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Behavior of *Escherichia coli* O157:H7, *Listeria monocytogenes*, and *Salmonella* Typhimurium in teewurst, a raw spreadable sausage

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† Mention of trade names or commercial products in this publication is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture.

Abstract

The fate of *Listeria monocytogenes*, *Salmonella* Typhimurium, or *Escherichia coli* O157:H7 were separately monitored both in and on teewurst, a traditional raw and spreadable sausage of Germanic origin. Multi-strain cocktails of each pathogen (ca. 5.0 log CFU/g) were used to separately inoculate teewurst that was subsequently stored at 1.5, 4, 10, and 21°C. When inoculated into commercially-prepared batter just prior to stuffing, in general, the higher the storage temperature, the greater the lethality. Depending on the storage temperature, pathogen levels in the batter decreased by 2.3 to 3.4, ca. 3.8, and 2.2 to 3.6 log CFU/g for *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes*, respectively, during storage for 30 days. When inoculated onto both the top and bottom faces of sliced commercially-prepared finished product, the results for all four temperatures showed a decrease of 0.9 to 1.4, 1.4 to 1.8, and 2.2 to 3.0 log CFU/g for *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes*, respectively, over the course of 21 days. With the possible exceptions for salt and carbohydrate levels, chemical analyses of teewurst purchased from five commercial manufacturers revealed only subtle differences in proximate composition for this product type. Our data establish that teewurst does not provide a favourable environment for the survival of *E. coli* O157:H7, *S.* Typhimurium, or *L. monocytogenes* inoculated either into or onto the product.

1. Introduction

Teewurst is a traditional sausage of Germanic origin, typically made from pork and beef, that is characterized by a soft spreadable texture. It is usually manufactured by small producers and sold under refrigeration as a raw spreadable meat *(USDA, 1993, USDA, 2005)*. At present, there is a general lack of criteria for both the manufacture and the compositional descriptions for fresh and raw spreadable sausages, including teewurst *(Islam and Jockel, 2005)*. Teewurst is grouped with other meat products such as mettwurst that display a relatively low acid content (e.g., pH 5.3-5.5) and high moisture content (e.g., $a_w \geq 0.95$) (*Brown, 2000)*. Processing and preparation of this product does not typically include any heat treatment or antimicrobial interventions other than the salts, spices, nitrites, and perhaps phenolics contributed by liquid smoke, that are added directly to the batter *(Brown, 2000)*. The teewurst manufactured in the USA is the same as the teewurst produced in Germany, unless it is cooked, as is practiced by some manufacturers. As USA regulations stipulate, true product names and a "safe handling statement" must be accurately affixed to the label to provide consumers with the ability to choose between "teewurst uncooked, cured meat spread" or "cooked teewurst", with the former being "raw".

The association of teewurst with foodborne illnesses in recent years is well documented (*Ammon et al., 1999, Werber et al., 2006*). In Germany, consumption of raw spreadable sausages, including teewurst, was identified as a risk factor for sporadic illnesses associated with Shiga toxin producing *E. coli* (STEC) in persons aged 10 years or older *(Werber et al., 2006)*. Similarly, a large outbreak (28 cases, 3 deaths) of haemolytic uremic syndrome (HUS) caused by a sorbitol-fermenting strain of *E. coli*

O157:H- was associated with consumption of teewurst, a raw pork product, and mortadella, a cooked pork product *(Ammon et al., 1999)*. Although teewurst is intended to be cooked by the consumer, its production includes ingredients such as nitrites that cause the raw sausage to appear as a ready-to-eat (RTE) product; therefore, teewurst is notoriously eaten without proper cooking, either by preference or by perception. In a survey conducted in Germany in 2001 related to knowledge and handling of raw meat, and in particular teewurst, ca. 50% of the 510 participants reported eating teewurst and, somewhat surprisingly, only ca. 36% of them recognised it as a raw meat product *(Bremer et al., 2005)*. Thus, this study was conducted to evaluate the behaviour of *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes* inoculated either into the batter or onto the surface of sliced teewurst that was subsequently stored under aerobic conditions at refrigeration and abuse temperatures. Proximate composition analyses of commercial teewurst produced by five relatively small processors were also conducted to address the potential variety and range of chemical traits, since a standard of identity does not currently exist for this product.

2. Materials and methods

2.1. Bacterial strains

The multi-strain cocktails of *L. monocytogenes* (MFS2, MFS102, MFS104, MFS105, and MFS110), *E. coli* O157:H7 (EC505B, C7927, and SLH21788), and *S.* Typhimurium (H3278, G7601, H3402, H2662, H3380, and G8430) used in this study were confirmed, cultured, combined, and/or maintained as described previously *(Porto-Fett et al., 2008a).*

2.2. Formulation and manufacture of teewurst

The formulation of teewurst batter, as purchased from a local manufacturer (Ernst A. Illg Meats, Inc.; Chalfont, PA), consisted of certified pork trimmings (60 lbs; fat-lean ratio 70%-30%), boneless beef plates (40 lbs; fat-lean ratio 70%-30%), and 3.83 lbs of the following non-meat ingredients: seasoning spices (First Spice Mixing Co., Long Island City, NY), sodium nitrite curing salt, liquid smoke flavoring, paprika, cardamom, and sugar. The manufacturing process for this brand of teewurst is shown in Figure 1. Chubs and slices of this brand of finished teewurst are shown in Figure 2.

2.3. Inoculation of teewurst batter

To simulate contamination at the processing plant, three batches (one batch per trial) of freshly-processed teewurst batter were separately inoculated with ca. 5.2 log CFU/g of each multi-strain pathogen cocktail. After inoculation, the batter was mixed at ambient temperature (22° ± 1°C) using a commercial countertop mixer (Univex SRM12; Salem, NH) for ca. 2 min to ensure for relatively even distribution of the inoculum. The batter was stuffed using a commercial (manual) stuffer (D-73779; Dick, Deizisau, Germany) into commercial 4.5 cm diameter artificial “fibrous” casings (F Plus; Walsroder GMBH, Germany) in portions of ca.

100 100 g. The resulting chubs were stored at 1.5, 4, 10, or 21°C for up to 30 days. In each of the
101 three trials two chubs were sampled at each sampling interval (N = 3 trials; n = 2
102 replicates/chubs per sampling interval per trial). It should be noted that the terms “batter” and
103 “chub” herein refer to teewurst inoculated prior to stuffing.

104 *2.4. Inoculation of the surface of teewurst slices*

105 To simulate post-process contamination in the home or in a food service establishment,
106 three batches (one batch per trial) of freshly-processed teewurst were obtained from our
107 collaborating manufacturer as above. Teewurst was transferred aseptically from the original
108 packages onto sterile styrofoam trays (1012S; Genpak, Glens Falls, NY) and sliced (ca. 20 g
109 each slice, ca. 5 cm diameter) with the aid of an ethanol-sterilized knife. Individual slices were
110 placed onto styrofoam trays (Genpak) and separately inoculated on the top surface of each slice
111 with 50 µl of each multi-strain pathogen cocktail. Cells were then distributed with the aid of a
112 sterile plastic cell spreader (Midsci; St. Louis, MO). The trays containing the inoculated
113 teewurst were placed into a biological safety cabinet and held for ca. 15 min at ambient
114 temperature (22 ± 1°C) to allow for the inocula to better attach to the meat slices. Next, the
115 slices were inverted and the process was repeated on the opposite side. The final concentration
116 of each pathogen was ca. 4.5 log CFU/g. Inoculated slices (one slice per bag) were then placed
117 into sterile polyethylene bags (Ziploc Brand Snack Bags; S.C. Johnson Products, Inc., Racine
118 WI). The bags were stored at 1.5, 4, 10, or 21°C for up to 21 days. In each of the three trials two
119 bags/slices were sampled at each sampling interval (N = 3 trials; n = 2 replicates/slices per
120 sampling interval per trial).

121 *2.5. Microbiological analyses*

Initial and final populations of total plate count (TPC) and total lactic acid bacteria (LAB) were enumerated on slices and in chubs as follows. A total of three slices (ca. 20 g each) or three chubs (ca. 5 g each), from each of the three trials/batches tested, were separately transferred into plastic two-chamber filter stomacher bags (Fisherbrand; Fisher Scientific, Pittsburgh, PA) containing 15 or 45 ml of 0.1% sterile peptone water (Difco, Becton, Dickinson and Co., Sparks, MD), respectively, and stomached for ca. 2 min (Stomacher 400; Seward, Cincinnati, OH). The TPC were enumerated by spread-plating 100 µl of the resulting slurry, with or without prior dilution in sterile peptone water, onto Brain Heart Infusion agar plates (BHI; Difco,) and aerobic incubation at 30°C for 72 h. For enumeration of LAB, appropriate dilutions of the slurry were spread-plated (100 µl) onto deMan Rogosa Sharpe agar (MRS; Difco) and incubated anaerobically at 37°C for 72 h (10.1% carbon dioxide, 4.38% hydrogen and the balance in nitrogen; Bactron IV Anaerobic/Environmental Chamber; Sheldon Manufacturing Inc., Cornelius, OR).

For enumeration of *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes* from teewurst, the inoculated slices and chubs were sampled at periodic intervals and treated as above and appropriate dilutions of the resulting slurry were surface-plated (100 µl) onto Modified Oxford agar for enumeration of *L. monocytogenes* (MOX; Difco), MacConkey sorbitol agar for enumeration of *E. coli* O157:H7 (SMAC; Difco), and xylose lysine tergitol-4 agar for enumeration of *S.* Typhimurium (XLT4; Difco). Typical colonies of each pathogen were counted after aerobic incubation of plates at 37°C for 48 h (MOX) or 24 h (SMAC and XLT4). When pathogen numbers in batter decreased to ≤1.0 log CFU/g by direct plating, their presence or absence were determined by enrichment as described *(Porto-Fett et al., 2008a).*

2.6. Physicochemical analyses

At both the beginning and at the end of storage, control (non-inoculated) teewurst samples [N = 3 trials; n = 3 slices (ca. 20 g each) or n = 3 chubs (ca. 5 g each) per trial] were analyzed for pH and a_w by using a model 6000P pH/temperature electrode and a model 5500 pH meter (Daigger, Vernon Hills, IL) and a water activity meter (Decagon Aqualab Model series 3; Decagon Devices, Pullman, WA), respectively, according to the manufacturer's instructions. For the market basket component of this study, two chubs from each brand were analyzed to determine the proximate composition of the teewurst purchased from five commercial processors as determined by a commercial laboratory using methods approved and described by the Association of Official Analytical Chemists *(McNeal, 1990)*.

2.7. Statistical analyses

Microbial counts were transformed to logarithms before means and standard deviations were computed, and counts were reported in terms of log CFU/g. When bacterial counts in teewurst batter decreased to below the threshold of detection (≤1.0 log CFU/g), a value of 1 was used for positive samples after enrichment for determination of the arithmetic mean. Statistical analyses were performed using the SPSS 12.0 software program for windows *(SPSS Inc., Chicago, IL)*. For each contamination scenario and pathogen an analysis of variance (ANOVA) was performed to evaluate the effect of storage time and temperature on pathogen viability. Differences in the proximate composition of teewurst manufactured by different processors were also evaluated using ANOVA. Least squares means separation was performed using the Tukey procedure at a significance level of $p < 0.05$.

3. Results

3.1. Microbiological analyses of teewurst

Direct plating of samples of control (non-inoculated) teewurst slices or control batter/chubs taken from each of the three trials/batches tested revealed the absence (≤0.2 and ≤1.0 log CFU/g for teewurst slices and batter/chubs, respectively) of *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes* (data not shown). These samples also tested negative for each of these same three pathogens following enrichment. The average initial TPC and LAB levels were 7.2 ± 0.7 and 5.7 ± 0.9 log CFU/g, respectively, for teewurst batter, whereas for teewurst slices the average initial TPC and LAB levels were 6.5 ± 0.7 and 5.5 ± 0.9 log CFU/g, respectively (Table 1). Average initial values of pH were 5.87 ± 0.25 and 6.18 ± 0.19 for teewurst batter and teewurst slices, respectively, while thereafter the pH decreased somewhat to about pH 4.39 and 4.78, respectively, at the end of storage for both batter and slices. Average inital a_w values were about 0.960 (SD ≤0.005) for both slices and batter, and a_w changed relatively little over the storage period. For both slices and batter, numbers of TPC and LAB were very similar at the end of the respective storage period (Table 1).

3.2. Viability of E. coli *O157:H7,* S. *Typhimurium, and* L. monocytogenes *inoculated into teewurst batter or onto the surface of teewurst slices*

Regardless of the storage temperature, numbers of all three pathogens inoculated into the batter decreased after 30 days of storage (Table 2). With the exception of storage at 21°C which generated the greatest overall lethality, the observed reductions were not appreciably different for the other temperatures tested. More specifically, when chubs inoculated with *E. coli* O157:H7 prior to stuffing were subsequently stored at 1.5, 4, and

10°C pathogen numbers decreased by 2.3, 3.2, and 3.0 log CFU/g, respectively, after 30 days of storage. When chubs inoculated with *L. monocytogenes* prior to stuffing were stored at 1.5, 4, and 10°C, pathogen numbers decreased by 2.2, 2.6, and 2.6 log CFU/g, respectively, after 30 days of storage. *E. coli* O157:H7 and *L. monocytogenes* levels decreased to below the level of detection by both direct plating (≦1.0 log CFU/g) and enrichment after 25 and 18 days of storage at 21°C, respectively. *S.* Typhimurium levels decreased below detectable levels by direct plating within 15, 18, and 11 days at 1.5, 4, and 10˚C, respectively. The absence of *S.* Typhimurium was confirmed by the inability to recover cells of this pathogen even by enrichment after 30 days at 1.5 and 4°C, after 21 days at 10°C, and after 11 days at 21°C. In general, *S.* Typhimurium was inactivated at a greater rate and to a greater extent (absent by enrichment within 11 days at 21°C) than *E. coli* O157:H7 or *L. monocytogenes* when inoculated into batter (Table 2).

Regarding survival on teewurst slices, pathogen numbers remained relatively unchanged after four days of storage for all temperatures tested (Table 3). Storage at 1.5, 4, 10, and 21°C for up to 21 days resulted in reductions of *E. coli* O157:H7 and *S.* Typhimurium from ca. 4.8 log CFU/g to 3.7, 3.7, 3.9, and 3.4 log CFU/g and from ca. 4.3 log CFU/g to 2.5, 2.8, 2.9, and 2.7 log CFU/g, respectively. When slices were inoculated with *L. monocytogenes* and stored at 1.5, 4, 10, and 21°C for up to 21 days, pathogen numbers decreased from ca. 4.5 log CFU/g to 1.8, 2.3, 1.8, and 1.5 log CFU/g, respectively. In general, *L. monocytogenes* was inactivated at a greater rate and to a greater extent than *S.* Typhimurium and *E. coli* O157:H7 at all temperatures tested. Moreover, the decrease in levels of *E. coli* O157:H7, *S.* Typhimurium, or *L. monocytogenes* when inoculated onto slices of teewurst was not appreciably affected by

the storage temperature (Table 3), that being, similar reductions in pathogen levels were observed at all temperatures tested for a given pathogen.

3.2. Proximate composition analyses

With possible exceptions of the carbohydrate levels that were not statistically ($p \geq 0.05$) different among the five brands and the salt level for brand A that was significantly ($p \leq 0.05$) lower compared to the others four brands, chemical analyses revealed only subtle differences ($p \leq 0.05$) for a given chemical trait among the five commercial brands tested. These findings establish that teewurst displays a range of compositional compounds and characteristics (Table 4).

3.3. Market basket survey

As a final component of this study, we conducted a market basket survey of commercially available teewurst. With reference to USDA/FSIS directive 7235.1 *(USDA, 1994)* for raw or partially cooked meat and poultry products, the labels from four of the five brands tested herein declared teewurst as an uncooked product and/or provided safe handling instructions, that being “Keep refrigerated” and/or “Cook thoroughly” (Table 5). A lack of uniformity in the listed ingredients and additives used by these five processors was also observed and subsequently confirmed by proximate composition analyses (Tables 4 and 5). Proximate composition analyses also revealed that teewurst in general has relatively low nitrite and salt levels and a relatively high moisture and high fat content, characteristics that typically do not provide a sufficient barrier to microbial persistence in such products.

4. Discussion

Teewurst is a very popular traditional/ethnic sausage, typically consumed raw, that remains in demand, albeit in the face of generally declining sales (Ernst K. Illg, personal communication). It is produced by a limited number of small plants that are located primarily in the northeast and upper midwest regions of the USA. From a public health perspective spreadable sausages such as teewurst are considered to be higher-risk products, presumably because consumers are not aware of the safe-handling requirements for teewurst as a product that may contain raw meat *(Bremer et al., 2005)* and/or due to their preference to consume it "as is". In recent years, consumption of teewurst has caused human illnesses due to its contamination with *E. coli* O157:H7 and *L. monocytogenes* and, therefore, such products may potentially be a vehicle for harborage and/or transmission of foodborne pathogens *(Brown, 2000; FAO, 2004; Goulet et al., 2002; Pichner et al., 2004; Timm et al., 1999).* If opened/sampled, teewurst has a refrigerated shelflife of ca. 1 *(Campbell-Platt, 1995; Ockerman and Basu, 2007)* to 5 days *(Brown, 2000)*, whereas if left unopened the shelf life could extend for up to 7 to 21 days at 4°C (Ernst K. Illg, personal communication). In the present study, however, visible mold-like spoilage was evident on teewurst slices within 21 days of refrigerated storage (1.5 and 4°C) or within 5 days of storage at abuse temperatures (10 and 21°C). Regardless, pathogen levels decreased during storage; however, in the event of post-process contamination with relatively high levels of these pathogens, as seen for other meat products, teewurst could possibly expose some consumers to a heath risk (*Gounadaki et al., 2007; Matargas et al., 20008; Yang et al., 2006*).

Levels of *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes* decreased appreciably in teewurst chubs during storage for 30 days (Table 2). Greater reductions in pathogens numbers were observed at 10 and 21°C as compared to 1.5 and 4°C. However, at the end of storage, with the exception of *S.* Typhimurium for which the most significant lethality was observed, surviving numbers of each pathogen were of similar levels. Moreover, in agreement with related studies conducted on salami and soudjouk (*Nightingale et al., 2006; Porto-Fett et al., 2008b*), *S.* Typhimurium inoculated into teewurst batter/chubs was less viable than *L. monocytogenes* and *E. coli* O157:H7. Lethality may be attributed to the presence of native LAB in addition to antimicrobial ingredients such as nitrites, since according to Rödel et al. *(Rodel and Scheuer, 2006)* inhibition of *E. coli* in short fermented raw spreadable sausages was enhanced due to the acidification of the product by LAB and ensuing reduction of $a_{w,}$ whereas the presence of sodium nitrite had only a weak effect. Similar findings were reported by Birzele et al. *(Birzele et al., 2005)*, who found that nitrite at levels of 0.5 or 0.9% incorporated into fresh spreadable ham and onion sausage inhibited growth of *Salmonella* Enteritidis, *E. coli*, and *Staphylococcus aureus*, as well as partially inhibited *L. monocytogenes*. The proliferation and metabolic activity of LAB are known to inhibit undesirable bacteria, mainly through the production of lactic acid and the subsequent pH reduction of foods, but also by the production of CO_2, hydrogen peroxide, ethanol, diacetyl, and/or bacteriocins *(Hugas, 1998)*. The batch-to-batch levels and diversity of LAB naturally present in raw meat and associated microbial interactions (i.e. chemical changes in product) could possibly explain the observed variability in lethality for each pathogen

among trials and between chubs and slices (*Comi et al., 2005; Kaya et al., 2004; Skandamis et al., 2007*).

The findings of the present study suggest that inclusion of a defined lactic starter culture(s) and perhaps a limited/controlled fermentation during manufacture would improve the reproducibility from batch-to-batch and enhance both the quality and safety of the finished product *(Calicioglu et al., 2001; Lucke, 2000).* As previously reported, fermentation of a German-style uncooked sausage (24°C/24 h) followed by smoking (22°C/20 h) resulted in a 2.0- to 3.0-log reduction of *L. monocytogenes (Farber et al., 1993).* In fact, fermentation of some spreadable raw sausages in Germany constitutes a critical element of the manufacturing process so as to insure that the final product is characterised by an appropriate flavour, colour, texture, and acidification level (≤pH 5.6; D-lactic acid ≥ 0.2 g/100 g; *Islam and Jockel, 2005*). In the case of teewurst, however, the addition of a starter culture and the ensuing production of organic acid(s) and other compounds could possibly have an untoward effect on product taste, that being too sour, and on product texture, that being too firm and, as such, less spreadable (Ernst K. Illg, personal communication, 2008). Thus, it may be prudent to consider adding food grade chemicals as an ingredient to further enhance the wholesomeness of teewurst. In fact, in prefatory studies we observed an immediate decrease of ca. 1.6 log CFU/g of *L. monocytogenes* in the presence of 5.5 ppm of nisin added directly to the teewurst batter; however, no further decrease in pathogen levels was observed during storage at 4 or 10°C over 10 days of storage (data not shown). Regardless, the need for a more precise standard of identity was evident from the differences among brands in the various physicochemical traits measured, as well as from differences in the information included

299 on product labels (Table 4 and 5). In the absence of any readily accessible and/or
300 published information, the data in Tables 4 and 5 may serve as a starting point for
301 assisting in the development of a list of ingredients and range of attendant concentrations
302 for defining a standard of identity for teewurst.

303 To our knowledge, there is limited scientific literature on the fate of *E. coli*
304 O157:H7, *S.* Typhimurium, and *L. monocytogenes* either "on" or "in" teewurst. This
305 study provides valuable information to small and very small plants producing teewurst
306 and to regulatory authorities overseeing its production for assessing product safety from
307 these foodborne pathogens. The need to establish both a standard of identity and
308 guidelines for its manufacture are critical given that teewurst is typically a raw rather than
309 RTE product, as well as given that despite labeling instructions to the contrary, this
310 product is commonly/openly ingested as raw without cooking. The data herein also
311 highlight the need to educate both producers and consumers as to the appropriate manner
312 to produce/handle and store teewurst so as not to introduce pathogens at any point from
313 production through to consumption.

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Figure legends.

Figure 1. Flow diagram describing the teewurst manufacturing process used in this study.

Figure 2. Teewurst, a raw spreadable sausage.

Figure 1.

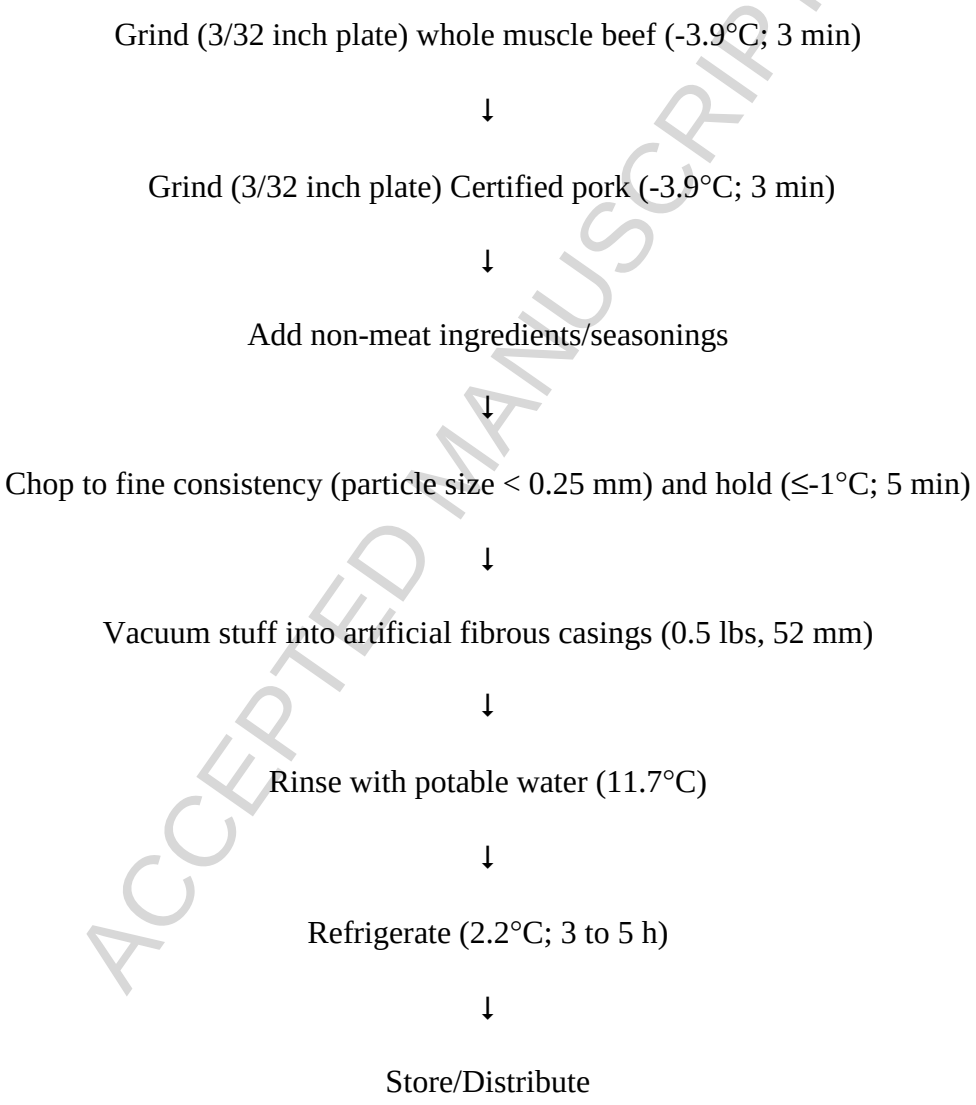

Figure 2.

Table 1. Evaluation of native biota, pH, and a_w of non-inoculated teewurst before and after storage.

Product Type	Storage time (days)	Temperature (°C)	TPC	LAB	pH	a_w
Teewurst chubs	0		7.2 ± 0.7[a]	5.7 ± 0.9	6.18 ± 0.19	0.957 ± 0.004
	30	1.5	6.5 ± 1.0	7.0 ± 1.5	4.85 ± 0.19	0.955 ± 0.002
		4	6.3 ± 0.7	6.6 ± 1.2	4.58 ± 0.15	0.953 ± 0.002
		10	6.6 ± 0.6	6.8 ± 0.5	4.45 ± 0.15	0.953 ± 0.003
		21	6.7 ± 0.3	6.6 ± 0.5	4.39 ± 0.10	0.945 ± 0.003
Teewurst slices	0		6.5 ± 0.7	5.5 ± 0.9	5.87 ± 0.25	0.960 ± 0.005
	21	1.5	7.0 ± 1.1	6.4 ± 1.2	4.66 ± 0.48	0.958 ± 0.004
		4	7.6 ± 0.7	7.2 ± 0.7	5.13 ± 0.81	0.958 ± 0.003
		10	8.3 ± 0.3	8.0 ± 0.2	5.51 ± 0.40	0.956 ± 0.003
		21	8.1 ± 0.1	8.0 ± 0.1	4.78 ± 0.76	0.952 ± 0.002

[a]Values are mean log CFU/g ± standard deviation (N = 3, n = 3).

Table 2. Counts (mean log CFU/g ± standard deviation; n = 6 chubs for each sampling interval) of *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes* inoculated into teewurst batter[a].

Microrganism/ Temperature (°C)	Storage time (days)								
	0	3	8	11	15	18	21	25	30
E. coli O157:H7									
1.5	5.3 ± 0.0^{aA}	4.9 ± 0.3^{abAB}	4.9 ± 0.1^{abA}	4.8 ± 0.1^{abA}	4.3 ± 0.6^{bcA}	3.6 ± 0.6^{cdA}	3.5 ± 0.4^{dA}	3.4 ± 0.5^{dA}	3.0 ± 0.6^{dA}
4	5.3 ± 0.0^{aA}	5.1 ± 0.1^{aAB}	5.0 ± 0.1^{aA}	4.7 ± 0.3^{aA}	4.0 ± 0.3^{bA}	3.7 ± 0.5^{bcA}	3.6 ± 0.3^{bcA}	3.3 ± 0.4^{cAB}	2.1 ± 0.7^{dA}
10	5.3 ± 0.0^{aA}	5.2 ± 0.1^{aA}	4.6 ± 0.4^{aA}	4.5 ± 0.7^{aA}	2.9 ± 0.7^{bB}	2.7 ± 0.4^{bAB}	2.3 ± 1.1^{bAB}	2.5 ± 0.8^{bB}	2.3 ± 1.1^{bA}
21	5.3 ± 0.0^{aA}	4.8 ± 0.3^{abB}	3.5 ± 0.4^{bcB}	2.4 ± 1.1^{cdB}	1.7 ± 0.6^{dC}	2.3 ± 1.4^{cdB}	1.9 ± 0.9^{cdB}	ND[b]	ND
S. Typhimurium									
1.5	4.9 ± 0.1^{aA}	4.2 ± 0.5^{aA}	3.2 ± 1.6^{abA}	2.0 ± 1.5^{bcA}	≤1.0 ± 0.0^{cA}	≤1.0 ± 0.0^{cA}	≤1.0 ± 0.0^{cA}	≤1.0 ± 0.0^{cA}	ND
4	4.9 ± 0.1^{aA}	4.3 ± 0.3^{aA}	3.1 ± 0.7^{bA}	2.5 ± 1.2^{bA}	1.2 ± 0.3^{cA}	≤1.0 ± 0.0^{cA}	≤1.0 ± 0.0^{cA}	≤1.0 ± 0.0^{cA}	ND
10	4.9 ± 0.1^{aA}	3.8 ± 1.0^{bAB}	1.6 ± 0.5^{cB}	≤1.0 ± 0.0^{cA}	≤1.0 ± 0.0^{cA}	≤1.0 ± 0.0^{cA}	ND	ND	ND
21	4.9 ± 0.1aA	2.6 ± 1.6^{bB}	1.1 ± 0.2^{cB}	ND	ND	ND	ND	ND	ND
L. monocytogenes									
1.5	5.4 ± 0.2^{aA}	5.3 ± 0.1^{aA}	4.5 ± 0.9^{abA}	4.2 ± 0.9^{abcA}	4.1 ± 0.8^{bcA}	3.5 ± 1.0^{bcA}	3.8 ± 0.3^{bcA}	3.6 ± 0.6^{bcA}	3.2 ± 0.5^{cA}
4	5.4 ± 0.2^{aA}	5.2 ± 0.1^{abA}	4.4 ± 0.7^{bcA}	4.6 ± 0.1^{bA}	3.7 ± 0.2^{cdAB}	3.5 ± 0.3^{dA}	3.1 ± 0.8^{dA}	2.9 ± 0.2^{dA}	2.8 ± 0.6^{dA}
10	5.4 ± 0.2^{aA}	5.3 ± 0.1^{aA}	3.9 ± 0.4^{bAB}	3.5 ± 0.6^{bcAB}	3.1 ± 0.3^{cdB}	3.4 ± 0.3^{bcdA}	3.1 ± 0.4^{cdA}	3.2 ± 0.4^{bcdA}	2.8 ± 0.6^{dA}
21	5.4 ± 0.2^{aA}	3.9 ± 0.9^{bB}	2.6 ± 1.0^{cB}	2.2 ± 1.4^{cB}	1.8 ± 0.6^{cC}	ND	ND	ND	ND

[a]Means with different lowercase letters within a row are significantly different ($p < 0.05$). Means with different uppercase letters within a column for each organism are significantly different ($p < 0.05$).

[b]ND; not detected by either direct plating or by enrichment.

Table 3. Counts (mean log CFU/g ± standard deviation; n = 6 slices for each sampling interval) of *E. coli* O157:H7, *S.* Typhimurium and *L. monocytogenes* inoculated onto teewurst slices[a].

Organism/ Temperature (°C)	Storage time (days)									
	0	1	2	4	6	8	11	13	18	21
E. coli O157:H7										
1.5	4.8 ± 0.2^{aA}	4.4 ± 0.2^{abA}	4.5 ± 0.2^{abA}	4.5 ± 0.1^{abA}	4.4 ± 0.1^{abA}	4.1 ± 0.3^{abcA}	4.4 ± 0.1^{abcA}	3.9 ± 0.7^{bcAB}	3.7 ± 0.4^{cA}	3.7 ± 0.5^{cA}
4	4.8 ± 0.2^{aA}	4.4 ± 0.3^{abA}	4.5 ± 0.1^{abA}	4.5 ± 0.1^{abA}	4.1 ± 0.2^{bcdA}	4.1 ± 0.2^{bcdA}	4.2 ± 0.3^{bcA}	4.1 ± 0.5^{bcdA}	3.7 ± 0.1^{cdA}	3.7 ± 0.3^{dA}
10	4.8 ± 0.2^{aA}	4.5 ± 0.3^{abA}	4.5 ± 0.2^{abA}	4.4 ± 0.2^{abA}	4.1 ± 0.1^{abA}	4.0 ± 0.3^{bA}	4.2 ± 0.3^{abA}	3.8 ± 0.0^{bAB}	4.5 ± 0.8^{abA}	3.9 ± 0.2^{bA}
21	4.8 ± 0.2^{aA}	4.6 ± 0.1^{abA}	4.6 ± 0.3^{abA}	4.2 ± 0.1^{abcB}	4.1 ± 0.2^{abcA}	3.8 ± 0.5^{bcdA}	3.7 ± 0.4^{bcdA}	3.0 ± 0.2^{dB}	3.4 ± 1.1^{cdA}	3.4 ± 0.5^{cdA}
S. Typhimurium										
1.5	4.3 ± 0.2^{aA}	4.1 ± 0.2^{abAB}	3.7 ± 0.2^{abcA}	3.7 ± 0.2^{abcA}	3.4 ± 0.4^{bcA}	3.2 ± 0.2^{cA}	3.1 ± 0.4^{cdA}	3.1 ± 0.2^{cA}	2.5 ± 0.4^{dA}	2.5 ± 0.5^{dA}
4	4.3 ± 0.2^{aA}	4.1 ± 0.2^{abAB}	4.0 ± 0.2^{abA}	3.6 ± 0.2^{abcA}	3.5 ± 0.2^{bcdA}	3.2 ± 0.1^{cdeA}	2.7 ± 0.3^{eA}	2.5 ± 0.2^{eAB}	2.9 ± 0.5^{deA}	2.8 ± 0.8^{deA}
10	4.3 ± 0.2^{aA}	3.9 ± 0.1^{abcB}	4.0 ± 0.1^{abA}	3.5 ± 0.3^{abcA}	2.9 ± 0.1^{cB}	2.9 ± 0.5^{cA}	3.1 ± 0.4^{bcA}	3.2 ± 0.1^{bcA}	3.1 ± 0.3^{bcA}	2.9 ± 0.6^{cA}
21	4.3 ± 0.2^{aA}	4.2 ± 0.2^{aA}	4.0 ± 0.3^{abA}	3.5 ± 0.1^{abA}	3.5 ± 0.5^{abA}	3.2 ± 0.5^{abcA}	2.6 ± 1.0^{bcA}	2.0 ± 0.6^{cB}	3.0 ± 1.4^{abcA}	2.7 ± 0.8^{bcA}
L. monocytogenes										
1.5	4.5 ± 0.1^{aA}	4.3 ± 0.5^{aA}	4.6 ± 0.2^{aA}	4.3 ± 0.3^{aA}	4.3 ± 0.2^{aA}	4.0 ± 0.8^{abA}	3.1 ± 1.3^{abcA}	$2.5 \pm 1.\ 6^{bcA}$	1.9 ± 1.1^{cA}	1.8 ± 0.8^{cA}
4	4.5 ± 0.1^{aA}	4.3 ± 0.5^{abA}	4.6 ± 0.1^{aA}	3.8 ± 0.8^{abcA}	3.9 ± 0.9^{abcAB}	3.5 ± 1.4^{abcA}	2.7 ± 1.1^{bcA}	2.6 ± 1.3^{bcA}	2.2 ± 1.2^{cA}	2.3 ± 0.9^{cA}
10	4.5 ± 0.1^{aA}	4.4 ± 0.5^{aA}	4.6 ± 0.5^{aA}	3.4 ± 1.2^{abA}	3.1 ± 1.2^{abAB}	3.0 ± 1.3^{abA}	3.7 ± 0.9^{abA}	2.2 ± 1.3^{abA}	2.4 ± 1.9^{abA}	1.8 ± 1.7^{bA}
21	4.5 ± 0.1^{abA}	4.3 ± 0.6^{abcA}	4.8 ± 0.5^{aA}	4.0 ± 0.3^{abcA}	2.3 ± 1.4^{cdB}	2.8 ± 1.0^{bcdA}	3.0 ± 1.7^{abcdA}	2.4 ± 0.1^{cdA}	1.8 ± 1.0^{dA}	1.5 ± 1.1^{dA}

[a]Means with different lowercase letters within a row are significantly different ($p < 0.05$). Means with different uppercase letters within a column for each organism are significantly different ($p < 0.05$).

Table 4. Proximate composition analyses of five brands of commercial teewurst[a].

	Teewurst A[b]	Teewurst B	Teewurst C	Teewurst D	Teewurst E
Phenolics(g/100g)	0.07 ± 0.00$^{ab;c}$	0.07 ± 0.01^{ab}	0.09 ± 0.00^{bc}	0.05 ± 0.00^{a}	0.11 ± 0.01^{c}
Salt (g/100g)	1.26 ± 0.16^{a}	2.10 ± 0.22^{b}	2.12 ± 0.10^{b}	2.16 ± 0.00^{b}	2.34 ± 0.00^{b}
Nitrite (mcg/g)	<1.00^{a}	4.03 ± 0.07^{b}	1.19 ± 0.26^{a}	1.61 ± 0.24^{a}	<0.10^{c}
Moisture (g/100g)	44.35 ± 0.21^{ab}	40.65 ± 0.07^{a}	52.60 ± 0.99^{c}	51.90 ± 0.14^{c}	50.10 ± 3.11^{bc}
Protein (g/100g)	12.35 ± 0.64^{a}	12.95 ± 0.21^{ab}	15.60 ± 0.71^{bc}	15.00 ± 0.28^{abc}	16.20 ± 1.13^{c}
Fat (g/100g)	39.00 ± 0.42^{a}	41.60 ± 1.84^{a}	26.15 ± 0.21^{b}	25.85 ± 0.21^{b}	28.80 ± 0.57^{b}
Acidity[d] (%)	0.35 ± 0.06^{a}	0.40 ± 0.13^{a}	0.67 ± 0.11^{ab}	0.94 ± 0.04^{b}	0.65 ± 0.06^{ab}
CHO[e] (g/100g)	1.77 ± 0.11^{a}	1.27 ± 1.65^{a}	2.75 ± 0.15^{a}	4.17 ± 0.75^{a}	1.70 ± 2.40^{a}
Ash (g/100g)	2.54 ± 0.11^{a}	3.57 ± 0.00^{b}	2.91 ± 0.22^{ac}	3.08 ± 0.11^{bc}	3.38 ± 0.10^{bc}
pH	6.11 ± 0.01^{a}	NT[f]	NT	5.69 ± 0.04^{b}	6.09 ± 0.03^{a}
a_w	0.956 ± 0.004^{a}	NT	NT	0.973 ± 0.001^{b}	0.967 ± 0.001^{b}

[a]Proximate analyses were performed on two samples from each processor (mean values ± standard deviation).
[b]*Teewurst* A (Ernst A. Illg Meats Inc.) product was utilized in all challenge experiments conducted in this study.
[c] Means with different letter within a row are significantly different ($p < 0.05$).
[d]*Acidity* titratable as acetic acid.
[e]CHO; carbohydrates.
[f]NT; not tested.

Table 5. Labeling information from 5 brands of commercial teewurst[a].

Ingredients/Other information	Teewurst A[b]	Teewurst B	Teewurst C	Teewurst D	Teewurst E
Pork	x	x	x	x	x
Beef	x	x	x		
Salt	x	x	x	x	x
Carbohydrates	x		x		x
Spices	x	x		x	x
Paprika	x	x	x	x	x
Oleoresin of paprika				x	
Rum		x		x	
Smoke flavor/natural smoke	x			x	x
Flavoring			x		x
Sodium nitrite	x	x	x	x	x
Sodium erythorbate			x	x	x
Sodium acetate			x		
"Uncooked product"	x	x	x		x
"Cook thoroughly"	x				x
"Keep refrigerated"	x		x	x	x

[a] According to the labeling information declared from processor.

[b] Teewurst A (Ernst A. Illg Meats Inc.) product was utilized in all challenge experiments conducted in this study.