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Review Article

Health and Safety Considerations of Fermented Sausages

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Fermented sausages are highly treasured traditional foods. A large number of distinct sausages with different properties are produced using widely different recipes and manufacturing processes. Over the last years, eating fermented sausages has been associated with potential health hazards due to their high contents of saturated fats, high NaCl content, presence of nitrite and its degradation products such as nitrosamines, and use of smoking which can lead to formation of toxic compounds such as polycyclic aromatic hydrocarbons. Here we review the recent literature regarding possible health effects of the ingredients used in fermented sausages. We also go through attempts to improve the sausages by lowering the content of saturated fats by replacing them with unsaturated fats, reducing the NaCl concentration by partly replacing it with KCl, and the use of selected starter cultures with desirable properties. In addition, we review the food pathogenic microorganisms relevant for fermented sausages *(Escherichia coli*, *Salmonella enterica*, *Staphylococcus aureus*, *Listeria monocytogenes*, *Clostridium botulinum*, and *Toxoplasma gondii)* and processing and postprocessing strategies to inhibit their growth and reduce their presence in the products.

1. Introduction

Meat is especially rich in proteins, vitamins, and minerals and is an important element in human diet [1]. Due to its perishable nature, meat historically had to undergo different methods of conservation. One strategy was mincing the meat with salt and spices and lowering the water content by drying. Fermented sausages were thus created and are treasured traditional foods. Nowadays, a large number of different sausages are produced using widely different recipes and manufacturing processes. In 1995, the production of fermented sausages in the EU was estimated to be about 750,000 tons [2]. Spain produces around 200,000 tons per year, while France produces another 110,000 tons [3]. The production figures for 2014 for Norway and Finland were 7300 tons and 7000 tons, respectively [4].

Traditionally, fermented sausages were considered healthy and safe foods. More recently, eating fermented sausages has been associated with health hazards caused by the high contents of saturated fats and NaCl, presence of nitrite and degradation products such as nitrosamines, and use of smoking which can lead to toxic compounds such as polycyclic aromatic hydrocarbons in the products. Hazards can also be both of direct microbiological nature, the sausages potentially being contaminated with food pathogens, and of indirect microbiological nature by metabolic activity of microorganisms causing presence of biogenic amines and mycotoxins.

Raw meat is an ideal medium for growth of many microorganisms due to its high moisture content (70–80%), and its abundance of proteins, peptides, and amino acids, growth factors, and minerals. In addition, it usually contains fermentable glycogen and has a pH favorable for many microorganisms. This is why raw meat is a highly perishable product and should be preserved. For fermented sausages, this preservation consists of a number of strategies (hurdles) working together. These include lowering of pH by fermenting sugars to mainly lactic acid, lowering of water activity (a_w) by salting, drying by evaporating water, inhibiting growth of aerobic bacteria by creating an anaerobic environment, inhibiting microbial growth by addition of nitrate or nitrite, and inhibiting surface growth by smoking or by addition of specific molds. Together these hurdles generally lead to a shelf-stable product. However, traditional fermented sausage

manufacturing processes do not ensure microbiologically safe products. Several foodborne outbreaks attributed to dry or semidry fermented sausages (DFSs) (see references below) have demonstrated that actions must be taken to ensure that these products are safe to consume. In most cases, the pathogen in question does not grow in the finished products but survive long enough in high enough numbers to cause disease.

Here we give an overview of the literature pertaining to health issues and microbiological issues for fermented sausages and strategies to produce healthier and microbiologically safer sausages.

2. Production of Fermented Sausages

The large variety of fermented sausages and fermentation processes that exist have been thoroughly described elsewhere [2, 5, 6]. Most often fermented sausages are produced from two-thirds of lean meat from animals such as pork and beef and one-third of fat, nearly always pork backfat. In short, meat is cut and mixed with fat, spices, salt, sugar, sodium nitrite (sometimes nitrate), and starter culture. Generally, the starter culture is a single species of lactic acid bacteria (LAB) or a LAB mixed with other bacteria such as *Staphylococcus xylosus* or *S. carnosus*. The mix is stuffed into natural or artificial casings of varying diameters and subjected to a fermentation procedure where the LAB grow and convert the sugar to lactic acid which leads to a pH decrease from around 5.8 down to 5.3–4.6, depending on the amount of available fermentable sugars and process conditions. The staphylococci, when present, will contribute to flavor development and reduction of nitrite and nitrate. Subsequently, the sausages are dried until the desired a_w is reached. Fermentation and drying steps are performed in smoke chambers and drying rooms with controlled temperature and humidity.

Fermented sausages can be either dry or semidry [7]. Generally, DFSs have $a_w \leq 0.90$, while, for semidry sausages, a_w ranges between 0.90 and 0.95 [8]. American type dry sausages such as Genoa salami, dry salami, and pepperoni contain 25–40% moisture, are heavily spiced, are not heated above 26.7°C, have a firm texture, and are usually shelf-stable. In Europe, these fermented sausages can be further divided into Northern and Mediterranean types [9]. Northern type products such as cervelatwurst, Westphalian salami, plockwurst, boerenmetworst, and Belgian salami often contain beef and pork and are characterized by relatively short ripening periods of up to 3 weeks and involve clearly separated fermentation and drying periods. Rapid acidulation to final pH values below 5 and smoking ensure microbiological safety and shelf-life. Mediterranean type sausages such as Spanish salchichón and chorizo and Italian salami are predominately pork products and involve longer ripening periods, up to several months, often without clear separation between fermentation and drying. Smoke is not applied and acidulation to final pH values above 5 is slower. Instead of smoking, the sausages are often covered with specific molds. Semidry sausages, such as summer sausage, cervelat, Lebanon Bologna, and Mettwurst are usually fermented at higher temperatures, 32.5–38.1°C, for more than 18 h to a final pH < 4.7. They have a moisture content between 45 and 50%, are heavily smoked, are lightly spiced, and are usually heated to an internal endpoint temperature between 43 and 65°C.

3. Sausage Ingredients Related to Health

3.1. Fat. Consuming a healthy diet throughout the life course helps prevent malnutrition in all its forms as well as a range of noncommunicable diseases and conditions [10]. The increased production of processed food, rapid urbanization, and changing lifestyles have led to a shift in dietary patterns. People are consuming more foods high in energy (fats and sugars). Energy intake (calories) should be in balance with energy expenditure. Evidence indicates that total fat should not exceed 30% of total energy intake to avoid unhealthy weight gain, with a shift in fat consumption away from saturated fats to unsaturated fats so that saturated fats contribute no more than 10% of the total energy intake [10]. Regarding polyunsaturated fatty acids, controlled feeding and cohort studies of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) intakes have demonstrated physiological benefits on blood pressure, heart rate, triglycerides, and likely inflammation, endothelial function, and cardiac diastolic function. Consistent evidence for a reduced risk of fatal coronary heart disease and sudden cardiac death at consumption of approx. 250 mg/day of EPA plus DHA was demonstrated [11]. In industrialized countries, approx. 36–40% of the total calories in the food supply come from fat, nearly half of which is from meat intake [12, 13].

A way to reduce the amount of fat in fermented sausages is to simply add less backfat to the batter. There are however limitations as to how large such a reduction can be before sensory and technological quality of the sausages are reduced since fats contribute profoundly to taste, texture, and mouth feeling. In Norway, one of the large producers of fermented sausages has a commercial product called "Extra Salami," which is produced with 20% less fat than in the standard salami recipe. An alternative strategy is to replace some of the pork backfat with more healthy unsaturated fats or oils. Again, several challenges are associated with substitution of animal fat for oils in comminuted meat products. Incorporating hydrophobic oils can be difficult as meat contains approx. 75% water and is hydrophilic. Also, increasing the content of unsaturated fatty acids increases the susceptibility to lipid oxidation, which reduces shelf-life [14]. By emulsifying or encapsulating the oil and by addition of antioxidants, this problem can in many cases be mitigated.

In a set of experiments with DFSs, 25% of the pork backfat was substituted for an emulsion with linseed oil [15]. No oxidation problems were detected during the ripening process in such sausages with butylhydroxytoluene and butylhydroxyanisole added as antioxidants. No substantial changes in odor, appearance, flavor, and oxidation status were observed. In Dutch-style fermented sausages, 15 or 30% of the backfat was replaced with pure commercial encapsulated fish oil, added either as such or as a preemulsified mixture with soy protein isolate [16]. Sausages with encapsulated fish oil appeared to retain the overall quality, and no clear effects

were found in the different sensory attributes when using 15 untrained assessors.

In most experiments where oil partly replaced backfat, the oil was added together with stabilizers. In low fat fermented sausages (total fat content 10%), 20% of the fat was substituted for preemulsified olive oil and added ι-carrageenan [17]. The application of vacuum packaging over the last two weeks of ripening improved the physicochemical characteristics of the sausages and resulted in sensory attributes equal to or better than the high fat control sausages with 30% backfat. Likewise, 32.8% of the fat could be replaced by a linseed oil carrageenan gelled emulsion without loss of sensory qualities [18]. In Pamplona-style chorizo, both low sodium ion and low fat (20% less than standard recipe) sausages were produced [19]. Here 58% of the NaCl was substituted for 20% KCl and 38% $CaCl_2$, and 50% of the backfat was replaced with an alginate emulsion consisting of 64% water and 30% olive oil. 5% inulin was also added to sausages. These sausages retained sensory notes similar to those of the traditional control chorizo and achieved a good acceptability rating. Fat can also be partly replaced with other compounds. When 50% of the pork backfat was replaced with konjac gel, a low-calorie ingredient with a high content of nondigestible fiber, the sausages had an overall acceptability similar to the control sausages [12]. A "Super Salami" with 45% less fat and with 10% canola oil encapsulated in alginate and guar gum is available on the Norwegian market. The finished sausages contain 20% fat, of which 25% is saturated fat, 60% is monounsaturated, and 15% is polyunsaturated. A review of approaches to healthier formulations of comminuted meat products in conjunction with fat and salt has been published by Bolger et al. [14].

3.2. Salt. Salt serves many important functions in fermented sausages, where it contributes to taste, texture, microbiological safety, and overall acceptability. High sodium ion consumption (>2 g Na^+/day, equivalent to 5 g salt (NaCl)/day) contributes to high blood pressure and increase of the risk of heart disease and stroke [20]. Most people consume too much salt, on average 9–12 grams per day, or around twice the recommended maximum level of intake. The principal benefit of lowering salt intake is a corresponding reduction in high blood pressure. WHO Member States have agreed to reduce the global population's intake of salt by a relative 30% by 2025. Reducing salt intake has been identified as one of the most cost-effective measures countries can take to improve population health outcomes. An estimated 2.5 million deaths could be prevented each year if global salt consumption were reduced to the recommended level. Meat and meat products contribute 21% to the sodium intake [21].

Fermented sausages contain high amounts of salt, which contributes to the microbiological safety and shelf-life by binding water and making it unavailable for microorganisms. Salt also has a profound impact on the technological properties of the meat and thus on the sausage texture. It facilitates solubilisation of myofibrillar proteins, increases binding properties of proteins to improve texture, and increases viscosity of meat batters [22]. As the Na^+ ions cause health issues, reducing the NaCl content and/or replacing some of it with other salts like KCl or $CaCl_2$ has been investigated. Potassium ions can give a bitter taste, which poses restrictions on to how much can be introduced in a product. No changes in organoleptic characteristics of fermented sausages were detected when KCl substitutions for NaCl were lower than 40% [23]. Corral et al. observed the same for slow fermenting sausages fermented and dried at 10–12°C for 57 days where 16% of the NaCl was replaced with KCl [24]. Although a slight reduction in aroma development was detected, the sausages were judged to have the same overall quality as the controls with 2.7% NaCl.

Dos Santos et al. produced fermented sausages with 50% reduction of NaCl (12.5 g/kg), sausages where 50% of the NaCl was substituted for KCl, $CaCl_2$, or a 1 : 1 mixture of KCl and $CaCl_2$ [25]. A 50% NaCl reduction and a 50% substitution of the NaCl for KCl did not influence the fermentation and maturation process. Sausages with $CaCl_2$ showed a decrease in pH, an increase in a_w, and lower lactic acid production. Overall, sensory acceptance decreased in sausages with reduced sodium content. However, preference mapping identified a group of consumers that existed for fermented sausages with 50% reduced NaCl substituted for KCl or a blend of KCl and $CaCl_2$. De Almeida et al. produced salami sausages with 60% reduction in NaCl and adding different amounts of a 1 : 1 blend of KCl and $CaCl_2$ [26]. The salt replacement mixtures did not affect the technological process, but the sausages had lower acceptability. The authors suggested to enhance the sensory perception by addition of spices and other flavor enhancers. This strategy was successfully used when sausages were produced with 25% or 50% of their NaCl replaced with KCl and supplemented with 2% yeast extract [27]. The increased volatile compounds from catabolism of the yeast extract suppressed the sensory quality defects caused by KCl introduction. KCl is considered safe and exhibits an antimicrobial activity similar to that of NaCl [28]. Replacing some of the NaCl with KCl should therefore not influence antimicrobial safety of the sausages. General implications of salt and sodium reduction on microbial food safety have been reviewed earlier [29].

3.3. Nitrite. In addition to its important preservative effect, nitrite is involved in development of the red curing color formation and flavor development and acts as an antioxidant [30, 31].

According to the Commission Regulation (EU) number 1129/2011, nitrates (sodium nitrate, E251; potassium nitrate, E252) and nitrites (sodium nitrite, E250, and potassium nitrite, E249) are listed as permitted food additives. Maximum dose authorized for use in cured meat products by the EU is 300 mg/kg nitrate (for some products 250 mg/kg nitrate) and 150 mg/kg K-nitrite (or 150 mg/kg Na-nitrite) measured as ingoing amounts [32]. Nitrate may be reduced by Gram+ catalase+ cocci (GCC+) to nitrite in the meat. Nitrate is less used nowadays and primarily employed in dry cured hams and dry sausages where long, slow curing processes necessitate a long-term reservoir for nitrite that is reduced to nitric oxide in several reactions, which can then react with myoglobin in the meat to give the red cured color [33–35]. For nitrite, the residual amounts will vary with the formulation of the product, especially if ascorbate (vitamin C) is added to

prevent oxidation and to improve the color of the product. According to EFSA, the ingoing amount of nitrite rather than the residual amount contributes to the inhibitory effect against microorganisms.

Flavor is a complex stimulus involving taste, odor, texture, and temperature. The meat, salt, lactic acid, and spices are major contributors to flavor. Nitrite contributes to the cured meat flavor. Several experiments with bacon, frankfurters, and hams produced with and without nitrite have been reviewed [36]. The results usually showed higher flavor scores for products produced with nitrite.

The antioxidant properties of nitrite will inhibit development of rancid off-flavors [37]. The antioxidant properties are caused by nitrite being oxidized to nitrate by sequestering oxygen, which is then not available for oxidizing fatty acids. Similarly, nitrogen oxide can easily sequester oxygen and be oxidized to NO_2 [34]. In addition, the stable complexes between nitrite-derived compounds and heme-bond iron inhibit the release of free Fe^{2+}, which is therefore not available for initiation of lipid peroxidation [38]. The antioxidant properties of nitrites have also been partly explained by nitrite and dinitrogentrioxides reacting with unsaturated lipids to form nitro-nitroso derivatives and thus stabilizing the lipids against peroxidation changes [39].

From a health perspective, nitrates are relatively nontoxic, but nitrites and nitrite metabolic compounds such as nitric oxide and N-nitroso compounds have raised concerns over potential adverse health effects [40]. The International Agency for Research on Cancer (IARC) has concluded that nitrates and nitrites are probably carcinogenic to humans under conditions favoring nitrosation where an NO group is covalently bound to carbon, sulphur, oxygen, or nitrogen atoms in an organic molecule. During curing in acidic environment, undissociated nitrous acid picks up a hydrogen ion and splits off a water molecule. The resulting positively charged nitrosonium ion may then react with amino groups to form N-nitrosamines. Some of these N-nitrosamines are carcinogenic. In meat, the most relevant nitrosamines are N-nitrosodimethylamine (NDMA), N-nitrosopiperidine (NPIP), and N-nitrosopyrrolidine (NPYR). Formation of these compounds is only possible when secondary amines are present, pH must be <5.5, and temperature must be >130°C (NPYR) or the product must be stored for a long time at room temperature (NDMA, NPYR) [38]. N-nitrosamines can also be formed from biogenic amines. In a survey of DFSs of both North and South European types in Belgium, N-nitrosamines were detected in 54 of 101 samples [41]. The total amount remained below 5.5 μg/kg except in one sample with 14 μg/kg. NPIP was the most prevalent N-nitrosamine present above limit of detection in 28% of the sausages. There was only a limited relation between N-nitrosamine content and residual level of $NaNO_3$ and no relationship with $NaNO_2$ level. The authors assumed that the amounts of N-nitrosamines were low because the median concentrations of residual $NaNO_2$ and $NaNO_3$ levels were lower than 20 mg/kg in the screened products. EFSA refers to several surveys on residual levels of nitrite in cured meat products [32]. The range varied considerably, but generally the average residue levels were low. For example, in France 74% of raw dried cured meat products tested were in the range 0–9 mg/kg. In Germany, 116 samples of cured meat products were tested, of which 85% were below 20 mg/kg. Some reduction of the total N-nitrosamine content in DFSs appeared to be possible through the addition of ascorbic acid [42]. A large number of agricultural food products, seafoods, meat products, vegetable oils, sauces, and seasonings contain N-nitrosamines in the range 0.2 to a few μg/kg [43]. A benchmark dose methodology for developing tolerable daily intakes (TDIs) has been developed based on a large lifetime cancer dose-response study of NDMA in drinking water given to rats [44]. Taking into account inter- and intraspecies differences, a TDI range of 4.0 to 9.3 ng/kg/day was calculated. From these considerations, intake of NDMA from DFSs will generally be well below the TDI.

Partly due to the health concerns in conjunction with nitrite, there has been a growing popularity of cured meats produced as "natural" and "organic" without addition of nitrate or nitrite [33, 45, 46]. These "natural curing" processes consisted of adding a natural source of nitrate along with a nitrate-reducing starter culture. Most often, the natural source was a concentrated vegetable extract of celery (*Apium graveolens* var. dulce) with about 3% nitrate. Sometimes the extracts are pretreated to convert the nitrate to nitrite before use. Others have been employing Swiss chard (*Beta vulgaris* var. cicla) powders. This product contains 3.0 to 3.5% nitrate. A benefit of this product compared with celery extracts is that it contains no allergens.

The World Health Organization estimates that the daily dietary intake of nitrate is usually between 40 and 172 mg [47]. A substantial amount of dietary nitrate comes via fruits and vegetables. For example, approximately 98% of the dietary intake of Swedish children originates from fruits and vegetables and only 2% from cured meat products [48]. In contrast, dietary nitrite amounts to less than 20% of the daily nitrite exposure. The remaining 80% results from endogenous bioconversion of dietary nitrate to nitrite in saliva. Humans generally consume 0.3 to 2.6 mg nitrite each day [47]. Some reports estimate that cured meat contributes 4.8% of the daily nitrite intake [49].

Nitric oxide is involved in regulation of blood pressure and in regulations of gastrointestinal, respiratory, and genitourinary tract functions and immunologic reactions [50]. The basal level of nitrate in blood is around 2 mg/kg and that of nitrite approx. 100-fold lower [50]. Lack of nitric oxide production can lead to a number of conditions like hypertension, atherosclerosis, and thrombosis and can be ameliorated by dietary nitrite interventions [51]. A number of case control studies have been conducted worldwide to determine if there is a link between gastric cancer and nitrate intake [49]. No such link has been found. Other studies trying to link nitrates and nitrites consumption to brain, esophageal, and nasopharyngeal cancers have been inconclusive.

In conclusion, one might argue that the positive effects of curing are overwhelming against the small possibility of the formation of low doses of nitrosamines. The intake of curing agents from meat products is small in comparison with other foods [34].

3.4. Smoke. Smoking is a traditional treatment of Northern type fermented sausages and is part of the conservation to inhibit growth of molds and bacteria on the product surface. In addition, smoking adds a desirable smoky flavor, delays lipid oxidation, and adds color from light lemon to dark brown depending on the kind of smoldering wood and the time/temperature regime of the process. Smoke develops from the charring of wood, usually beech, oak, alder, hickory, or maple as well as fruit trees. The wood is normally cut into shavings or saw dust. The thermal composition of the wood followed by oxidation generates hundreds of different compounds, mainly H_2O, CO, CO_2, alcohols, carbonyl compounds, carboxylic acids, esters, hydrocarbons, nitrogen oxides, and phenols [52, 53]. Most smoke compounds would not be allowed by law to be added to foods in pure form; however, since the toxicity and concentration in the products are very low, smoking is generally regarded as safe. Many of the phenols such as guaiacol and its derivatives, cresol, pyrocatechols, and pyrogallol, show high antimicrobial activity. The content and distribution of these compounds in smoked meats are related to their solubility in lipid and water phases of the products. It is not yet possible to predict exactly the concentration of smoke phenols that is necessary to inhibit bacteria. The inhibitory concentration of smoke phenols for *Listeria monocytogenes* is in the range of 10–100 μg/g, which is in the same range as that found when mini-salamis (20 mm diameter) were smoked with beech (35–75 μg/g) [54]. The desirable smoky flavor is predominately from phenols such as syringol, 4-methylsyringol, 4-allylsyringol, guaiacol, 4-methylguaiacol, and trans-isoeugenol [52].

Some hydrocarbons formed in smoke are hazardous to human health, namely, the polycyclic aromatic hydrocarbons (PAHs). These are highly hydrophobic compounds consisting of two or more fused aromatic rings, mainly of hydrogen and carbon atoms. Compounds with four or more rings are less volatile and adsorb on soot and other combustion particles. There are 15-16 PAHs that are considered by the IARC and the European Union due to their carcinogenic and mutagenic properties [55, 56]. They are classified as carcinogenic, probably carcinogenic, possibly carcinogenic, and not classifiable. Benzo(a)pyrene (BaP) is the only compound in the carcinogenic group. Special attention has been given to a group of eight of the PAHs (PAH8), which were used in previous cancer studies and in EFSAs risk evaluation [55]. The PAH compounds convert to diol epoxides and bind covalently to DNA and cause errors in replication, mutation, and tumor genesis. BaP, when administered by the oral route, has been reported to produce tumors of the gastrointestinal tract, liver, lungs, and mammary glands of mice and rats and has also been associated with several other cancers [57].

For nonsmokers the main source of PAH is foods. The median dietary exposure across European countries was calculated both for mean and for high dietary consumers and varied between 235 ng/day (3.9 ng/kg body weight (b.w.) per day) and 389 ng/day (6.5 ng/kg b.w. per day), respectively, for benzo(a)pyrene alone, and 1168 ng/day (19.5 ng/kg b.w. per day) and 3078 ng/day (51.3 ng/kg b.w. per day), respectively, for PAH8. The two highest contributors to the dietary exposure were cereals and cereal products and seafood and seafood products. A number of products contain PAHs with undetectable levels of BaP. The EFSA therefore concluded that benzo(a)pyrene is not a suitable indicator for the occurrence of PAHs in food, and one should rather use a specific group of four (PAH4) or eight PAHs (PAH8) based on the available data relating to occurrence and toxicity. The EU Commission has in the Commission Regulation (EU) 835/2011 established an upper limit of BaP and PAH4 for smoked meat and smoked meat products. As of Sept. 1, 2014, the limit for BaP is 2 μg/kg, and the total amount of PAH4 is 12 μg/kg [58]. The accumulation of PAHs in different smoked meat products is related very significantly to the parameters of smoking and the kind of wood used for smoke generation and even on the location of the product in the kiln which affects the temperature and the flow rate of the smoke [52]. Codex Alimentarius Commission code of practice CAC/RCP 68/2009 specifies ten variables that need to be controlled to minimize and prevent PAH contamination of meat products during smoking [59]. These variables are fuel type, smoking or drying method (direct or indirect), smoke generation process (temperature, airflow, friction versus smoldering, liquid smoke), distance between the food and the heat source, position of the food in relation to the heat source, fat content of the food, duration of smoking, and direct drying, temperature during smoking and direct drying, cleanliness and maintenance of equipment, and finally design of the smoking chamber and the equipment used for smoke/air mixture (which influences the smoke density in the smoking chamber). The importance of these factors has been reviewed by Ledesma et al. [53].

The content of PAHs in smoked meat products is usually well below the maximum level set by the EU Commission [52]. The greatest amount of BaP is deposited on the meat product casing, and only a minor fraction then migrates into the product [53]. The content of PAH in sausages will depend on the type of casing used. Both for dry fermented Petrovská kolbása sausages from Serbia and traditional DFSs from Portugal, the PAH contamination level was lower when collagen casings were used [60, 61].

One option to reduce PAH in meat products is by using liquid smoke. This is an easier, more rapid, and reproducible process [53]. Liquid smoke is produced by chilling and thereby condensing wood smoke. The liquid smoke is then refined and filtered to remove toxic and carcinogenic impurities containing PAH. Use of liquid smoke is therefore generally considered to be of less health concern than traditional smoking.

3.5. Starter Cultures. In a traditional process for producing fermented sausages, bacteria, yeast, and fungi contribute to various degrees to the final product. However, it is generally accepted that LAB play the most prominent role, since the initial acidification is essential, both technologically and from a safety perspective [62]. Low pH and organic acids will inhibit contaminant spoilage flora and potential pathogens and ensure preservation. Acid conditions also aid in texture formation due to meat protein coagulation and in color formation through the reactions of nitrite and nitrogen monoxide with myoglobin [62]. Although LAB also contribute to aroma formation, mainly through organic acid production,

other bacterial groups appear to be more important. These are the Gram-positive catalase-positive cocci (GCC+), in particular the coagulase-negative staphylococci (CNS). CNS convert amino acids and free fatty acids to potent aroma compounds essential for taste notes of fermented sausages. In addition, CNS also possess highly active nitrate reductase and catalase, which contribute to color formation by producing nitrite from nitrate [35] and the limitation of lipid oxidation that may cause rancidity, respectively [63, 64]. Traditional production of fermented sausages is based on spontaneous fermentation; that is, endogenous microorganisms present in the raw material will perform the microbial transformation of the material. However, it has long been known that better reproducibility could be obtained by adding a small portion of a previous successful batch when starting a new, the so-called "back-slopping" technique [65]. This is the forerunner to the use of starter cultures, that is, the intentional addition of premade microbial cultures to a fermentation process, either single or mixed, in order to control and standardize the process. The first-generation starter cultures for fermented sausages were developed in the 1940s in the USA. However, these were not based on the dominating microorganisms found in spontaneous fermentation, or even isolated from meat, but rather on their technological feasibility, for example, surviving freeze-drying, and their fast acid production rate. These cultures, primarily strains of the genera *Pediococcus*, were useful for the particular products produced in the USA, that is, "summer sausages" with very short production and maturation times [62]. However, they were less suitable for products of the European tradition with longer fermentation and maturation times. Research in the 1960s, 1970s, and 1980s, also confirmed in many later studies, revealed that these types of sausages were dominated by *L. sakei* or the related species *L. curvatus* and, to some degree, *L. plantarum* [62, 66–68]. The second-generation LAB starter cultures, now widely used, are often based on these [69]. Molecular characterization by, for example, genome sequencing and comparative genomics has shown that strains of *L. sakei* isolated from meat and meat fermentation have evolved to be perfectly adapted to this particular environment [70–72]. *L. plantarum* lacks this specific adaptation but is a fast-growing, highly flexible bacterium with the largest genome size of the lactobacilli. Some specific nonstarter LAB ("house flora") strains of *L. plantarum* have been shown to outcompete commercial starters based on *L. sakei* or *L. curvatus* in industrial sausage production [73]. GCC+ strains were isolated from fermented meat products in the early 1900s and their role in aroma formation and color stability was established in the 1950s [2, 69]. They were subsequently suggested for use as starter cultures for sausage production, first as single cultures, but later mixed cultures were shown to be superior to both a single GCC+ culture or a single LAB culture [2, 63, 64]. The success of these mixed cultures is likely because they reflect the course and dynamics of a spontaneous fermentation better than a single culture and thereby retain the aroma and taste of the traditional products [63, 74, 75]. The GCC+ strains most often found in spontaneous fermentation and also used as starters are CNS and belong to the species *Staphylococcus carnosus*, *S. xylosus*, and *S. saprophyticus* [64, 74].

Mold growth on the external surface of DFS is desirable on some types of fermented sausages in many European countries, especially around the Mediterranean, but also in, for example, Hungary and Belgium. The distinct grey-whitish appearance of these products is an attractive feature. In the traditional manufacture of these products, the process relies on the fortuitous inoculation of the maturing sausages by spores resident in the air. The different factories have their own distinct "house flora," which are adapted to the process and will eventually dominate the surface growth and ensure some reproducibility of the product quality. The surface molds contribute to the taste and aroma of the sausages by lipolytic, proteolytic, and lactic acid oxidizing activities, enhance general quality parameters through oxygen consumption, which counteract rancidity development, and improve color. The mold surface layer also modifies the drying rate and thus prevents excessive drying of the sausages [76]. The specific conditions prevailing on the sausage surfaces, for example, temperatures from 10 to 20°C and relative humidity starting at 90–95% and decreasing during the ripening period, select for certain genera of molds, in particular *Penicillium* and occasionally *Aspergillus*. Common species are *P. nalgiovense*, *P. chrysogenum*, and *P. nordicum* [76–78]. Mold starter cultures have been developed, most often consisting of spores of *P. nalgiovense* [78, 79]. The main selection criteria for these cultures are their low potential for mycotoxin production (see below) and their ability to outcompete the "house flora" while retaining the ability to produce sausages of acceptable taste, aroma, and appearance [76, 78–80].

Fungal surface colonization of maturing sausages starts with salt and acid tolerant yeast species, such as *Debaryomyces hansenii*. However, along with the decrease in a_w, there is generally a shift in the mycobiota towards molds [81]. Although the role of yeasts in sausage fermentation is not equally well known as for bacteria or molds, it can be significant in some products [82, 83]. Lipolytic, proteolytic, and lactate oxidation activities account for this effect [81–83]. Starter cultures containing *D. hansenii* have been developed, sometimes in combination with mold spores [81].

All starter cultures are, by definition, "functional," since their activities contribute to the transformation of the raw material and to the appearance and quality of the final product. However, the description of a starter culture as "functional" often pertains to one (or several) additional function(s), beyond the normal properties of a starter culture. Several such additional functions have been described, for example, properties that enhance food safety (see also below) or have a technological advantage [64]. In recent years, in accordance with trends in consumer demands, functionality for enhanced health properties has been studied. Probiotic starter cultures have been one of the main themes in this research [84]. The term "probiotics" was coined in the 1950s as an antonym to "antibiotics." The term subsequently developed into a scientific concept and was defined as "live microorganisms that, when administered in adequate amounts, confer a health benefit on the host" by FAO/WHO in 2001. This definition was later reinforced as adequate and sufficient [85]. LAB, especially bacteria belonging to

the genus *Lactobacillus*, are recognized as common inhabitants of the human gastrointestinal tract and have received considerable attention in the last decades for their health-promoting properties and use as probiotics. The use of probiotic strains in fermented products was first employed in the dairy industry, and milk-based products are still the most common vehicles for delivery of probiotics [86]. However, being products where LAB proliferate and dominate, fermented sausages are also potential carriers for delivery of probiotic LAB strains [64, 87, 88]. There are some significant challenges in using fermented sausages as probiotic products in comparison with dairy products. The most important are as follows: (i) the meat raw material is not sterilized or pasteurized before the fermentation process and a probiotic bacterium must therefore be as competitive as any starter culture normally used for the fermentation to outcompete the endogenous flora; (ii) the mature sausage constitutes a harsh environment with low a_w and containing salt and nitrate; thus, survival of the probiotic after fermentation should be validated; (iii) the numbers of the probiotic after maturation and storage must be very high since the serving size and daily consumption of fermented sausage product are generally less than a comparable dairy product; and (iv) the probiotic should produce an acceptable product with regard to taste and quality [89, 90]. There are two main alternatives in the research and development of probiotic fermented sausages. The first is to select strains based on their probiotic properties and subsequently investigate the suitability of the strain(s) in the production of fermented sausages. Using this strategy, already commercial probiotic strains have been studied. The perhaps most well-documented probiotic strain, *Lactobacillus rhamnosus* GG, has been used in several studies for this purpose, with varying success [91–94]. Although the GG strain can perform the fermentation, there seems to be a balance between inoculum size, off-taste (due to excessive acid), and enough survival in the finished product, which is difficult to achieve [94]. Similar problems were encountered using another well-documented strain, *L. plantarum* 299v [95]. A better outcome was obtained with a new *L. rhamnosus* strain, isolated from human intestine and with potential probiotic properties [95]. The disadvantage of using such a strain is that it is not possible to use the wealth of previous documentation, which a well-known strain might have, in promoting the product. The second strategy that has been used for developing probiotic meat products is to use strains isolated from successful meat fermentation, or even meat starter cultures [73, 96]. Such strains have to be assessed for potential probiotic properties but are usually well adapted to the meat fermentation environment. These strains will also suffer from the fact that their probiotic properties will be poorly documented in comparison to well-known documented strains. There have been attempts to launch probiotic meat products commercially in Germany and Japan [97], but the outcome in commercial terms is unclear. An obstacle in the development of probiotic products in general is also that EFSA has so far rejected all health claims of probiotics using a very strict assessment in their approval process [85, 98].

4. Microbial Hazards Associated with Fermented Sausages

Although historically considered as safe, the characteristics of DFSs can provide survival and even growth of certain pathogens in these products. Surveys have shown the presence of pathogenic *Escherichia coli*, *Salmonella* Typhimurium, *Staphylococcus aureus*, and *L. monocytogenes* in dry fermented sausages. *Clostridium botulinum* and *Toxoplasma gondii* have also been reported as potential microbial risks for consumers of DFSs.

Pathogenic microorganisms can be introduced through contaminated raw materials or through cross-contamination from equipment or personnel during processing or at retail. Conditions during sausage processing and pathogen characteristics determine the ability for pathogen growth and survival and also determine possible strategies for pathogen elimination to ensure product safety.

4.1. E. coli. Pathogenic *E. coli* belong to various pathotypes, with verocytotoxigenic *E. coli* (VTEC), (synonymous to Shigatoxigenic *E. coli* (STEC)) predominantly associated with meat. VTEC strains produce Shiga-toxins 1 and/or 2. They may carry different virulence factors responsible for variations in clinical manifestations. A subgroup of VTEC causing severe infections of enterohemorrhagic colitis and possibly hemolytic uremic syndrome (HUS) characterized by acute renal failure and anemia is designated enterohemorrhagic *E. coli* (EHEC). More than 150 different serotypes of VTEC have been associated with human diarrheal infections. Serotype O157:H7 strains have been the most known disease causing VTEC. Non-O157 have emerged with the serotypes O26, O45, O103, O111, O121, and O145, also known as the "big six," being most frequently associated with human disease [99]. Raw meat ingredients contaminated through the slaughtering process are regarded a primary source of VTEC in DFSs. Cattle are regarded a primary O157:H7 VTEC reservoir although other animals such as sheep, swine, goat, and deer can also be carriers of VTEC. In outbreaks caused by contaminated DFSs, VTEC serogroups of O157, O26, O111, and O103 have been causative agents [6]. Low cell numbers (10–1000) are sufficient to cause disease [100, 101] and levels lower than 1 cell (EHEC O111:NM) per 10 g were reported in a salami outbreak from Australia. Although growth of pathogenic *E. coli* during initial phases of fermented sausage production can occur, combinations of low pH and a_w inhibit growth of *E. coli* in finished products [88]. However, extensive pathogen survival in finished products has been reported [102–105]. Strategies for effective VTEC elimination in DFSs are a challenge for producers. It has been suggested that serotype O157:H7 strains have enhanced tolerance to acids compared to other serotypes and that this may have a role in their capacity to cause outbreaks via low pH foods like DFSs, for example [106, 107]. However, within this and other serotypes, strains variations in acid resistance exist. The low infectious dose, the serious outcome of EHEC infections, and several reported outbreaks linked to VTEC contaminated DFSs highlight VTEC as the most serious safety risk in DFSs.

Effective strategies for VTEC reduction/elimination during the whole farm to fork chain are therefore required.

4.2. Salmonella. *Salmonella* are important zoonotic pathogens with high economic significance in animals and humans. As foodborne pathogens, the two *S. enterica* serovars Epidermidis and Typhimurium are dominating among human cases. Serovar Epidermidis is associated with eggs and poultry while Typhimurium is linked to meat of pork and bovine origin [108]. Most salmonellosis infections are self-limiting, yet severe and life-threatening complications (e.g., sepsis) can follow. Infected animals are the primary source of *Salmonella* where transmission to environments and foods likely occurs through fecal contamination and cross-contamination. According to EFSA, 2.8% of the samples taken from minced meat and meat preparations from other species than poultry intended to be eaten cooked tested positive for *Salmonella* in the EU in 2010 [109]. In foods, such as minced meat and meat preparations intended to be eaten raw, 1.8% of samples were *Salmonella* positive. A coordinated approach has led to a significant reduction of human cases of salmonellosis in the EU in the last decade. Still, *Salmonella* were the most common causative agent of foodborne outbreaks reported in EU in 2013 [108]. *Salmonella* have been implicated in several outbreaks linked to consumption of DFSs where contaminated meat ingredients are a common source. Reported outbreaks seem to be dominated by fermented sausages produced from pork meat contaminated with *S.* Typhimurium although other serovars (e.g., Montevideo, Goldcoast) have also been causative agents [110–113]. The infectious dose can be low where 10–1000 cells are sufficient to cause disease [114]. Studies have shown *Salmonella* to be more sensitive than *E. coli* O157:H7 and *L. monocytogenes* to at least certain DFS manufacturing process parameters [103, 115, 116]. As for reduction of other pathogens, use of starter cultures has a positive effect on *Salmonella* reductions, for example [117, 118]. Reported differences in *Salmonella* reductions are influenced by variations in recipes, processes, and strains and direct comparisons between studies are difficult. At higher contamination levels, complete elimination through traditional processing is difficult.

4.3. S. aureus. *S. aureus* is common on skin and mucosal membranes of humans with estimates of 20–30% persistent and 60% for intermittent colonization [119]. The bacterium is also found on food animals. *S. aureus* produces a range of staphylococcal enterotoxins (SEs) of which some show emetic activity [120]. SEs are a major cause of food poisoning, which typically occurs after ingestion of foods, particularly meat and dairy products, that have been contaminated and stored at elevated temperatures where *S. aureus* have grown and produced toxins. Symptoms are of rapid onset, due to the preformed toxins in the food, and include nausea and violent vomiting, with or without diarrhea. The disease is usually resolved within 24–48 hours. Staphylococcal toxin SEA is the most common cause of staphylococcal food poisoning worldwide. The SEs belong to a group of superantigen toxins, which bypass conventional antigen recognition by interaction with major histocompatibility complex class II molecules on antigen presenting cells and with T-cell receptors on specific T-cells [121]. SEs are also able to penetrate the gut lining and activate immune responses, thereby leading to vomiting [122]. The level of *S. aureus* present in the foods causing disease in an English survey ranged from no viable *S. aureus* detected to 1.5×10^{10} cfu/g with a median of 3.0×10^{7} cfu/g [123].

S. aureus does not compete well with the indigenous microorganisms in foods and will grow better in processed foods where the competing flora has been destroyed, for example, in products contaminated after a heat treatment or when the food process gives *S. aureus* a selective advantage. This can be the case for cured meats, since *S. aureus* can tolerate high amounts of salt and grow down to $a_W = 0.86$. *S. aureus* is able to grow in a wide range of temperatures (7° to 48°C) with an optimum 37°C and pH (4 to 10), with an optimum of 6 to 7 [124]. These characteristics enable *S. aureus* to grow in a wide variety of foods.

Although *S. aureus* can tolerate high salt and low pH and is often implicated in meat outbreaks (ham, pork, and sausages), few incidences on food poisoning from fermented sausages are reported [123, 125–129]. Outbreaks caused by *S. aureus* are usually old, of which some have been registered by Center for Disease Control [130–134]. *S. aureus* is frequently found in fermented sausages, but generally at levels too low to produce enterotoxin amounts sufficient to cause illness. Although *S. aureus* can tolerate salt and nitrite, it is a poor competitor under anaerobic conditions, at low pH and low temperatures. If sausages are fermented at no higher than 25°C for 2 to 3 days and the initial count of *S. aureus* is below 10^4 cfu/g, the risk of enterotoxin formation is low [2]. For semidry sausages, fermentation up to 43°C is common in the US, and a rapid pH drop during manufacture will ensure inhibition of *S. aureus*. Consequently, the American Meat Institute in 1982 specified the maximum time allowed to reach pH 5.3 [2]. Apparently, the use of appropriate process controls and starter cultures has significantly reduced the incidence of "summer sausages" outbreaks of *S. aureus* food poisonings in the US [2]. North Carolina State University Meat lab has proposed in their HACCP program that to ensure safety products should be fermented to pH 5.3 or below within 1200 degree hours [135].

When chorizo was inoculated with *S. aureus* and without a starter culture and fermented at 30°C, the pathogen grew well. *S. aureus* growth was, however, reduced by using starter culture, lower fermentation temperature (20°C), and higher concentrations of spices, nitrites, nitrates, and ascorbate [136]. In addition, no enterotoxin A was detected in the latter sausages after drying. Both strategies using specific starter cultures and starter cultures in combination with bacteriocins have been shown to reduce the presence of *S. aureus* [137–139]. *S. aureus* growth in Italian dry salami was affected by the initial pH, initial levels of *S. aureus*, lactic acid bacteria, day of fermentation, and interactions between these parameters [140, 141].

Other species of staphylococci (CNS) are frequently found in foods. Some are also used as starter cultures in DFS. Of a set of 129 such different strains, only one strain carried an enterotoxin gene, and 78% of the strains did not carry

decarboxylases for biogenic amine formation. Although 78% of the strains possessed at least one gene encoding antibiotic resistance, these CNS were considered to pose a low safety hazard [142].

4.4. L. monocytogenes. Foods contaminated by *L. monocytogenes* can cause listeriosis, infections varying from mild flu-like symptoms to life-threatening disease with a high fatality rate in vulnerable populations. Ready-to-eat (RTE) products consumed without prior heat treatments and containing higher than 100 cells/g are considered to pose a direct risk to human health. *L. monocytogenes* is ubiquitous in nature [143] and contamination of DFSs can occur through contaminated ingredients, preferably raw meat. The important role of contaminated processing equipment and environments as a source of *Listeria* in DFSs has been indicated in several studies [144–147]. Thus, *L. monocytogenes* are commonly found in DFSs with reported prevalence up to 40% [148]. Prevalence in beef is usually in the range 0–10%, but with generally higher prevalence reported on pork meat [149, 150]. Nevertheless, only one outbreak in Philadelphia, USA, in 1986/1987 with possible epidemiological association to fermented meat is known. Fermented sausages have been evaluated to be products of low to moderate risk associated with listeriosis. This is due to usually low levels of *L. monocytogenes* in these products and that a high minimum infectious dose ($>10^4$ cells) is normally required for illness. Some growth of *L. monocytogenes* can occur in the initial phase of DFS processing, but the combinations of low pH (5.3–4.6) and a_w ($\leq$0.90) generally restrict growth of the bacterium in the fermented sausage products. The extent to which DFSs can be considered safe is primarily dependent on the fermentation and drying process. With the wide specter of fermented sausages produced, not all sausage recipes and processing conditions may ensure products where the levels of *L. monocytogenes* are compliant with the microbial criterion of $\leq$100 colony-forming units per gram [151]. It is therefore important for the DFS producers to gather information on the safety of their products in terms of *L. monocytogenes* contamination and growth and implement processing parameters to assure food safety.

The effects of using starter cultures for increased pathogen reductions have been shown in several studies, for example [152–154]. In general, enhanced reductions were obtained in products with low pH and low a_w and stored under ambient conditions [103, 116, 155]. Reductions of *L. monocytogenes* during fermentation and drying in fermented sausages are dependent on many factors, including strain differences in their ability to tolerate and adapt to DFS conditions that are also dependent on recipe and processing conditions [147, 156].

4.5. C. botulinum. *C. botulinum* is a strictly anaerobic spore forming bacterium. Spores of *C. botulinum* occur in the soil and may enter the meat from contaminated hides. The botulinum neurotoxins are produced in growing vegetative cells after the spores have germinated. The toxins can cause nausea, vomiting, fatigue, dizziness, dryness in mouth and throat, paralysis of muscles, double vision, respiration problems, and death. The toxins bind irreversibly to peripheral nerve endings and block the release of nevrotransmitters. An overview over reported outbreaks associated with meat and fish has been given previously [157]. The rapid alert system for food and feed (RASFF) for the years 2010–2015 does not report any outbreaks of *C. botulinum* from fermented sausages. *C. botulinum* that can affect man are often grouped into proteolytic and nonproteolytic strains. The proteolytic strains are the most hardy ones and can grow down to a pH of 4.6 or at 10% NaCl and down to a_w of 0.94. They also have spores that can withstand boiling for extended periods. The combination of low pH, high NaCl, and low a_w ensures that *C. botulinum* will not grow in matured fermented sausage. In addition, nitrate or nitrite is added to the sausage batter to inhibit growth of *C. botulinum* and other pathogens. Nitrate is reduced by GCC+ in the batter to nitrite. The mechanism by which nitrite inhibits *C. botulinum* is uncertain. Nitrite has been reported to inhibit the phosphoroclastic system of *C. botulinum* [158]. This could be of importance for inhibiting *C. botulinum* the 2-3 initial days of sausage production where the water activity is high and before the fermenting lactic acid bacteria have lowered the pH.

Hospital et al. produced two types of Mediterranean fermented sausages, salchichón and fuet with final pH of 5.0 and 5.2, respectively [159]. a_w was between 0.88 and 0.90. One batch contained the maximum ingoing dose allowed by the EU 150 mg/kg $NaNO_3$ and 150 mg/kg $NaNO_2$. They also made sausages with 25 and 50% nitrate and nitrite reductions and control sausages without nitrate/nitrite. In no cases was toxin production detected from spores added to the sausages, even though the conditions for growth of *C. botulinum* remained acceptable for 8–12 days during manufacture. Cell free extracts from a meat isolate of *Staphylococcus sciuri* have been shown to inhibit *C. botulinum* in vitro and may show some potential in inhibiting *C. botulinum* in fermented sausages [160].

The *C. botulinum* concern in conjunction with cured product is more relevant for nonfermented products which could support growth than for fermented sausages. The use of nitrite in fermented sausages, the conditions in the sausages not being able to support growth of the bacterium, the number of *C. botulinum* spores generally being very low if present, and the lack of registered outbreaks from fermented sausages together point to a low risk of food poisoning from these products.

4.6. Toxoplasma gondii. *T. gondii* is an obligate, intracellular parasite, which is widely distributed in the world. Conventionally it is associated with handling cats and cat litter; however, Center for Disease Control and Prevention, USA, now estimates that 50% of toxoplasmosis is foodborne and that foodborne toxoplasmosis causes 327 deaths annually and is the leading cause of death from foodborne pathogens after *Salmonella* in USA [161, 162]. Consuming undercooked meat products has been considered the major risk factor. Healthy adults generally have no symptoms, whereas severe illness can occur in infected fetuses, newborns, immunocompromised individuals, and transplant patients. Nitrite and nitrate, spices, low pH, and cold storage have no effects on

the viability of *T. gondii* cysts [163]. The cysts do not survive freezing for longer than 4 hours. Using frozen meat for the sausage batter will thus reduce the risk of infection. Regarding DFS production, duration of the fermentation is critical to *T. gondii* survival. Tissue cysts remain viable in fermented sausages after 12 h of treatment even in presence of 2% curing salt. When fermented sausages were produced containing experimentally contaminated goat meat, no viable cysts were detected in the final sausages after 12 days [164]. These and other risk evaluations conclude that fermentation over long periods reduces the risk of infection [163].

5. Other Microbiology Related Health and Safety Concerns

5.1. Biogenic Amines. Biogenic amines (BAs) are basic, nonvolatile low-molecular weight, nitrogenous compounds, common in living organisms where they perform various functions on, for example, the nervous, gastric, and intestinal systems and on regulation of blood pressure [165]. They are formed as a result of normal metabolic activities in humans, animals, plants, and microorganisms, generally through decarboxylation of the corresponding amino acids. BAs are of considerable food safety concern as they may be present in various foods and when ingested in excessive amounts may cause certain diseases, or disease-like conditions, due to a disturbance of the normal physiological concentrations. Symptoms of intoxication include headaches, flushes, nausea, cardiac palpitations, and increased or decreased blood pressure. The most important BAs in foods are histamine, putrescine, cadaverine, tyramine, tryptamine, phenylethylamine, spermine, and spermidine [166]. Of these, histamine and tyramine are the most toxic. Presence of some of the other BAs may enhance the effects of histamine or tyramine [166]. Normal physiological concentrations of BAs are carefully regulated in the human body. For instance, the amines can be oxidized by monoamine oxidases (MAO) or diamine oxidases (DAO). Hypersensitivity for BAs in some humans may be caused by decreased activity of these enzymes due to deliberate inhibition (MAO inhibitor drugs) or genetic disposition [167]. Definitive toxicity levels or limits are therefore difficult to determine [165]. Amino acid decarboxylases are the enzymes responsible for the formation of BAs. These enzymes are widely present in spoilage microorganisms, but also ubiquitous in desirable microorganisms, such as bacteria important in fermented sausages, that is, LAB and CNS [168].

High levels of biogenic amines may occur in foods such as fish, fish products, and fermented foods (meat, dairy, some vegetables, beers, and wines). Generally, the potential of BA formation increases with the protein content of the raw material as the breakdown of proteins provides the amino acid precursors for BAs. Fish and cheese are the most implicated products in foodborne BA intoxication. No cases of BA poisoning have implicated fermented sausages as the cause, although measured amounts of BAs have in some instances reached similar levels as in fish related outbreaks [165].

The most important BAs present in fermented sausages of food safety concern are tyramine, phenylethylamine, and histamine, with tyramine usually being the most abundant [168]. Contaminant Gram-negative enterobacteria and/or pseudomonads, present in the raw material, are the most important BA producers before the onset of the fermentation by LAB. High BA content of food products is often considered an indication of spoilage or hygiene failure in the handling of the raw material [165, 168]. Good hygienic quality of the meat and a rapid pH reduction in the initial stage of the sausage production process are essential for inhibition and control of BA production by these contaminants [169]. Salt and nitrite tolerant Gram-positive bacteria, such as LAB and CNS, will initiate the fermentation and eventually dominate the microflora. Prominent tyramine producers among LAB relevant for sausage fermentation are *L. curvatus* and many enterococcal strains found in artisanal sausage manufacture in southern Europe [170]. Histamine producers are very rare among sausage LAB and histamine, when present in sausage, is considered to be produced by mainly contaminant enterobacteria [169]. However, specific strains of, for example, *L. buchneri* and *L. parabuchneri* harbor the histidine decarboxylase enzyme and are considered spoilage organisms in cheese [171, 172]. Although never dominating a sausage fermentation, such lactobacilli may be present as contaminants [169]. Other LAB relevant for sausage fermentation, such as *L. sakei* and *L. plantarum*, are generally nonaminogenic [168, 170, 173]. Amino acid decarboxylases are uncommon in the most common CNS relevant for sausage fermentation, for example, *Staphylococcus xylosus*, *S. saprophyticus*, and *S. equorum* [173]. However, occasional strains of *S. carnosus* and *S. equorum* may show BA production [142, 173].

Different strategies have been investigated to control and minimize BA formation in fermented sausages. The addition of specific inhibitory agents to the meat batter, such as wine [174] or plant essential oils [175], is an example. Such additions reduce the initial contaminating flora, thereby reducing BA formation, but may also change the product taste and appearance. Methods have been suggested for the removal of BAs after their formation, such as the use of fermentative bacteria with amine oxidase activity [176] or the use of gamma radiation [177]. However, such procedures are considered inappropriate since it may disguise incidents of hygienic malpractice and/or spoilage [169]. The generally recommended and most efficient way of reducing and/or controlling BA formation in fermented sausages seems to be the use of nonaminogenic starter cultures [165, 168, 175, 178–182]. The use of a LAB starter culture results in a more rapid pH decrease than a spontaneous fermentation, thereby inhibiting contaminant Gram-negative bacteria and thus the potential for BA formation at the initial stages of the process. The dominance of nonaminogenic LAB during the fermentation ensures minimal BA production. Nonaminogenic CNS will contribute to the effect. Mixed cultures of both nonaminogenic LAB and CNS have been shown to perform better than single starters, probably because each starter controls and dominates different parts of the microflora [169, 178]. To ensure dominance of the selected starters, the use of so-called autochthonous starter cultures is recommended [168, 173, 183]. These are bacterial strains isolated from the particular products they subsequently should be used in

as starters. Such starters are potentially better adapted to each specific process than commercial cultures and will also preserve the quality and taste of the original product. If commercial cultures remain the only option, they should be tested for performance since highly competitive nonstarter LAB may dominate the fermentation, despite the use of starter cultures [73].

In conclusion, the selection of starter cultures, especially LAB, for use in fermented sausage production, should use the absence of amino acid decarboxylase activity as a basic criterion.

5.2. Mycotoxins. The surface colonization of dry fermented sausages by fungi is nearly inevitable. The conditions are ideal for, for example, *Penicillium* species, unless specific measures are taken to minimize fungal growth, such as mechanical removal or the use of dipping regimes with antifungal compounds, for example, sorbate solutions. Smoking may also inhibit the growth of fungi to some extent. One or more of these measures are often used in the Northern European, especially Scandinavian, tradition of fermented sausage production where mold growth is undesirable. However, as mentioned, mold growth on the surface is a desirable and characteristic feature of many products in some countries. A safety concern with regard to surface growth of molds on fermented sausages is mycotoxin production. Most *Penicillium* species are capable of producing one or more mycotoxins [184, 185], the most important being ochratoxin A (OTA), patulin, citrinin, cyclopiazonic acid, and roquefortine. In surveys of molds isolated from fermented sausages, potentially toxigenic *Penicillium* strains are commonly found [79, 186]. Actual production of mycotoxins in the products has also been shown, though to a lesser degree [79, 80, 187]. *P. nalgiovense* strains were early selected as starter cultures due to their apparent low toxigenic potential and useful technological properties [78, 188]. This seems still to be the best choice as more recent studies confirm low toxigenic potential [76, 79].

Fungal starter cultures alone may not always be able to outcompete resident house flora, which has adapted over long time. Other measures may be necessary to control mycotoxin production. OTA represents the most important mycotoxin produced by different molds relevant for sausage production, that is, *Penicillium* strains [80]. *P. verrucosum* and *P. nordicum* are capable of producing OTA when they grow on the sausages surface during both ripening and storage [187]. OTA is undesirable because it is classified by IARC into "Group B" as a molecule with possible carcinogenic activity in humans [189]. Ozonated air has been suggested as a method for preventing the growth of OTA producing molds [187]. Protective yeast cultures (*D. hansenii* and *Saccharomycopsis fibuligera*) were recently shown to inhibit OTA producing fungi in a fermented meat product [190]. It is unclear if this technique can be applied to fermented sausages where a mold coat is desired. Another biocontrol approach is the use of nontoxigenic molds producing small, cysteine-rich antifungal proteins (AFPs). These strains, or the purified AFPs, have been suggested as useful for controlling growth and mycotoxin production by toxigenic fungi on dry-ripened foods [191, 192]. A more practical approach is to carefully choose the environmental parameters during ripening, especially with regard to a_w and temperature, in order to favor colonization of starter cultures against OTA producing fungi [193].

5.3. Antibiotic Resistance. The growing level of resistance to antibiotics in bacteria presents a serious concern to human and animal health and presents significant financial and societal costs. Antibiotic resistance (AR) in food bacteria is of concern because they may act as reservoirs for AR genes. Even if the relative amount of antibiotic resistant bacteria in a particular fermented food product may be low, the absolute number can nevertheless be significant because large amounts of living bacteria are ingested when the food is consumed. Food bacteria may carry transferable AR, which could be transferred to commensal or pathogenic bacteria in the gastrointestinal tract. The presence of transmissible AR genes should therefore be an important safety criterion in the selection of starter cultures [180]. Enterococci are generally not used as starter cultures for fermented sausages but may be involved in spontaneous fermentation. Enterococci have been thoroughly investigated with regard to AR because of their clinical significance. AR is also frequently detected among food enterococci [194]. Because enterococci harbor different gene transfer mechanisms (e.g., pheromone-responsive plasmids, conjugative and nonconjugative plasmids, and transposons), they may acquire these determinants from other enterococcal strains and transfer them to potential pathogens [195]. This represents a possible risk related to the use of enterococci as probiotics or starter cultures [194, 195]. Thus, no enterococcal strains are currently included in the QPS (qualified presumption of safety) list of EFSA (European Food Safety Authority) [196].

Lactobacilli have a long history of safe use in fermented food, which supports their GRAS (generally recognized as safe) and QPS status granted by FDA (US Food and Drug Administration) and EFSA, respectively. Many *Lactobacillus* species are intrinsically resistant to a number of antibiotics, for example, streptomycin and vancomycin [180, 197]. However, transmissible AR has frequently been detected, also in strains isolated from fermented sausages [180, 198–200]. Tetracycline resistance, mediated by the *tetM* gene, and the *ermB* erythromycin resistance gene seem to be the most common [180, 199]. In vitro experiments have shown that AR determinants can be transferred from meat associated LAB to other LAB and to pathogens [201, 202]. A similar pattern exists in CNS [180, 203, 204], showing that most AR genes are shared in nearly all meat associated Gram-positive bacteria [180]. This may reflect the (mis)use of antibiotics in animal husbandry for decades, leading to a large pool of AR genes present in the microbial population, spreading also to bacteria in the food chain [205]. To minimize the potential risks associated with the intentional use of microorganisms in food (e.g., starter cultures and/or probiotics), including transfer of AR, EFSA has regulated the industrial use of bacteria as starter cultures through the QPS system [196]. In addition, guidelines have been developed for assessing AR in relevant strains [206].

6. Reduction of Microbial Hazards

Reported outbreaks and disease history have shown that main microbial pathogens in DFSs include VTEC and *Salmonella*. As a food safety hazard in DFS, *L. monocytogenes* is regarded less relevant although their presence throughout the manufacturing processes of DFS is well documented [207–210]. Nevertheless, *L. monocytogenes* is a significant pathogen where its presence in ready-to-eat products is troublesome. Its elimination from DFS products is therefore important. Strategies for control and elimination of pathogens in DFS include optimization of recipe and process parameters and eventually use of postprocess treatments of finished sausages to ensure safe products. Several outbreaks caused by VTEC contaminated fermented sausages lead the US Food Safety and Inspection Service to establish a lethality performance standard requiring 5-log reduction of *E. coli* during DFS processing. In Canada, a 5-log reduction is recommended, while in Australia, the required reduction is 3-log units [211].

There are limitations in how much different parameters in recipe and process can be varied without negatively affecting the characteristics and sensory quality of these products. Combination of parameters in recipe and process according to the "hurdle concept" for optimal reduction of pathogens while maintaining the sensory quality of the products has been one approach. More recently, the effects of more novel technologies for, for example, meat batter decontamination and postprocess treatments of finalized DFS have been evaluated [212].

An overview of reported processing and postprocess strategies for elimination of pathogens in DFS, with particular focus on VTEC, is provided below.

6.1. Reductions of Pathogens in Raw Meat Ingredients. Contaminated raw meat and possibly nonmeat ingredients can provide important sources of VTEC and *Salmonella*. Freezing of raw meat prior to be used in DFS production is not uncommon. Bacteria in the meat can be damaged by a freeze/thaw process, and this has been shown to provide an extra 0.5-1-log reduction of *E. coli* O157:H7 in the final salami product [213]. Another strategy, commercially used in the USA, is heat treatments of raw meat ingredients by lactic acid–hot water (80–90°C). The process provided 3.6–3.9-log reductions of *Salmonella* and *E. coli* O157 in final DFS, though with some negative sensory influences [214]. Use of high pressure processing (HPP) of meat trimmings for DFS affected the physiochemical properties of the meat batters and had negative effect on the sensory properties of the DFS [215]. Irradiation in the range 1.5–4 kGy of raw meat/fat ingredients prior to production of DFS delivered a 5-log reduction of *E. coli* O157:H7 but was less effective in reducing *L. monocytogenes* [216, 217]. Irradiation resulted in products with quality indicators closely resembling those of traditional dry sausage [216, 218].

6.2. Reductions of Pathogens through Changes in Recipe and Process Parameters. There are large variations in the reductions of pathogenic *E. coli*, *Salmonella*, and *Listeria* in different processes and products of DFS. This is expected due to the broad range of DFS products varying in pH, salt content, a_w, recipe, and production process like fermentation temperature and maturation time. Parameters important for VTEC reductions have been reviewed previously [6, 7]. Reduction of VTEC in traditional production processes of salami, pepperoni, and some other types of DFS was generally 1-2 log, although some higher reductions were also reported [6]. Comparable reductions are often reported for *Salmonella* while inactivation of *L. monocytogenes* is generally lower, typically <1 log [103, 116, 152, 219–222]. Reduced inactivation of *L. monocytogenes* is probably due to their overall high tolerance to acid, high salt, and low a_w environments [223]. In several studies, ingredients or production parameters (i.e., nitrite concentration, fermentation temperature, final pH, degree of drying, and ripening time) have been varied systematically to enhance the safety of DFS [6]. Our group studied the potential for VTEC reductions by combining recipe and process parameters within limits that would give acceptable products of two types of DFS, salami, and Morr [104, 224]. The factorial designed experiments showed that high levels of salt and curing salt (NaCl and $NaNO_2$) and glucose (lower final pH in the sausages) along with fermentation at elevated temperature provided enhanced VTEC reductions. High fat and large casing diameters gave the opposite effect. The importance of a_w for VTEC reductions in DFS was documented. High and optimal fermentation temperature were important to ensure growth and activity of the starter culture, with subsequent lactic acid production, pH drop, moisture loss, and a_w reduction over time. In line with other studies, approximately 3-log reductions were obtained compared to 1.5-log reductions for standard recipe DFS [104]. Higher reductions have been reported but seem difficult to obtain within levels relevant to producing high quality DFS [6].

A meta-analysis of 44 separate studies investigated the relative effects of temperature, pH, and a_w on the survival of *E. coli* during manufacture of fermented meats. The study indicated that temperature (fermentation, maturation, and storage) accounted for 61% of the variability in the data while pH and a_w accounted for less than 8% [225]. Similarly, in a meta-analysis including 13 studies on inactivation of *L. monocytogenes* in fermented sausages, temperature explained 60% of the data variability while pH and a_w explained only a small part [226].

The above studies show that elevated temperatures in the range 25–47°C, although not lethal to *E. coli* and *L. monocytogenes* per se, would be effective for pathogen inactivation in the processing of DFS under conditions where the bacteria are unable to grow. Increased inactivation of relevant pathogens, including VTEC, *L. monocytogenes*, and *Salmonella*, with increasing temperatures has been shown in several studies [102, 103, 222, 225–227]. For effective inactivation of pathogens it is crucial to obtain conditions preventing pathogen growth (low pH, a_w), but once these conditions have been reached, it is the factors of time and temperature that most dramatically improve the microbial safety of the product. Overall, optimal combinations of hurdles and control strategies during DFS processing could enhance the safety of DFS but finished products could still

contain surviving pathogens. No single parameter appears to enhance VTEC reduction enough to entirely eliminate pathogens. Consequently, application of several measures to reduce risk should be taken.

Changes in recipe or process parameters do not necessarily lead to enhanced reduction of pathogens. For example, when semidry reduced fat (20% less than control) Italian salami was spiked with *E. coli*, *S.* Typhimurium, and *L. monocytogenes*, the reductions during manufacturing were similar to those of other typical Italian salami [228].

Application of novel technologies combined with traditional hurdles (e.g., low pH, a_w, and temperature) in the production process of DFS also presents an interesting venue for enhancing the quality and safety of fermented meat products [212]. For optimal combinations of control strategies, it is important to consider bacterial stress tolerance and cross-protection scenarios ranging from possible antagonistic to additive to synergistic effects that can be obtained by combining different treatments and hurdles; see, for example, Gayán et al. [229].

Overall, optimal combinations of hurdles and control strategies during DFS processing could enhance the safety of DFS; however finished products may still contain surviving pathogens.

6.3. Importance of Starter Cultures for Safety. The importance of using starter cultures for effective reduction and inactivation of pathogens of *E. coli*, *Salmonella*, and *Listeria* in DFS is well documented [7, 64, 105, 230]. Different starter cultures may vary in their abilities to reduce these pathogens [64, 153, 231, 232]. Combinations of starters may give increased reduction in *E. coli* during sausage production [233, 234]. The performance of *Lactobacillus sakei* in sausage fermentation was shown to be improved by heat, cold, and salt stress prior to inoculation [235]. Selection criteria for lactic acid bacteria used as starter cultures in fermented sausage were reviewed by Ammor and Mayo [236]. The growing interest in artisanal products of fermented sausages has also identified a need for the isolation and use of appropriate starter cultures that could provide increased food safety and maintain the characteristics of such products. These sausages are often produced following traditional practice in small processing units, with no use of starter cultures and less control of temperature and humidity during fermentation and ripening compared to industrial production [237, 238].

The main preservative effect of starter cultures for fermented sausages is production of organic acids, mainly lactic acid, by LAB [239]. It has long been recognized that LAB may produce additional antimicrobial compounds [240, 241]. Of these, the bacteriocins have received the most attention. Bacteriocins are antibacterial peptides or proteins that kill or inhibit the growth of closely related bacteria. For many LAB bacteriocins, the inhibitory spectrum includes only other LAB likely to be present in the same ecological niche, thus giving the bacteriocin producer a competitive advantage [242–244]. However, some LAB bacteriocins have a somewhat larger spectrum of inhibition and may be active towards a broader panel of Gram-positive bacteria, including foodborne pathogens, such as *L. monocytogenes*, *Bacillus cereus*, *S. aureus*, and different clostridia. The use of bacteriocin-producing LAB as starters for fermented sausages therefore shows potential for natural enhanced safety of these products [64, 87, 242]. The so-called class IIa bacteriocins, sometimes referred to as "pediocin-like" (after the first discovery of this class, pediocin PA-1), are particularly potent against *Listeria* species, including *L. monocytogenes* [245]. Class IIa bacteriocins are relatively small amphiphilic peptides of 3.5–5 kDa and the mode of action is permeabilization of the cell membrane of susceptible cells, mediated via a membrane-located receptor protein [246]. Production of class IIa bacteriocins is a relatively common trait among LAB species relevant for fermented sausages, that is, *L. curvatus* and *L. sakei* [245, 247–251]. Bacteriocinogenic strains of these species have therefore been tested as starter cultures in several fermented sausage experiments and their antilisterial effect has been evaluated [239, 252–259]. Generally, bacteriocinogenic *L. curvatus* and *L. sakei* starters could reduce the *L. monocytogenes* numbers to some degree in the finished product compared to controls with nonbacteriocinogenic cultures. However, the effect varied between barely significant to a 2-log cfu/g reduction, depending on strain and recipe. This rather modest effect compared to the promising inhibitory potential as measured in in vitro experiments can be explained by interaction of the bacteriocin with the sausage matrix, for example, fat adsorption or proteolytic degradation [260]. Moreover, the potential for bacteriocin production by the producer strain may be inhibited to some degree in the sausage environment [64]. The most common LAB bacteriocins used for sausages, such as those of class IIa, also have some general drawbacks. They have no activity whatsoever on some of the main pathogens relevant for the product, *Salmonella* and EHEC [239]. In addition, *L. monocytogenes* strains may develop resistance to some bacteriocins, especially class IIa, at relatively high frequencies in vitro [241]. Whether this occurs in a food product is currently unclear. In conclusion, bacteriocin-producing starters may enhance food safety to some degree but can never replace good manufacturing practices [64].

6.4. Preservatives for Enhanced Safety. The addition of various compounds with antibacterial effects has been evaluated as ingredients in DFS for improved safety. Microencapsulated allyl isothiocyanate (AIT) at 500 ppm gave 4.75-log reductions of *E. coli* O157:H7 in DFS 28 days after processing, >3 log more than control DFS [261]. Deodorized mustard powder, containing AIT as an antimicrobial ingredient, provided 5-log reduction of *E. coli* O157:H7 28 days after processing when used at 4% in DFS [262–266]. However, mustard levels needed to cause the required inhibition of *E. coli* O157:H7 reduced consumer acceptability of the sausages [265]. Other ingredients tested include the use of lactoferrin [234] and diacetyl [267]. The former was shown to provide mainly nonlethal injury of *E. coli* O157:H7, while an extra 1-log reduction was obtained by addition of 300 ppm diacetyl to the sausage batter. The antibacterial activity of essential oils from herbs and spices were recently demonstrated in DFS. At concentrations of 0.005% and 0.05%, decreases of *Salmonella* and *L. monocytogenes* were >2 log and significantly higher than in control sausages. However, the sensory impact of

essential oils is a factor limiting their application in DFS [268].

6.5. Postprocessing Treatments. Storage of DFS at elevated temperatures (≥20–25°C), short-term heat treatments, and freezing/thawing regimes are the most widely applied postprocess measures. In the review of Holck et al., reductions rates of *E. coli* O157:H7 showed large variations but generally increased with lower pH, lower a_w, and higher storage temperatures [6]. Storage at low temperatures (4°C) for up to two months usually gives marginal reductions [105, 227], whereas storage at 20–25°C may result in considerable reductions.

Including a storage step at ambient temperatures in addition to the production process itself may not be enough to achieve the 5-log reduction required in some countries. Heat treatments may be effective to reduce the numbers of pathogens in sausages, also taking into account the fact that *E. coli* O157:H7 show reduced tolerance to heat in low pH meat products compared to higher pH meat products [269–271]. Total reductions of >5 log were obtained for several combinations of products and storage/mild heat treatment regimes. More recent data from our group have shown heat treatments of 43°C, 24 h to provide >5-log total reductions for 11 *E. coli* strains including different VTEC serotypes. Similar reductions were obtained by freezing at −20°C for 24 h combined with 1 month of storage at 20°C [272]. Higher resistance to heat has been observed for *L. monocytogenes* compared to *E. coli* and *Salmonella* in DFS [221]. Others have reported that heat treatments providing >5-log reductions of *E. coli* in Lebanon Bologna were sufficient for similar reductions of *L. monocytogenes* [273]. The studies illustrate that inactivation of *L. monocytogenes* is dependent on the same parameters as inactivation of *E. coli* and *Salmonella* but that lethal effects on pathogens are product dependent.

Different freezing/thawing and storage/mild heat treatment regimes of DFS showed negligible sensory effects on treated DFS [274]. Other studies have reported variable quality and sensory effects, ranging from unacceptable to improved sensory scores, due to heat treatments at higher temperatures (≥approx. 50°C) [105, 221, 275, 276]. Combinations of high temperature and reduced treatment times may be regarded as most feasible in industrial production. Optimal treatment regimes are likely to differ between products with different characteristics [102, 221, 273, 274, 276].

High pressure processing (HPP) has been employed in many areas of food production [277]. In DFS products, HPP has potential for postprocess reduction or elimination of *L. monocytogenes* in the final products in compliance with the requirements (9 CFR part 430; the *Listeria* Rule) for *L. monocytogenes* control of such RTE products, as issued by FSIS. HPP is recognized by the FDA as a method for achieving the 5-log VTEC reduction in DFS processing that are required in USA [278] and Canada [279]. DFS products having a texture that is less susceptible to changes during HPP compared to raw meat products are suitable for HPP. The DFS color is barely affected even at very high pressure levels, and the in-package pasteurization by pressure is an advantage as possible recontamination is avoided. As a postprocessing method, it also has the advantage that it can be performed at low temperatures. Several consumer trials have revealed that the sensory quality of HPP treated RTE products is maintained after a storage period [280–282]. However, there can be some differences between HPP treated and nontreated DFS during the storage period. Raw meat ingredients are less suited for HPP treatments. Omer et al. found that the organoleptic properties of DFS made from HPP treated meat trimmings changed substantially and were less favored after 2 weeks of storage compared with the nontreated ones [215]. When frozen raw materials were used, the sensory differences between treated and nontreated samples were reduced.

Very high pressure levels, up to 600 MPa, are often used for DFS. Several studies have shown high initial reductions of microorganisms after HPP [280, 282]. Gill and Ramaswamy showed that the *E. coli* O157 numbers were reduced by greater than 4-log cfu/g by HPP (600 MPa, 3 min) and remained static after processing in Hungarian salami but increased in All Beef salami during storage at 15°C [280]. They also showed that increasing the holding time to up to 9 min did not give additional reductions. In a HPP study of Norwegian type DFS, treatment at 600 MPa for 10 min gave reductions of 2.9-log cfu/g of *E. coli* O103:H25, and treatment in cycles (600 MPa for 200 s, 3 cycles) gave a somewhat higher reduction of 3.3-log cfu/g [282]. The same study showed that elevated levels of dextrose, NaCl, and nitrite gave lower reduction (2.7-log cfu/g) compared with the standard recipe. Porto-Fett et al. tested treatments of DFS added pathogens with several pressure levels between 483 and 600 MPa for 1–12 min [220]. The reduction varied from 1.6 to 5.8-log cfu/g depending on pressure conditions and bacteria (*Listeria*, *E. coli*, and *Salmonella*). During storage, additional reductions were observed for all bacteria tested.

Differences in pathogen reductions obtained in the various studies of pressurizing DFS can be related to variation in the recipe, fermentation regime, and water activity level. The production process of DFS is shown to give a reduction of about 2-log cfu/g of VTEC [282]. With the additional reduction of 3-log cfu/g due to HPP, this will provide the desired 5-log reduction that is often required.

7. Mathematical Models for Predicting Survival of Pathogens in DFS

Predictive modeling has developed as an adjunct to traditional microbiological techniques. Essentially, the survival and/or growth of an organism of concern may be predicted on the basis of a mathematical relationship between microbial growth rate and environmental conditions [283]. A large number of mathematical models to predict the population kinetics of *E. coli* and other bacteria in foods are publically available, such as the ComBase Predictor (CP) [284], the Pathogen Modelling Program (PMP) [285], and Meat and Livestock Australia (MLA) *E. coli* inactivation model in fermented meat [286]. These models have limitations as they primarily focus on the static effect of a_w, $NaNO_2$, pH, and temperature. The MLA model considers dynamic changes, however only those related to temperature in the sausage environment during production [225, 286]. A simpler version of the MLA model calculates

the reduction of *E. coli* as a function of temperature and time during fermentation and maturation, available at http://www.foodsafetycentre.com.au/fermenter.php. Specifically, the inactivation of *E. coli* O157:H7 has been modeled as a function of pH and a_w in Soudjouk-style fermented sausages during the process of fermentation and drying, available at https://pmp.errc.ars.usda.gov/PMPOnline.aspx [222]. The software THERM predicts growth of *E. coli* O157:H7, *Salmonella*, and *S. aureus* as a function of the time-temperature history of raw meat products [287].

A dynamic model to predict VTEC concentration throughout manufacturing and storage of fermented raw meat sausages has been developed by Quinto et al. [288]. The model is implemented in a tool called *E. coli* SafeFerment (EcSF), available at http://www.ifr.ac.uk/safety/EcoliSafeFerment. EcSF integrates growth, probability of growth, and thermal and nonthermal inactivation models to give the predictions of VTEC concentration under constant or fluctuating environmental conditions. The tool can be applied for the evaluation of the impact of modifications, interventions, or unexpected events during the manufacturing process and/or storage period on VTEC survival. Recently, Gunvig et al. developed three models for predicting survival of VTEC, *L. monocytogenes* and *Salmonella*, taking into account the dynamics of the sausage environment and maturation of fermented sausages [289]. Based on challenge experiments under production conditions of dried and semidried sausages, the models covered dynamic changes related to various pH decreases, weight losses during maturation, $NaNO_2$ concentrations, and a_w. Their "ConFerm" tool is available in a user-friendly interface at http://dmripredict.dk. Predictive models can be useful for estimating pathogen reduction, however, for processes within the ranges of the variables used for the development of the specific model. They also need to be interpreted with caution due to their wide confidence intervals of the fitted equations, which corresponds to an uncertainty in predictions.

8. Concluding Remarks

Fermented meats are unique products often with elements of culinary heritage and identity. The preservation role of the nutritious meat has become largely obsolete after the introduction of the cold chain. Yet, fermented sausages remain very popular and are produced in large amounts in an immense variety. Fermented sausages comprise a relatively small fraction of the total meat consumption. For example, in Germany the annual per capita consumption of fermented sausages has been estimated to 4.5 kg, which is 7% of the total meat consumption [2]. Due to their high fat, salt, nitrite, and smoke content, health considerations are still relevant. We have discussed several health and microbiological issues related to consumption of fermented sausages. Additional information may be found in the book *Fermented Meat Products: Health Aspects*, which considers the safety of fermented meat products through a whole food chain approach [290].

A topic not covered in the present review is the suspected connection between meat in itself and cancer. A working group of the IARC recently classified processed meat as "carcinogenic to humans" and red meat as "probably carcinogenic to humans" for colorectal cancer, appealing to critically consider the future role of meat in a healthy diet. Considerations around meat and cancer and possible mitigation strategies have been summarized previously [291]. Groups of consumers claim personal health motives for reducing or banning the consumption of meat [292]. A response to negative perception related to meat products embraces an innovation agenda [293]. However, the borderline between innovation and tradition appears complex since traditional products tend to be perceived more basic and natural [294]. The benefits and risks associated with red and processed meat consumption should not necessarily cause dilemmas, if these meats are produced to ensure optimal microbial safety and consumed in moderate amounts as part of balanced diets [291].

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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