Validation of the QDS Process® of *Pepperoni* sausages through a challenge test with *E. coli* 0157:H7 surrogate

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The consumption of pepperoni worldwide has increased steadily in the last decades, mainly due to the use of this meat product as an ingredient in sandwiches and its widespread use for topping on pizzas (Technomic, 2012). Considered even as an appetizer, its many different recipes always include paprika, a high content of fat and – depending on the producer – a high proportion of spices and herbs. All these factors, together with a deep acid flavour due to the fermentation phase it undergoes before drying, confer to the final product a tasty flavour and juicy texture.

During this drying phase the product hangs for several weeks (depending on the calibre, composition and parameters used) and loses enough quantity of water until a moisture:protein content ratio of no more than 1.6:1 is achieved. This drying phase is the most time consuming process in traditional manufacturing of fermented sausages and many attempts to shorten this process have been made by increasing temperature, reducing thickness or adding specific additives or ingredients. The Quick dry slice (QDS) process® is a technology based on the application of convective air that provides for accelerated drying of slices, immediately after the desired pH have been reached (Comaposada et al., 2004). Slices can be dried to the same level as traditional products in a significantly shorter drying time (30-60 min. vs 1-2 months) (Comaposada et al., 2009).

Food safety of pepperoni sausages

Most of the pepperoni produced in the U.S. and worldwide contains a certain amount of beef meat in its composition, which is a type of meat that has been linked to different *Escherichia coli* 0157:H7 cases (EFSA, 2012) and recalls (http://www.fsis.usda.gov/fsis_Recalls/index.asp). This bacterium is also known as enterohemorrhagic *E. coli*, or EHEC, and can produce the Haemolytic Uremic Syndrome (HUS). For this reason the FSIS (2001) asks for at least 5-log reduction in dry and semi-dry fermented beef products according to its guide (USDA, 2005). The achievement of this bacterial load reduction must be validated performing a Challenge test with the in-plant process.

In the final report on food safety published by the Meat & Livestock Agency of Autralia (Predicting Escherichia coli inactivation in uncooked comminuted meat products) a full literature review was done on the inactivation of Escherichia coli, and it could be concluded that few processes could reliably achieve greater than a 2-log kill without a heating step or extended time or elevated temperature steps. Actually, the different published challenge tests done with E. coli 0157:H7 surrogates indicate that a 5 log reduction can be achieved only when a heat treatment is done at more than 50ºC in the core for not less than 1 hour, with a final pH lower than 4.7 after fermentation (Health Canada, 2000).

The National Advisory Committee on the Microbiological Criteria for Foods (2000) recommends the use of surrogate microorganism in place of target pathogens for in-plant inactivation studies. Commenting on the utility of surrogate microorganisms, the U.S. Food and Drug Administration (FDA) in their Glossary of Kinetics of Microbial Inactivation for Alternative Food Processing Technologies stated that surrogates enable biological verification of a given antimicrobial process or treatment without introducing pathogens into a food (FDA, 2000). The FDA (2000) also states that a microorganism described as a surrogate bacterium must be "a non-pathogenic species and strain responding to a particular treatment in a manner equivalent to a pathogenic species and strain."

Therefore, microbial intervention challenge studies performed with a surrogate bacterium that has not been validated, or one that is demonstrably more sensitive than the pathogen to a particular treatment, may discredit results that are intended to model the reduction of a targeted pathogen.

Escherichia coli 0157:H7 surrogate selection

In order to validate the application of the QDS process® technology in the production of Pepperoni a non-pathogenic surrogate whose behaviour closely resembled that of the pathogen *Escherichia coli* 0157:H7 was selected with the final aim of the validation of the QDS *Pepperoni* manufactured sausages.

Two independent batches of Pepperoni model sausages were manufactured. Each batch was prepared by mixing the following ingredient: Pork lean 90:10, fatty pork meats 50:50 and fatty beef meat 50:50. Salt, dextrose, corn syrup, mustard, paprika, oleoresin of paprika, garlic, sodium erythorbate, tricalcium phosphate, ice water, starter culture (*Pediococcus* spp.) and nitrite were also part of the formula. Meats were ground until the desired particle size and mixed under vacuum with the rest of the ingredients.

Each batch consisted of 3 lots of *Pepperoni* batter. Each one was inoculated under a Biohazard cabin (Telstar BIO-II-A) with a culture of selected *E. coli* strain to reach ca. 10⁷ cfu/g. The strains used belong to different culture collections (CECT Spanish Type Culture Collection, CTC own collection a meat isolate, ATCC American Type Culture Collection): *E. coli* 0157:H7 CECT4267, E.coli 0157:H7 CTC1058, *E. coli* CTC1079 (ATCC25922, CECT434). The inoculated batters were distributed in Petri dishes with lid. One additional Petri dish containing non-inoculated batter for recording pH and temperature evolution during fermentation and cooking process was prepared. All the samples were fermented and cooked in a laboratory incubator (Heraeus) according to a common industrial process, achieving a pH after fermentation lower than 4.8 and with a thermal treatment of 128ºF during 1 hour in the core.

Microbiological determinations and physicochemical analysis

E. coli counts were determined after inoculation (Time 0) in double and after fermentation-cooking process (Time 1) in triplicate. Fifteen grams of meat batter were homogenized (1/10 dilution) with 0.1% Bacto Peptone with 0.85% NaCl in a Masticator Classic (IUL, Barcelona, Spain), serially diluted and poured plated onto chromogenic media (CHROMagarTM 0157). Plates were incubated at 37ºC for 24 to 48 h. Counts were expressed as log cfu/g. The detection limit was 10 cfu/g. Bacterial inactivation was assessed in terms of logarithmic reductions as the difference between counts after the thermal treatment and the initial counts, i.e Log/N/N0).

The pH was measured with a portable pHmeter (Crison pH25) at Time 0 and after fermentation. The water content, fat and protein content of meat batters were determined using Foss Foodscan Near-Infrared Spectrophotometer following the method described by Anderson (2007).

The results obtained in the first part of this study are shown in figures and tables below.

TABLE 1 Chemical composition (%) of meat batters.

	Batch 1	Batch 2
Fat	36.96	31.97
Protein	11.15	11.91
Moisture	45.67	49.25
Collagen	3.20	1.73

▲ Table 1. Chemical composition (%) of meat batters.

▼ Table 2. Evolution of pH during the process.

TABLE 2 Evolution of pH during the process.

	Time 0 (meat batter)	After fermentation
Batch 1 ↑*	5.77	4.68
Batch 2 ↑**	5.76	4.71

↑ non inoculated samples
*10 hours and 15 minutes of fermentation and 4 hours and 45 minutes to reach 54 ºC of core temperature.
**8 hours and 45 minutes of fermentation and 3 hours and 15 minutes to reach 54ºC of core temperature.

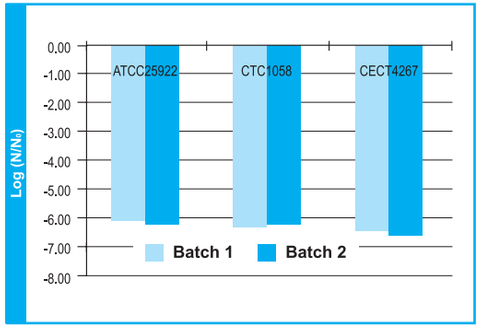
TABLE 3 Counts of *E.coli* in Pepperoni model sausages.

Batch	Time 0* (inoculated batter)	Time 1* (after thermal treatment)
Batch 1 inoculated strains		
ATCC25922/CECT434	7.01±0.09	<1
CTC 1058 (O157:H7)	7.26±0.10	<1
CECT4267 (O157:H7)	7.40±0.18	<1
Batch 2 inoculated strains		
ATCC25922/CECT434	7.13±0.16	<1
CTC 1058 (O157:H7)	7.20±0.04	<1
CECT4267 (O157:H7)	7.47±0.01	<1

▲ **Table 3.** Counts of *E.coli* in *Pepperoni* model sausages.
*Microbial counts are expressed as Log cfu/g ± S.D

The inactivation of *E.coli* strains on *Pepperoni* model sausages achieved after the fermentation-cooking process is shown in Figure 1, in terms of logarithmic viability reduction, Log (N/N_0), with N_0 and N being the initial number of cells after the inoculation and the final number of survivors, respectively. The fermentation-cooking process resulted in 6-Log reductions for *E.coli* ATCC25922 or even more for the *E.coli* 0157:H7 strains used, in both independent assays (batch 1 and batch 2). Therefore, the non-pathogenic *E.coli* ATCC25922/CECT434 was selected as a useful surrogate for *E.coli* 0157:H7 for the QDS process validation of *Pepperoni* sausages.

FIGURE 1 Inactivation of E.coli in Pepperoni model sausages



▲ **Figure 1.** Inactivation of *E.coli* in *Pepperoni* model sausages.

Validation of QDS process of Pepperoni sausages through a challenge test

The manufacturing for *Pepperoni* used in the challenge test was performed by mixing the same ingredients, and in the same proportion, as the ones used for the *E.coli* surrogate selection. The same fabrication process was used.

Inoculation with the non-pathogenic *E.coli* ATCC25922/CECT434 was done during the mixing phase, before the filling into 80-mm diameter collagen casings. All pieces were hung on sticks and transferred to smokehouse. The dry bulb (DB) and wet bulb (WB) temperature within the vapour oven, and one core sausage temperature were recorded every 10 seconds with a Logger 177-T4 instrument. At the end of the cooking process, half of the sausages were vacuum packed in polyamide and polyethylene (PA/PE) bags and stored at 0 ºC for 24 hours until the analyses, while the rest of the sausages were vacuum packed and frozen at -4 ºC for drying by QDS® process.

Drying process

Slices of 7.55 ±0.09 g for QDS process® were obtained from the centre of frozen *Pepperoni* sausages. A total of 3 drying processes were carried out (n=3). Drying time was 34 ±1 min at 25 ºC, 3.5 m/s air speed and 30-33 % of relative humidity until 26.7 ±0.1 % of drying weight loss was reached. The initial temperature of the slices was 1.7 ±0.5 ºC, while at the end of the drying process it was 19 ±1ºC. The average weight of the dried slices was 5.5 ±0.07 g. After drying, the slices were vacuum packed 3 PA/PE bags each sausage and cooled up to 4 ºC until analysis.

Microbiological determinations and physicochemical analysis

Fifteen grams of sausage were homogenized (1/10 dilution) with 0.1% Bacto Peptone with 0.85% NaCl in a Masticator Classic (IUL, Barcelona, Spain), serially diluted and poured plated onto agar Coli ID (BloMérieux). Plates were incubated at 37ºC for 24 to 48 h. Lactic Acid Bacteria counts were also determined in MRS agar (Merck), incubated in anaerobiosis at 37ºC for 48h. Counts were expressed as log cfu/g.
The detection limit was 10 cfu/g. Bacterial inactivation was assessed in terms of logarithmic reductions as the difference between counts after the thermal treatment and the initial counts, i.e Log/N/N_0).
Microbial counts were performed after preparing the meat batter (Time 0), after fermentation-cooking process (Time 1), after QDS drying (Time 2), at 45 d (Time 3), and 90 d (Time 4) of refrigerated storage (4 ºC). At each sampling time 3 different samples were analysed except time 0 (duplicate).

Physicochemical analysis (Anderson, 2007) and pH (Crison GLP 21, Crison Instruments, S.A., Alella, Spain) of the stuffed meat batter were determined. The pH was determined after fermentation and after QDS process with a portable pHmeter 13 (Crison pH25). The water activity (aw) was determined with an Aqualab 3TE device (Decagon Devices).

Results

The results obtained in the challenge test with *E.coli* ATCC25922/CETC434 in *Pepperoni* sausages are shown in tables and figures below.

TABLE 4 Chemical composition (%) of *Pepperoni* sausages at different steps of QDS process.

%	Moisture	Protein	Fat	Collagen	Salt	HU/prot.
Time 0	52.19	14.90	31.06	2.12	-	3.64
Time 1*	50.84±0.74	13.99	27.44±1.61	1.57±0.17	3.79±0.41	3.63
Time 2*	30.74±0.54	20.45±0.08	39.86±0.42	3.31±0.17	5.01±0.06	1.50

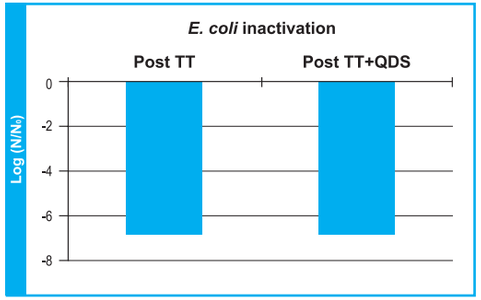
▲ **Table 4.** Chemical composition (%) of *Pepperoni* sausages at different steps of QDS process.
*means ± SD of three samples of each sausage.

TABLE 5 Microbial counts, pH and water activity (Aw) of *Pepperoni* sausages at different steps of QDS process.

Time	*E. coli* *	Lactic acid bacteria *	pH *	Aw *
0 (meat batter)	7.96±0.10	8.05±0.01	-	-
1 (after fermented-cooking process)	<1.00	6.75±0.43	4.61±0.01	0.949±0.002
2 (24h after QDS)	<1.00	6.46±0.19	4.54±0.03	0.895±0.003
3 (45 d of storage at 4ºC)	<1.00	5.71±0.34	-	-
4 (90 d of storage at 4ºC)	<1.00	5.12±0.13	-	-

▲ **Table 5.** Microbial counts, pH and water activity (Aw) of *Pepperoni* sausages at different steps of QDS process.
*Microbial counts are expressed as Log cfu/g ± SD and are the average of duplicate (time 0) or triplicate samples (time 1, 2, 3 and 4).

FIGURE 2 Inactivation of *E.coli* in *Pepperoni* sausages.



▲ Figure 2. Inactivation of *E.coli* in *Pepperoni* sausages.

The inactivation of *E.coli* in *Pepperoni* sausages achieved after the fermentation-cooking process (Post TT) is shown in Figure 2, in terms of logarithmic viability reduction, log (N/N_0), with N_0 and N being the initial number of cells after the inoculation and the final number of survivors, respectively. The fermentation-cooking resulted in 7-Log reduction of *E.coli*. No additional inactivation was observed after the QDS process® (Post TT+QDS). The inactivation achieved was maintained after 90 days of storage at 4ºC, no further recovery of *E.coli* was observed.

CONCLUSION

Inactivation studies may be used to determine whether a particular process provides adequate Log reduction of a target pathogen as defined by regulations or government policy (e.g. FSIS requirement for a 5-Log kill of *E.coli* 0157:H7).

With the results obtained in this study it can be concluded that the inactivation achieved by *E.coli* ATCC25922, previously selected as appropriate surrogate, fulfil the required target reduction pointing out the safety of QDS manufactured *Pepperoni* sausages, according to the FSIS regulations.
As previously stated, these results are consistent with the published studies on *E.coli* 0157:H7 surrogates inactivation, because greater than 6-Log kill is achieved after heat treatment phase of more than 50ºC in the core for not less than 1 hour, combined with a pH of 4.7 or lower after fermentation.

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