

## Dry and Semi-Dry Fermented and Direct Acidified Sausage Validation

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### Introduction

In 1994, an outbreak of *E. coli* O157:H7 infection was linked to dry, fermented, pork and beef salami. In response to this first implication of a dry fermented sausage product, the United States Department of Agriculture/Food Safety Inspection Service developed guidelines requiring sausage manufacturers to validate that their processes achieve a five-log reduction of *E. coli* O157:H7. Various validation studies have shown that *E. coli* O157:H7 is able to survive in sausages that are fermented and then dried to various moisture-to-protein ratios of 2.3, 1.9, or 1.6:1. Additional thermal processing methods or longer fermentation processes were utilized to achieve 5-log reductions (Getty et al. 2000).

### Definition of Dry and Semidry Sausages

Fermented sausages can be either dry or semidry. The most well-known dry sausages, such as Genoa salami, dry salami, and pepperoni, originated from Italy (Ricke and Keeton, 1997). In general, dry sausages have a final pH of 5.0-5.3, lactic acid percent of 0.5-1.0% and an MPR of <2.3:1. The moisture loss is between 25-50% and the final moisture percent on average is <35% with water activity (aw) ranging between <0.85 to 0.91.

However, these values may be different due to government and company specifications. German and hard salami are required by FSIS to have an MPR of 1.9:1, with the exception of Genoa salami, which is required to have an MPR of 2.3:1 (FSIS, 1986). The pH range for these products is 4.7-4.9, whereas, pepperoni has a slightly lower pH range of 4.5-4.8 and a lower MPR of 1.6:1 as required by FSIS (FSIS, 1986). The moisture content for these products ranges from 25-39%. All of these products, because of their moisture-to-protein ratios, are considered shelf stable (FSIS, 1986; Ricke and Keeton, 1997).

Semidry sausages such as summer sausage, cervelat, and Mettwurst typically have a final pH between 4.7-5.1, a lactic acid percent of 0.5-1.3%, and an MPR of >2.3:1 but <3.7:1. The moisture loss ranges from 8-15% and the moisture percent ranges from 45-50%. The water activity range is 0.90-0.94. Again, values will vary depending upon the government and company specifications. For instance, a summer sausage will have a final pH <5.0, a lactic acid percent of 1%, and an MPR of 3.1:1 with a moisture percent of 41-51%. Lebanon bologna is unique in that it contains a higher moisture content of 56-62% (Ricke and Keeton, 1997). Due to the higher moisture-to-protein ratios, semidry sausages are required to be refrigerated.

### Processing Parameters

Fermented sausage ingredients generally include: a comminuted meat blend of lean and fat, herbs, spices,

salt, sugar, sodium nitrite, and lactic acid bacteria (Bell and Kyriakides, 1998). Sodium nitrate is commonly used in European fermented sausages. U.S. processors of European-style sausages may also incorporate sodium nitrate into the formulation.

The basic steps for producing fermented sausages are:

1. grinding or chopping the meat ingredients;
2. blending the meat and nonmeat ingredients;
3. incorporating the lactic acid bacteria;
4. developing proper conditions for lactic acid bacteria growth (often referred to as fermentation or ripening);
5. smoking and final heat processing; and
6. drying (Buege and Cassens, 1980; Rector, 1993).

Steps five and six will be dependent upon the product produced. Semidry sausages generally are smoked, and a final heat treatment often is applied. However, dry sausages normally are not smoked, receive little heat processing, but are dried (Buege and Cassens, 1980; Ricke and Keeton, 1997).

Semidry products usually are incubated/fermented at a temperature between 32.5-38.10C and a 90% relative humidity for 18h or more (dependent upon sausage diameter and pH drop) to a final pH of <4.7. The pH value may range from 4.7-<5.3 depending upon the type of product and the manufacturers' specifications (Ricke and Keeton, 1997).

Dry sausages are processed in a similar manner, but the fermentation parameters are different. Typically, products are fermented at lower temperatures between 15-26°C and at a 90% relative humidity for approximately 72h to an endpoint pH of <4.7. However, fermentation times and temperatures may vary depending upon the optimum temperature of the starter culture or cultures used. In the United States, fermentation temperatures are often increased (37.8-43.3°C) and the time may be less than 24 hours depending upon the diameter of the sausage. The pH value also may range from 4.7-<5.3 because of differences in sausages and the manufacturers' specifications (Ricke and Keeton, 1997). Smoke may be applied at the end of the fermentation process.

Semidry or dry sausages are dried after fermentation and/or heat processing. Semidry sausages usually are placed in a drying chamber set between 12.9-15.7°C and at 65-75% relative humidity. The drying period may be 12 days or more depending upon sausage diameter and the required MPR. Dry sausages are dried at a lower temperature between 10-11.2°C and a slightly higher relative humidity of 68-72%, and the drying period lasts for 21 days or more (Bacus, 1986; Lücke, 1985).

### **Outbreaks of *Escherichia coli* O157:H7 and O111:NM**

In 1994, an outbreak of *Escherichia coli* O157:H7 infection was linked to a dry, fermented, presliced pork and beef salami product from delicatessen counters (CDC, 1995a). The salami outbreak involved 20 individuals in Washington (age range, 23 mo-77 yrs; median age, 6 yrs; 3 hospitalized; one 6-year-old with HUS) and three individuals in California (age range, 4-75 yrs; 3 hospitalized; one 4-year-old with HUS).

In 1995, another outbreak of HUS in Australia was linked to semi-dry (uncooked) fermented sausage called Mettwurst. This outbreak was attributed to *E. coli* O111:NM, a serotype that produces a SLT, and involved 23 patients (age range, 4 mo- 12 yrs; median age, 4 yrs; 23 with HUS) (CDC, 1995b). In addition, an outbreak of *E. coli* O157:H7 infection and recall were linked to a Hungarian fermented salami (Anonymous, 1999; Canadian Food Inspection Agency, 1999). These outbreaks could have been due to contamination of the raw meat mixes, inadequate process control or capability to kill *E. coli* O157:H7, or cross-contamination of finished products (Bell and Kyriakides, 1998).

### **FSIS Approaches for Controlling *E. coli* O157:H7**

Following the 1994 outbreak of *E. coli* O157:H7 infection linked to a fermented sausage product (salami), the FSIS issued guidelines for processors to conduct validation studies (Reed, 1995). The initial intent was that all fermented sausage processes achieve at least a five-log reduction in the pathogen. After validation research was conducted (Nickelson et al. 1996), the FSIS broadened its approach to how processors could meet the five-log reduction or take additional steps to assure the safety of fermented sausages. These options included: 1) achieving a five-log kill using a heat process (63°C for 4 min); 2) developing and validat-

ing individual five-log inactivation treatment plans; 3) conducting a hold-and-test program for finished product that would involve sub-sampling 15-30 individual chubs per lot; 4) using combinations that demonstrate a collective five-log reduction; and 5) initiating a hazard analysis critical control point system that would include raw batter testing and a two-log reduction during fermentation and drying (Freeman, 1996). However, the FSIS guidelines for conducting a validation study remained the same.

### **FSIS *E. coli* O157:H7 Challenge Study Design**

The main FSIS validation requirement was that a five-strain mixture of *E. coli* O157:H7 be used to inoculate the meat. The mixture must contain both meat and human isolates including the isolate from the salami outbreak (Reed, 1995). Acid adaptation of *E. coli* O157:H7 strains is required by FSIS. It is achieved by growing the strains in TSB plus 1% glucose (Reed, 1995). The addition of glucose to TSB ensures that cells have maximum acid tolerance before being inoculated into a fermented meat system that relies on acid production to achieve elimination of pathogens (Buchanan and Edelson, 1996; Leyer et al., 1995).

Harvesting during the stationary phase was recommended for the *E. coli* O157:H7 strains to be inoculated into meat for the FSIS validation study (Reed, 1995). Arnold and Kaspar (1995) observed that acid tolerance increased when *E. coli* O157:H7 strains were grown to the stationary phase. The validation study should use direct plating procedures for enumeration of *E. coli* O157:H7. This is achieved by starting with an initial inoculum level in the meat batter of 7.3 log cfu/g and then spread plating after fermentation and thermal processing or drying to obtain a detection limit of <1.0 log cfu/g. These procedures would allow for a five-log reduction to be determined (Reed, 1995).

MacConkey sorbitol agar (MSA) was the medium defined for enumerating and detecting *E. coli* O157:H7 (Reed, 1995). This is a selective medium that allows sorbitol-negative *E. coli* O157:H7 colonies to be detected at 24h of incubation (Okrend et al. 1990a).

### ***E. coli* O157:H7 Fermented Sausage Validation Studies**

Various *E. coli* O157:H7 validation studies have been conducted on fermented sausages. They have been conducted in test tubes (Ellajosyula et al., 1998); model systems (Tomicka et al., 1997); environmental chambers (Glass et al., 1992); and commercial-type smokehouses and drying chambers (Hinkens et al., 1996; McCauley, 1997; Getty et al., 1999). These studies have shown that *E. coli* O157:H7 is able to survive in sausages that are fermented and then dried to various MPRs of 2.3, 1.9, or 1.6:1 (Faith et al., 1997, 1998ab; Glass et al., 1992; Hinkens et al., 1996; McCauley, 1997; Nickelson et al., 1996). Additional thermal processing methods or longer fermentation processes had to be utilized to achieve five-log reductions. The total pH change and salt and nitrite content also contributed to the ability of *E. coli* O157:H7 to survive during fermentation and drying (Riordan et al., 1998).

Shortly after the 1994 outbreak of infection from salami, the University of Wisconsin conducted various validation studies following the FSIS Challenge Study requirements (Nickelson et al., 1996). The purpose of the research was to determine processes that could be used by industry for controlling *E. coli* O157:H7 in dry and semidry fermented sausages.

Overall processes did not achieve a five-log reduction as required by FSIS, if sausages were fermented at various temperatures to either high (5.0-5.3) or low (4.4-4.6) pH levels and then dried to MPRs of 2.3, 1.9, or 1.6 or held at fermentation temperatures for a period of 7d. Five-log reductions could be achieved by using alternative thermal processes or longer fermentation times.

### **Pepperoni**

Hinkens et al. (1996) observed a 1.2 log cfu/g reduction of *E. coli* O157:H7 when pepperoni (55mm casings) containing 75% pork and 25% beef with a fat content of 32% was fermented at 36°C and 85% relative humidity (RH) to a pH <5.0 and then dried at 13°C and 65% RH to an MPR of <1.6:1. To achieve a 5 to 6 log CFU/g reduction, a heat treatment of 63°C internal or 53°C internal for 1h had to be applied. In addition, only 1 to 2 log reductions were observed by Faith et al. (1997, 1998b) and Riordan et al. (1998) for typical pepperoni processes such as defined by Hinkens et al. 1996. However, Riordan et al. (1998) noted that sodium nitrite levels, total pH change, and salt levels had significant effects on *E. coli* O157:H7 survival.

## Lebanon Bologna

Validations of two semidry sausages; Lebanon bologna (all beef product) and beef summer sausage also have been conducted. Ellajosyula et al. (1998) evaluated the survival of *E. coli* O157:H7 and *Salmonella typhimurium* in Lebanon bologna using a current commercial process. The Lebanon bologna batter was placed in test tubes, which were sealed and then placed in a water bath with a programmable microprocessor-controlled temperature. A <2-log reduction was observed when the batter was fermented for 12h at 26.7°C followed by a period of time at 37.8°C until a pH of 5.2 or 4.7 was reached. A >7-log reduction was achieved using a combination of fermentation and low temperature heating to internal temperatures of 43.3°C for 20h, 46.1°C for 10h, or 48.9°C for 3h.

Getty et al. (1999) also observed a >5-log reduction of *E. coli* O157:H7 in both large- (115 mm) and intermediate-diameter (90mm) Lebanon-style bologna processed in a commercial smokehouse. The long-time, low-temperature process consisted of 8h at an internal temperature of 26.7°C, then 24h at 37.8°C, followed by 24h at 43.3°C; heat-injured cells were taken into account.

## Summer Sausage

In a validation study of a beef summer sausage (11% fat and 64 mm casings), fermentation was conducted in a staged process starting at 29°C until a final temperature of 41°C (approximately 13h) and a pH of 4.6 or 5.0 were reached (Calicioglu et al. 1997). Fermentation for both pH levels was able to achieve only a 1.39 log cfu/g reduction of *E. coli* O157:H7. When product with a pH of 4.6 was heated instantaneously to an internal temperature of 54°C over 3.6h, a >7 log cfu/g reduction occurred. However, when the pH level was only 5.0 an additional heating period of 30-60 min at 54°C was required to achieve a 5- or 7-log reduction.

## Salami

For the salami validation, raw batter containing 75% pork, 25% beef, and 20% fat was inoculated with *E. coli* O157:H7 and stuffed into 104mm casings (Faith et al. 1998a). Product then was stored at various combinations of freezing and refrigeration, fermented at 24°C and 90% relative humidity to pH <4.8, and dried to an MPR of <1.9:1. Regardless of whether the raw product was stored frozen and thawed; tempered, frozen, and thawed; or refrigerated only, the reductions after drying ranged from 1.1-2.1 log cfu/g.

## Direct Acidified Sausages

Direct acidified sausages are similar to fermented sausages with the exception that encapsulated citric or lactic acid are used in place of lactic acid bacteria. These products may be a potential source of *E. coli* O157:H7 due to their low temperature processing procedures and product properties. Research is being conducted by Getty, K.J.K. to determine the efficacy of processes for controlling *E. coli* O157:H7 in direct acidified snack sticks and summer sausage.

## Regulatory Performance Standards

FSIS has proposed performance standards for ready-to-eat (RTE) products such as fermented sausages (Federal Register, 2001). These standards require a 6.5-log<sub>10</sub> reduction of *Salmonella* for RTE meat products and a 7.0 log<sub>10</sub> reduction of *Salmonella* for RTE products containing poultry. Fermented products that contain beef must also meet a 5-log reduction of *E. coli* O157:H7 throughout processing. Draft compliance guidelines for ready-to-eat meat and poultry products are available through FSIS (FSIS, 2001). Various fermented sausage processing parameters from research studies are outlined that achieve the required reductions for *Salmonella* and *E. coli* O157:H7.

## Validation Design

USDA/FSIS and members of industry determined fermented sausage processes/steps that were at high and low risks of allowing *E. coli* O157:H7 survival (Nickelson et al. 1996). Processes/steps listed as being high risk included: high pH, beef ingredient in the product, high initial coliform counts in the ingredients, and a low fermentation temperature. Low risk processes/steps were heating and low aw or MPR products. In the FSIS Pathogen Reduction/HACCP (Hazard Analysis and Critical Control Point) rule "every estab-

ishment shall validate the HACCP plan (Federal Register, 1996).” Validation is often based upon specific parameters (critical limits) in a defined critical control point (CCP) of a HACCP plan. A CCP is any point, step or procedure at which control can be applied and a food safety hazard can be prevented, eliminated or reduced to an acceptable level. In fermented sausage production, these critical limits may be defined as a pH level, time and/or temperature for fermentation, additional thermal processing times/temperatures, relative humidity during drying and percent weight loss to achieve a specific MPR.

Validation studies need to be conducted in a pilot plant dedicated to pathogen research and the equipment should have commercial capabilities. Inoculation procedures need to be developed that adequately and evenly distribute the selected pathogen throughout the meat batter. Initial pathogen inoculation levels should be in the range of 8 to 9 logs CFU/g to account for the complete log reduction.

When selecting strains of pathogens for the study, the following factors need to be addressed:

1. characterizing the range of acid tolerance responses and mechanisms among isolates;
2. identifying the most resistant strains that are likely to be encountered; and
3. having available a means for ensuring that the isolates are in their most resistant state (Buchanan and Edelson, 1996).

Therefore, the use of specific strains such as the salami outbreak strain and ATCC 43895, growing cultures in glucose, and harvesting during the stationary phase allow optimum survival of *E. coli* O157:H7 during the fermentation and thermal process of a fermented sausage product.

Instrumental devices should be available to accurately measure: internal temperature of product; dry and wet bulb temperatures; and times at different temperatures. Sampling of product in the smokehouse should be at random to ensure that product has been collected from both hot and cold spots. Product for microbial testing should also be sampled from the coldest spot (generally the geometric center) of the sausage link.

In addition, microbiological methods for detecting acid- and heat-injured cells should be taken into account during enumeration so that log reduction values are not overstated. For instance, Getty et al. (1999) observed approximately a 1.5-log difference between phenol red sorbitol agar and MacConkey sorbitol agar for detecting acid and heat-injured cells in a fermented sausage process. Besides enumeration, it is important to conduct enrichment procedures to obtain presence/absence data to fully determine a log reduction if actual counts are below the detection limit.

A validation study also should include proximate analysis testing such as protein, moisture, fat, and salt content. This data is important because studies have shown that *E. coli* O157:H7 is able to survive better in products with a higher moisture and fat and lower salt content. The pH and water activity levels should be measured along with the weight loss amount. This allows for a correlation of weight loss to the MPR and water activity readings.

Lastly, it is important to consider two methods of experimental replication:

1. three individual batches placed in smokehouse at three different times; or
2. three individual batches placed in smokehouse at the same time.

Option one is generally preferred to achieve best smokehouse variation but may not be economically feasible.

## Validation Studies to Control Other Pathogens

Other pathogens of concern in fermented sausages are *Salmonella*, *Listeria monocytogenes*, and *Yersinia enterocolitica* for products containing pork. Ellajosyula et al. (1998) observed a greater than 7 log CFU/g reduction of *Salmonella typhimurium* for a Lebanon bologna process that utilized a long fermentation time and heating at 43.3°C for 20h, 46.1°C for 10h or 48.9°C for 3h. Two other research studies have shown that a fermentation process alone for salami reduces *Listeria monocytogenes* by approximately 1 log CFU/g (Kang and Fung, 2000; Johnson et al., 1988). Ceylan and Fung (1999) observed a 5.0 log CFU/g reduction of *Y. enterocolitica* in a fermented Turkish dry sausage (Soudjuk) that utilized a low temperature fermentation and drying process.

## Validation Assistance and Resources

Various universities such as Kansas State University, Iowa State University, and the University of Wisconsin have pilot plant facilities to conduct pathogen validation research. Extension meat and food science specialists at land grant universities have access to scientific literature that would assist in determining acceptable processing parameters for controlling pathogens in fermented sausage products.

The FSIS draft compliance guidelines for RTE meat and poultry products provide various guidelines on fermented processes that achieve 6.5 or 7.0 log reductions of *Salmonella* and 5 log reductions of *E. coli* O157:H7 (FSIS, 2001). In addition, a computer modeling program entitled "Pathogen Modeling Program" is available on the internet at <http://www.arserrc.gov/mfs/> from the USDA Agriculture Research Service. This modeling program allows a processor to input in product characteristics (moisture, protein, fat, and salt content and pH and water activity level) and processing parameters and will predict the growth and/or survival of various pathogens.

However, FSIS published a draft notice that states "it is not possible or appropriate to rely solely upon a predictive modeling program to determine the safety of foods and processing systems. Determining pathogen growth or survival and controlling it in food products often requires complete and thorough analysis by an independent microbiology laboratory, challenge studies, and surveys of the literature (FSIS, 2002)."

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