VALIDATION OF MANUFACTURING PROCESS TO CONTROL PATHOGENIC BACTERIA IN TYPICAL DRY FERMENTED PRODUCTS

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Abstract

Safety is the prime consideration and food manufacturers must ensure that products pose a minimum hazard to the consumer. The required safety must be achieved by preventing growth of pathogens during production and by reducing the remaining contamination. to the lowest possible level.

Dry and semi-dry fermented sausages are generally regarded as one of the most shelf-stable and safest meat products; they have rarely been implicated in food poisoning but sausage makers must ensure that their products don't harbour any pathogen bacteria. To ensure that processing is sufficient to eliminate any biological hazard present in the product, procedures must be validated to demonstrate that they are able to achieve a specified reduction in terms of pathogenic bacteria.

Keywords: safety, pathogenic bacteria, dry fermented sausages, manufacturing process.

Introduction

While manufacturers must provide consumers with products posing a minimum hazard, there is no available sampling plan nor laboratory practice ensuring total microbial safety, and the current way to minimize food hazard is to address prevention through process control and quality assurance. In recent years, food operators have been urged to develop food hygiene procedures based on the principles of HACCP and good manufacturing practices, requiring that the safety of final products be demonstrated prior to marketing. Several national or international branch organizations have also developed codes of practice for ready-to-eat products.

With most meat products, safety is generally achieved by controlling or preventing growth of pathogens during the process and reducing contamination to the lowest possible level.

Among meat derivatives, dry fermented sausages are ready-to-eat products, whose safety is essentially gained by 1) decrease of water activity to below the growth limit of most pathogens, and 2) pH fall, enabling more efficacy bacterial control in a 'hurdle technology' concept. While lower water activity (a_w) is reached by combined effects of salt uptake and meat shrinkage during ripening, pH decrease results from the primary process of microbial fermentation.

Dry and semi-dry fermented sausages are generally regarded as shelf-stable, safe meat products and they have rarely been implicated in food poisoning. A reason for this might be that during drying and ripening any pathogens, if present, are likely inhibited, while at combined a_w and pH values of the end product pathogenic bacteria cannot grow even at ambient temperature.

Although processing techniques generally used with ripened sausages or dried salami appear to be effective in pathogen control, there is evidence that raw materials are still a major source of bacterial contamination, and current sanitation and cleaning procedures may fail to prevent such pathogens as *Listeria monocytogenes* and *Salmonella* from entering the production line. To increase safety, efforts are being made by modifying the process to ensure lower a_w (extended ripening) or reduced pH (use of acidifying starter bacteria, GDL) or using substances with antibacterial effect (organic acids, bacteriocins). However, such approach has to be carefully investigated for possible adverse effects on the sensory properties of end products. This is particularly true for those meat products having to meet regulatory standards or established sensory traits, as happens with a variety of South European dry sausages whose production is strongly linked with consumer habits and their expectations.

Dry fermented meat products from a technical viewpoint.

Although all types of fermented meats base their stability on the same preserving factors, they may differ even substantially in the way they are obtained, hence in their final sensory traits. In principle, there is no limit to the use of raw meats from different animal species, and dried salami made from or added with beef, poultry, turkey, horse, goose and deer meat in addition to pork may be a common occurrence in meat and deli stores. However, pork is by far the prime source of raw material for most sausage processors worldwide.

Dry sausage from a technical standpoint results from a continuous sequence of events whose proper occurrence is key to successful outcome. They include grinding of meat and fat chunks of variable size and shape into uniform mince of given particle size (typically 0.8-10 mm) followed by mixing of the blend and salt adjuncts in a mixer generally operated under vacuum or modified atmosphere. After cold storage the mince is stuffed into casings which determine the product shape and size, then the encased mass is tied with thread or fastened with metal clips and submitted to drying in a drying room or a smokehouse operated under controlled temperature, humidity and air flow conditions. Next is the ripening or ageing stage, where sausages are kept until the fermentation process is accomplished. Depending on size, type of casing, drying temperature and, possibly, local regulation, the time required for the process to be completed may be of few days or months.

As a rule, fast fermented sausages as prepared in North America and increasingly in Northern Europe can undergo relatively high drying temperatures (>25°C) in order to enhance growth of added lactic bacteria and pH fall. This will result in accelerated texture and flavour development and increased stability as a consequence of low pH (4.5-5.0) which makes this sausage class ready to eat even in a few days and in spite of a relatively high a_w (>0.92). In contrast, high-pH (>5.5) dried salami as manufactured in Mediterranean countries due their stability to long term ripening which may be of months, leading to an a_w of less than 0.90. In the latter sausage class, reduced lactic acid formation, typically ranging from 1-1.5% is the main cause for brighter redness, lower gumminess on mastication and limited sour taste that are common perception with a variety of Italian, Spanish and Greek salami items.

The mechanism of acidification

Sausages classified as fermented undergo a controlled lactic acid-type fermentation, usually through the action of commercially produced starter cultures added to the meat batter. The extent of acidification may change according to sausage type, being greater in vacuum-filled and large diameter salami, where oxygen is limited and the growth of lactic acid microflora enhanced. Moreover, the meat particle size seems to play a role in this context, as finely ground (<3 mm) minces are more susceptible to increased acid formation.

However, pH decrease may be inhibited when excessive grinding or improperly made comminution results in smeared batter making it impossible for fat and lean particles to link together.

The pH of raw meat is an additional factor capable of affecting the final pH value, and sausages made from high pH meat are more likely to result in lower acidity as happens with DFD pork. This is also the case with sausages made only with specified cuts, such as shoulders, whose pH may be greater than values found in other carcass cuts, e.g. leg trimmings. Additional causes for variation in pH can be ascribed to inadequate control of the drying process, where the onset of an external crust as result of excessive drying is likely to result in abnormally high external pH compared with values in the sausage core.

To get rid of undesired changes of pH and, more important, minimize the occurrence of abnormally high pH values, producers have increasingly practiced addition of commercial lactic bacteria starter cultures plus simple sugars such as dextrose that promote lactic acid bacterial growth by serving as a fuel. The use of starter cultures is critical to the successful sausage fermentation; cultures usually consisting of lactic acid bacteria and *Micrococcus* or *Staphylococcus* strains (Deuschel, 1993; Hill, 1995). Although starter cultures are commonly added to rapid fermentation products and less to low acidity salami as prepared in Southern Europe, there is no evidence that the two types of products differ in terms of foodborne illnesses related to sausage consumption.

In other words, it seems that both classes achieve good safety properties through their own processing pathways. This enables the following basic points to be established as far as microbiological safety is concerned.

- Regardless of the manufacturing practice followed in sausage formulation, a major objective for the meat industry is to reduce pathogens, if present, to below the threshold generally considered as safe for human consumption.
- As the occurrence of pathogens in the end product cannot be excluded *a priori*, nor are on-line analytical techniques available to select uncontaminated meats, the possibility for raw materials to be actual sources of contamination has always to be taken into account. This is particularly true for a comminuted meat product, where chunks from a variety of carcasses and cuts make it impossible to efficiently check meat suppliers even on a individual batch basis control.
- Additives, though adequately used, and in compliance with current regulations, are not likely to determine pathogen inactivation
- Therefore, the ripening or fermentation process has to be regarded as the major control point in sausage production. It encompasses the drying and ripening (or ageing) stages, with the former being prevalent in importance.

Pathogens and typical dry fermented products

Occurrence of pathogens has been sometimes reported in fermented sausages. *E. coli* O157:H7, an contaminant in ground beef and non-intact beef product, survived fermentation and maturation when initially present in high number in American-type sausage (Glass, Loeffelholz, Ford & Doyle, 1992). In dry cured Canadian sausages, 10 out of 42 samples were positive for *L. monocytogenes* before fermentation and five of them remained positive even after the maturation period (Farber, Tittiger & Gour, 1998). *L. monocytogenes* has also been found to survive the initial fermentation in beaker sausages and in the storage of pepperoni (Glass & Doyle, 1989). *Salmonella* species have been found to cause food poisoning in sausages prepared without starter cultures and with a short fermentation period (Pontello *et al.*, 1998).

However, estimating the overall magnitude of the pathogen problem is imprecise. Fermented meats may differ markedly in their production process and, as a consequence, the risk associated with the survival of contaminating pathogens is likely to differ. Although fermented meat products can be generally considered a low risk product, growth of *Staphylococcus aureus* and survival of other pathogens cannot be excluded. Depending on strains and anatomic cuts, the presence of pathogenic bacteria in raw meat may differ extremely, with *E. coli* O157 appearing practically absent in pork meat (Caprioli, Minelli, Morabito & Tozzi, 1997). Minced meat can be contaminated with *Salmonella* with a frequency ranging from 0.4 to 12% of samples (Cantoni & Soncini, 1999).

Listeria, an ubiquitous micro-organism, can resist many food preservation methods (Lou & Yousef, 1999) and has the ability to colonize meat plants and survive under unfavourable conditions (Farber & Peterkin, 1999; Samelis & Metaxopoulos, 1999). Its prevalence in raw materials intended for the manufacture of meat products is relatively frequent as is its detection in final products such as medium-acid salami (Barbuti, Pancini & Dellapina, 1995). The contamination level of these products is generally low (<100 listerias/g); moreover, *Listeria* cannot grow in cured meat products because of the presence of a typical microflora in combination with physico-chemical properties of cured meat. Therefore, the health risk associated with the occurrence of *Listeria* in cured meat products can be regarded as low. Nevertheless, food laws require that the bacterium be absent in ready-to eat products. To the end, sausage makers must ensure that their products are not contaminated by pathogens. They have to control the fermentation process, and make appropriate use of the smoking and drying processes in order to reduce any pathogens potentially present.

Several studies have addressed the inhibition of pathogens in fermented sausages and the process conditions to be applied accordingly. In theory, the hurdle effect appears an effective means to cope with pathogens. Inhibition of *S. aureus* is achieved at pH value below 5.3 (Genigeorgis, 1976); nontyphoidal *Salmonella* can be eliminated from dry sausages by combined use of fermenting cultures, increased ripening temperatures and careful check of the salt in the mince and pH in the final product (Tietjen & Fung, 1995; Turantes & Unluturk, 1991). This treatment would likewise positively affect sausages when exposed to contamination from *E. coli*.

Recent outbreaks due to Gram negative food borne pathogens in fermented meat products have raised questions about the safety of this class of foods. An outbreak of *E. coli* O157:H7 was linked to consumption of fermented salami in western United States (Center for disease control, 1995a). In 1995, an Australian outbreak of *E. coli* O111:NM was attributed to consumption of semi-dried fermented sausage (Center for disease control, 1995b), while in Italy two outbreaks of salmonellosis were ascribed to the presence of *Salmonella* in sausage-like products. The first of such cases was caused by *Salmonella choleraesuis* (Marazza & Crespi, 1963) and the second to *Salmonella typhimurium* (Pontello *et al.*, 1998).

In conclusion, epimediological data, though rather limited, evidence that foodborne disease from dry sausages cannot be underestimated and efforts should be made to control contamination at slaughter level and bacterial growth at processing stage. Based on significant contamination rates in raw meats, the inhibitory effects provided by manufacturing processes appear to be the means processors are presently relying on to achieve microbial safety.

Validation of manufacturing process

To ensure that the fermentation and drying process are efficient to reduce or eliminate pathogens, procedures should be validated to demonstrate that they achieve established reduction for specific organisms.

Food Safety and Inspection Service (FSIS) published a proposed rule "Performance Standards for the Production of Processed Meat and Poultry products" (FSIS, 2001). Except for thermally-processed meats the performance standards for lethality for ready-to-eat

(RTE) items require 6.5 log_{10} reduction of *Salmonella* in finished meat products and 7.0 log_{10} in those made with poultry. In addition, for beef-based RTE fermented products the process is required to provide 5 log_{10} reductions of *E. coli* O157:H7. Also, the same agency requires the use of a validated manufacturing process for RTE dry and semidry fermented acidified sausages. FSIS clarified the definition of a "validated manufacturing process" as a process that:

- Applies one of the heat treatments prescribed in regulation 9 CFR 318.17 or 9 CFR 318.23.
- Applies a 5 D process (5 log reduction in pathogenic populations)
- Includes checking of the product for *E. coli* O157:H7 (30 samples per lot with zero positives).
- Uses raw ingredients prepared under HACCP that verified less than or equal 1 *E. coli* O157:H7/125 grams (95% confidence level) and applies a validated 2 D process.
- Uses other methods that would ensure equivalent safety.

A process microbiological challenge testing (MCT) is applied to preclude that a potentially hazardous organism survives a certain process. Interactions of environmental factors affecting bacterial growth and survival can be safely studied by use of the MCT (Rose, 1987). MCT is a laboratory technique simulating what would happen to a product during production. This involves inoculation of the product with relevant microrganisms in order to assess the risk of food poisoning. The aim of MCT is to simulate the fate of relevant organisms during processing, when inoculated at levels higher than normal.

Using unusually large contamination levels in fermented sausages is an aid to understand and model pathogen inhibition by drying and fermenting, as the natural degree of contamination is very low in these products. In other terms, following the fate of naturally occurring low levels of contaminants in dry fermented meats would require an impracticably high number of samples to be analysed. Therefore, the MCT procedure is generally regarded as a means to make sure that a potentially hazardous organism will not survive the process (Notermans & in't Veld, 1994).

To design a correct MCT Notermans, in't Veld, Wijtzes & Mead (1993) produced a user's guide comprise four-sections addressing experimental design, use of microbial strains as inoculants, test procedures and interpretation of results.

A critical point associated with MCT of fermented meats is the choice of the inoculum size. Trials based on huge inoculation of other bacteria than those responsible for fermentation would be misleading, since they might swamp the preservative mechanism of the product; in contrast, too few organisms might give false negative results.

Examples of MCT: safety of dry fermented sausages

Unlike their semidry counterparts, dry fermented sausages are never exposed to pathogen inactivating temperatures. Consequently, dry sausages have attracted more attention, as shown by several studies that examined the fate of *E. coli* O157:H7, *Salmonella* and *L. monocytogenes* during the fermentation, slicing and storage of hard salami, pepperoni and several others sausages.

Challenge test studies of salami documented that enteric pathogens can be reduced in number during the process. Smith *et al.* (1975) assessed the survival of *Salmonella dublin* and *S. thyphimurium* during the manufacture of pepperoni showing that the use of starter cultures (*Pediococcus cerevisiae* and *Lactobacillus plantarum*) significantly enhanced their destruction. Goepfer & Chung (1970) studied the behaviour of *S. thyphimurium* in a cured sausage emulsion model stored at 30°C in presence or absence of starter culture. While the former mixture resulted in 2-log decrease of *Salmonella* after 24 hours of incubation, the latter enabled increase of the same strain from 39/g to 10^5/g within 14 hours.

More recently, Barbuti (1998) used the MCT approach to evaluate the inactivation of *S. typhimurium* during the ripening of Italian salami. In the presence of a culture starter made up with *L. curvatus*, *L. plantarum* and *Staphylococcus xylosus*, inoculated *Salmonella* (10^5ufc/g) decreased by 1-log after 14 days of fermentation while on the 58th day no *Salmonella* could be recovered .

Studies have been conducted to assess the survival of *E. coli* O157:H7 in dry fermented sausages (Reed, 1995; Faith, Parniere, Larson, Lorang & Luckansky, 1997; Nissen & Holck, 1998; Riordan *et al.,* 1998; Arinder & Borch, 1999; Blair *et al.* 1999; Duffy, Riordan, Sheridan, Whiting, Blair & McDowell, 1999), showing it is difficult to achieve a 5 log reduction as required by USDA FSIS.

Investigation at Food Research Institute of the University of Wisconsin into the effects of fermentation temperature, pH changes, drying and pasteurisation on *E. coli* O157:H7 (Anon., 1996) in meat batters inoculated with a five strain cocktail at $>10^7$ cfu/g showed that a reduction lower than 5 log was observed in salami produced without a cooking stage after fermentation. Another study showed that in a standard pepperoni process, initial contamination levels of log 6.89/g remained fairly constant during the fermentation stage. Levels decreased during the drying phase and the final levels were log 5.69 cfu/g, with a total decrease of less than 2 log cfu/g (Hinkens *et al.*, 1996).

It is to be noted that while relatively large validation data have been reported for such fermented sausages as pepperoni and summer sausages, mostly concerning *E. coli* O157:H7, there have been comparatively few studies dealing with the same pathogen in other sausage types.

Colombo, Boni & Bonometti, (1997) evaluated the survival of VTEC O157 to the acid conditions developing in Italian salami "Milano". Three inoculum levels, 3.60, 6.30 and 7.30 log cfu/g were used, under the following processing conditions: 1st drying stage for 6 hours at 24 – 26°C, free RH; 2nd drying for 7 days at 12°C, RH 30 – 90%; ripening (ageing) for 60 days at 9 – 12°C, RH>60%. The recovery of *E. coli* at different times showed a variable decrease (3 – 5 log cfu/g reductions) in relation with the inoculum level.

Calicioglu, Faith, Buege & Luchansky (2001) studied the fate of *E. coli* O 157:H7 during the manufacturing process of fermented, semidry Turkish Soudjouk. A cooking step (54.4°C/ 60 min) after fermentation and drying reduced pathogen numbers below a detectable level by conventional detection methods.

Conclusion from the abovementioned studies was that most tested processes would result in a reduction of less than 5 log cfu/g as required by Food Safety Inspection Service, so the implementation of a control program ensuring high quality raw material and statistically significant sampling programme of raw material and finish products are necessary. The inclusion in the pepperoni production of a mild heating step has been suggested as an option for reducing pathogen number in RTE meat (Hinkens *et al.,* 1996).

As many of the outbreaks from *E. coli* O157:H7 have been associated with beef products, there is evidence that the nature of the meat directly affects the inherent risk associated with several product items. As variable quantities of beef are often included in salami production, the exclusion of this type of meat is likely to reduce the risk of *E. coli* foodborne infection.

Barbut & Griffiths (2002) developed validation models for *E. coli* O157 inactivation in dry fermented sausages. Three main models were developed to describe the log reduction of *E. coli* O157:H7 in uncooked, semi-dry, fermented sausages. Results showed that the ability of *E. coli* O157:H7 to survive the process decreases as the pH decreases and the fermentation time/temperature function increases.

The presence of salt and sodium nitrite and the growth of starter bacteria to significant amounts, and the concomitant production of organic acids and pH reduction coupled with reduction in moisture content may result in inhibition of *L. monocytogenes*. Dry sausages have a good safety record concerning *L. monocytogenes*, with no reported outbreaks of listeriosis from such products. However, epidemiological data seem contradictory with respect to the prevalence of *L. monocytogenes* reported in published surveys of salami, which would demonstrate that the process is probably not capable of completely destroying *Listeria* though it might reduce levels of contamination in raw meat.

Several authors tried to develop strategies toward *Listeria* inactivation. Natural or artificial additives such as sodium benzoate, propionate, nitrite, ascorbate, lactate and acetic acid were tried to this end (Buchanan, Golden & Whiting, 1993; Young & Foegeding, 1993; Barbuti, Schiaretti, Pancini, Dellapina, Mutti, Quintavalla, 1998). More recently, bacteriocin-producing antagonist strains were also tested (Campanini, Pedrazzoni, Barbuti & Baldini, 1993). Often bactericidal substances were incompatible with chemical and sensory requirements of the product and /or useful concentrations could not be used as not in compliance with current regulations.

Whiting & Masana (1994) reported a 4 log reduction in experiments with a type of salami prepared with high fermentation temperature. Farber, Daley, Holley & Usborne (1993) examined the survival of *L. monocytogenes* during production of uncooked German, American and Italian-style fermented sausages. Results placed emphasis on to the importance of using starter cultures and relying on a HACCP plan designed for each type of sausages in order to obtain 5 log reduction. Glass & Doyle (1989) reported inactivation of *Listeria* in pepperoni undergoing normal processing and using a heating step (51.7°C/4h) after fermentation.

Conclusion

Salami and dry fermented meat products are very traditional products based on manufacturing processes that can be hardly modified without adversely affecting the qualities of end products, hence consumer acceptance. Accordingly, the preserving factors inherently involved in the technological process should be thoroughly known and practiced in order to achieve the fundamental goal of microbiological safety. Studies into the effects of the process on the inhibition of pathogenic bacteria suggest that such factors as pH reduction, generation of lactic and organic acids, moisture loss, a_w decrease and competitive microflora play a major role in the control of undesired bacteria.

Because the mentioned factors are rather specific for each sausage type, as happens with strongly acidified-rapid fermentation pepperoni, compared with low acidity, long ripened Mediterranean salami, validation procedures are needed for individual sausage classes, in agreement with current USDA guidelines.

Efforts should be encouraged to reduce the extent of contamination at the abattoir level, by improving hygiene standards and control procedures. Innovative washing equipments should be implemented as regulatory agencies enable use of safe antimicrobial cleaning substances. Finally, research dealing with newly developed technologies, including pulsed electric fields and hydrostatic pressure has to be followed for potential benefits to the fermented meat industry, when their compatibility with established product properties and regulatory requirements is demonstrated.

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