good flavor formation in the lactic food

It is evident that viable lactic acid bacteria in large number have to be administered if it is aimed at reasonably high number in the intestine that can compete other microbes already well adapted. In recent investigations strains selected for application in probiotics are tested also for pathogen inhibition and adhesion to intestinal human cell linings (Mogensen and Friis, 1997). Testing of all the properties listed above is advisable if we want the probiotics do their duty (Velazquez and Feirtag, 1997). The detrimental effects of gastric fluid on probiotic bacteria can be hampered when these microbes are consumed with food, because this way pH is raised. It has been suggested that adherence and colonization

can take place or at least has greater chance if to a human subject his own bifidobacteria has been administered. It is interesting to note that even if the administered bifidobacteria survive the effect of gastric juice and bile effect and have good adherence ability they may not necessarily colonize the colon (Bouhnik *et al.*, 1992), yet their transient presence and their significant biochemical influences *in vivo* can exert the beneficial effect expected (Savage, 1977). Whether this favorable effect comes from production of acid, bacteriocin, some other factors or from their combination is not clear. Bacteriocins have usually a narrow spectrum of inhibition, mostly against closely related bacteria (other lactobacilli). For this reason selection of probiotic strains for bacteriocin production and their application can have a negative effect by inhibiting or displacing native 'desired' lactobacilli, not pathogens or putrefactives in the gastrointestinal system (Sanders, 1993).

DISCUSSION

Considering the resistance of EHEC strains to low pH and relatively low water activity as well as the fact that the presence of 10–100 bacteria can cause serious illness the challenge seems to be great and the real solution somewhat remote for several reasons:

- It is evident from the experimental data, that manufacturing of fermented sausages according to common practice with some weeks ripening time will bring about only 1–2 log unit reduction in number of the pathogen, a value far less than required by USDA-FSIS.
- Changing common practice to the extreme (lowering pH, raising salt and nitrite) results in somewhat higher reduction of the pathogen but the product is of dubious value from sensory point of view.
- Most of the experimental data suggest that *heating* is the only effective and reliable method for a 5-log reduction in number of enterohemorrhagic coli. The question is then: can these sausages be considered as *raw* fermented products? To what extent will be the aroma affected adversely?
- Even if meat industry does its utmost for supplying consumers with safe products, they still can run the risk of EHEC infection if fermented sausages are sliced at retail outlets or at home inadvertently: an action well beyond the responsibility of manufacturer.

What solution seems then acceptable?

With *short ripened* products, if USDA-FSIS requirements have to be met, heating is probably the only choice. It can not be excluded of course that multi-target preservation and metabolic exhaustion (Leistner, 1995) will help in solving this problem, promising results are shown by Nissen *et al.* (1997) and Yu and Chou (1996) in this respect, yet more extensive research has to be done in order to meet safety requirements. Fashionable field of research on bacteriocins (De Vuyst and Vandamme, 1994; Samelis *et al.*, 1994) may also contribute offering effective alternatives. With sausages of *longer ripening* period option 5 (HACCP system including raw batter testing and a 2-log inactivation in fermenting and drying) seems an adequate solution.

Traditional, *long ripened and dried* sausages and salamis represent a special group of products not only in terms of technology, pH value and richness in flavor and aroma (Incze, 1987; Nagy *et al.*, 1988; Incze, 1992), but also regarding the low risk as vehicle of EHEC infection. If these products are heavily contaminated during slicing operations, they can cause illness, just as *any other food.* If on the other hand the sausage batter is

contaminated with enterohemorrhagic *E. coli*, that can occur also in case of GMP, they most likely die off during ripening and drying, hardly influenced by pH-changes typical to these group of products. According to the results of Gareis (1997) EHEC strains are rather resistant to pH-values common with dry sausages, nevertheless decrease in a_w-values do have a lethal effect on these organisms, mainly if the effect is long lasting. Keeping in mind the results of Nissen *et al.* (1997) where they stated that longer time and relatively high temperature favours the destroying effect, we can expect an adequate safety with traditionally dried sausages also with respect to enterohemorrhagic *E. coli*, since all these factors are available during manufacturing and storage.

Traditionally manufactured and dried sausage with long ripening time is thus a group of products that can be characterised not only by its unique flavor, aroma and stability but also by its safety and these products will most likely have a fairly promising future, too.

As for fermented sausages as probiotics several possible advantages as well as further research need have been listed in this article. The future looked very promising, since apathogenic micro-organisms of intestinal origin with good starterculture abilities have been successfully isolated (Arihara *et al.*, 1996, 1997) and tested experimentally. With the emerging of EHEC nevertheless a real, almost unsurmountable problem has arisen. It has been shown that in general probiotics can be applied with expected effect only if micro-organisms are administered in large number and with high viability. Considering the potential risk of EHEC infection, and fulfilling the requirement of USDA-FSIS the possible choices are as follows:

- heating the sausage which then will not be suitable as probiotics
- drying the sausage according to 'good old' traditions in case of which micro-organisms in question are most likely inactive
- trying to meet Option 5 and hoping (evidently supported by research) that in this case all the requirements concerning food safety, sensory value and effectivity of probiotics will equally be fulfilled.

As a conclusion, we might say that if somebody has the intention to find the way of elaborating a probiotic in form of fermented sausage, he must be aware of stepping on a narrow path which is rough and needs a good piece of research and ingenuity in order to reach the goal.

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