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## Research Note

# Combined Steam-Ultrasound Treatment of 2 Seconds Achieves Significant High Aerobic Count and *Enterobacteriaceae* Reduction on Naturally Contaminated Food Boxes, Crates, Conveyor Belts, and Meat Knives

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## ABSTRACT

Food contact surfaces require rigorous sanitation procedures for decontamination, although these methods very often fail to efficiently clean and disinfect surfaces that are visibly contaminated with food residues and possible biofilms. In this study, the results of a short treatment (1 to 2 s) of combined steam (95°C) and ultrasound (SonoSteam) of industrial fish and meat transportation boxes and live-chicken transportation crates naturally contaminated with food and fecal residues were investigated. Aerobic counts of 5.0 to 6.0 log CFU/24 $cm^2$ and an *Enterobacteriaceae* spp. level of 2.0 CFU/24 $cm^2$ were found on the surfaces prior to the treatment. After 1 s of treatment, the aerobic counts were significantly ($P < 0.0001$) reduced, and within 2 s, reductions below the detection limit (<10 CFU) were reached. *Enterobacteriaceae* spp. were reduced to a level below the detection limit with only 1 s of treatment. Two seconds of steam-ultrasound treatment was also applied on two different types of plastic modular conveyor belts with hinge pins and one type of flat flexible rubber belt, all visibly contaminated with food residues. The aerobic counts of 3.0 to 5.0 CFU/50 $cm^2$ were significantly ($P < 0.05$) reduced, while *Enterobacteriaceae* spp. were reduced to a level below the detection limit. Industrial meat knives were contaminated with aerobic counts of 6.0 log CFU/5 $cm^2$ on the handle and 5.2 log CFU/14 $cm^2$ on the steel. The level of *Enterobacteriaceae* spp. contamination was approximately 2.5 log CFU on the handle and steel. Two seconds of steam-ultrasound treatment reduced the aerobic counts and *Enterobacteriaceae* spp. to levels below the detection limit on both handle and steel. This study shows that the steam-ultrasound treatment may be an effective replacement for disinfection processes and that it can be used for continuous disinfection at fast process lines. However, the treatment may not be able to replace efficient cleaning processes used to remove high loads of debris.

The materials found in food processing plants include a comprehensive list of various equipment and items *(24)*. Materials such as conveyor belts, food boxes, crates, and cutting tools are highly subject to contamination due to their frequent use and are important contributors to cross-contamination through direct contact with food products *(3, 11, 13, 15, 18, 27)*. Controlling microbial contamination on such materials often requires different cleaning and disinfection approaches. The generally accepted order of events for cleaning and disinfection is rinse, clean, rinse, and disinfect *(17, 24)*. Cleaning is the removal of food debris by appropriate detergents. Modern cleaning agents for food industries are a mixture of chemicals that target different components in food debris. Examples are alkaline detergents, which are effective against fat and protein-based food residues, while mineral-based residues require acid cleaners *(16, 17, 24)*. An optimized disinfection process has to reduce microorganisms to levels considered safe from a public health of view and to reduce the count of quality-damaging microorganisms *(16, 17)*. General types of disinfection methods may include thermal disinfection, using a hot water or/and steam treatment at a specific treatment time and temperature, or a chemical disinfection with a specific concentration and surface contact time *(2, 7, 9, 16, 17, 20, 28, 30)*. Chlorine is among the most common food industry disinfection agents that target many types of bacteria and molds. These agents are relatively inexpensive and tolerate hard water *(16, 24, 30)*. The downside of these disinfectants is that they can be very corrosive to equipment and may form organochlorine by-products of environmental concern *(24)*. If cleaning procedures are not followed properly, biofilm-producing bacteria may survive and adapt to the environment, which makes them even harder to remove through conventional cleaning methods *(5, 7, 22, 29)*.

Thermal disinfection methods, such as steam processes, are being recognized for their many advantages. Steam reaches difficult areas in production lines, and surfaces are left dry after disinfection. No environmental concerns and no microbial adaptation can be found with steam processes *(2, 8)*. Steam treatments are roughly divided into two

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categories; saturated steam (moist steam) and superheated dry steam. The type of material and the success criteria to be achieved, in regard to microbial reduction and sensory changes, determine what type of steam process to use. Microbial death by these two processes is different. While death by superheated steam is a result of dehydration, death by moist heat is caused by deactivation and coagulation of essential proteins *(2, 14).* The major steam process limitation is the high cost of heating, which is noticeably higher than that of chemical sanitizers. Steam treatments are usually performed at high temperatures or/and long treatment times *(14).* Long treatment times are a result of the boundary sublayer that is around any object and restricts heat transfer. As a consequence, longer treatments are needed to disrupt this layer in order to reach the surface *(1, 6, 10, 21).* This study investigated an environmentally friendly and cost-effective decontamination system that uses a combination of steam and ultrasound (SonoSteam, FORCE Technology, Brøndby, Denmark) for rapid and continuous treatment of different food contact material surfaces *(1, 6, 21).* The ultrasound is able to disrupt the boundary sublayer of air and thereby allow steam to reach the surface much faster. Unlike most steam treatments, the steam-ultrasound treatment can achieve bacterial reductions ranging from significantly large to complete within only 2 s of treatment at 95°C, corresponding to a steam consumption of less than 30 g/s *(12, 21).* The materials investigated were two different industrial meat transportation boxes, one type of live-chicken transportation crate, three different types of conveyor belts, and industrial cutting knives, all precontaminated at industrial food process lines.

## MATERIALS AND METHODS

**Food contact materials.** A contaminated red meat transportation box (reusable, polypropylene, working temperature −30 to +75°C, Færch Plast, Hosltebro, Denmark) and two identical fish transportation boxes (reusable, high-density polypropylene, working temperatures −30 to +75°C; LogiCon Nordic, Kolding, Denmark) were received directly from process lines at Danish meat and fish plants. The boxes were naturally contaminated with fish and meat residues. Two identical crates (reusable, high-density polyethylene; Dow Plastics, Midland, MI) used for transporting live chickens to slaughterhouses were naturally contaminated with fecal contaminants. The crates were received in large black unsterile plastic bags from a chicken slaughterhouse after they were rinsed at the plant. Different types of conveyor belts were also investigated. One type was a structured modular plastic belt with open hinges for drainage (polypropylene 31002, blue 35040, operational temperatures 40 to 105°C; IntraLox LLC, Savage, MD) (Fig. 1A) that was received from a Danish cheese processing plant. A large piece of the conveyor belt was cut off at the end of the line and wrapped in sterile plastic bags. The belt was visibly contaminated with cheese residues. Two different types of conveyor belts were received from a meat processing plant. One type was a flexible plastic modular conveyor belt with a flat surface and closed hinges (polypropylene, Uni MPB C POM-DI white, operational temperatures up to 100°C; Ammeraal Belttech, Vejle, Denmark) (Fig. 1B). A large piece was cut off at the end of the line. The second type was a flat rubber belt (urethane impregnated, E 3/2 U0/U2 hazard analysis and critical control point [HACCP]-FF blue, operational temperatures −30 to 100°C; Forbo Siegling Transilon, Hannover, Germany) (Fig. 1C). This conveyor belt was also cut off at the end of the line. Both conveyor belts were visibly contaminated with meat residues. The belts were wrapped in sterile plastic bags for transportation. Meat cutting knives (Raadvad Professional, Lyngby, Denmark) were received from another Danish meat processing plant. Three knives were received precontaminated with meat residues and wrapped in sterile plastic bags. During transportation to the laboratory, all materials were kept under aseptic and cooled conditions. In all cases, the transportation time was less than 3 h.

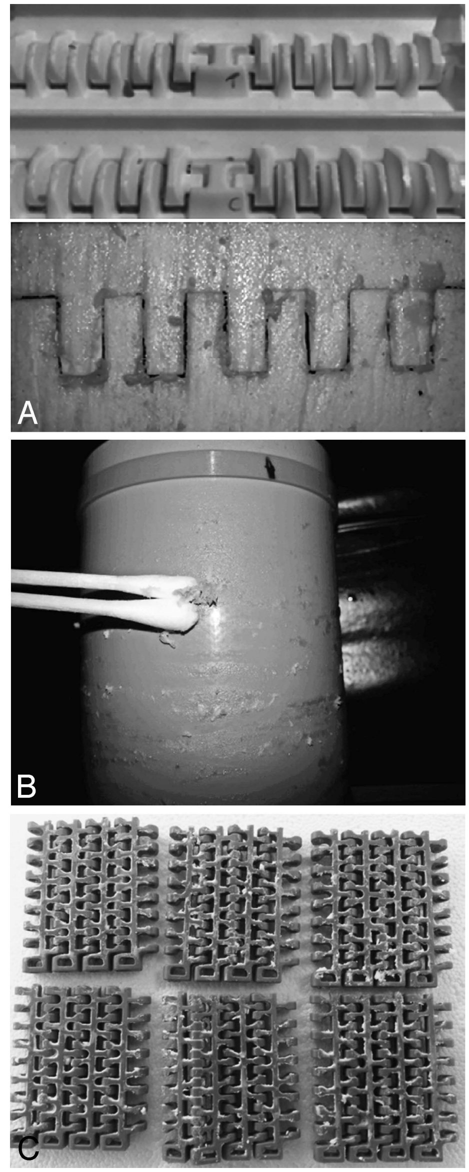


FIGURE 1. *(A) Flat and flexible modular conveyor belt contaminated with meat residues is shown on both sides. (B) Rubber conveyor belt wrapped around a tube. The belt is contaminated with meat residues that were swabbed during sampling. (C) Structured modular conveyor belt contaminated with white cheese residues.*

**Steam-ultrasound treatment.** All materials were treated with pilot-scale steam-ultrasound demonstration equipment at the FORCE Technology laboratories, Brøndby, Denmark. This equipment is built to perform testing of continuous disinfection. Steam-ultrasound (patent EP1381399-B1, March 2001, Niels Krebs) uses only the combination of steam at 95°C at 2 bars and ultrasound at 20 to 40 kHz, both produced simultaneously by specially designed nozzles. The nozzles are optimized to produce a limiting steam consumption of less than 30 g/s. The nozzles are installed inside the treatment chamber of the equipment designed to test various materials. All materials were treated for 1 s, with a maximum of 2 s.

**Sampling.** Due to the small scale of the steam-ultrasound demonstration equipment, transportation boxes were cut into smaller pieces that were used to perform repetitive treatments. The cutting was performed under aseptic conditions using a cleaned multicutter blade for each cut. The bottom of each of the two fish transportation boxes was cut into nine plates. The bottom of the meat transportation box was cut into 3 plates, and the bottom of the live-chicken transportation crate was cut into 12 plates. The rubber conveyor belt was cut into long plates (15 by 10 cm). For sampling on flat surfaces, two cotton swabs were briefly soaked in tryptone salt broth (Oxoid-ThermoFisher, Greve, Denmark) and swabbing was performed on an area of 12 by 2 cm on opposite sides of the plates before and after the treatment. Swabs were transferred back to the tryptone salt broth in a 10-ml tube after sampling. Swabbing on a knife was also performed with two cotton swabs, before and after the treatment, on opposite sides of the knife. The knife handle and knife steel were analyzed separately. In all cases where swabbing was performed, effort was put into scraping the surface for food and fecal contaminants. The 10-ml tryptone salt tubes (Oxoid-ThermoFisher) containing the swabbed samples were used for further dilutions. The tubes were vortexed carefully for 10 s in order to release entrapped bacteria on swabbed particles.

Plastic modular conveyor belts were cut into smaller pieces of 50 $cm^2$ to fit a 400-ml stomacher bag (Oxoid-ThermoFisher). Modular conveyor belts were transferred to a 400-ml stomacher filter bag (Oxoid-ThermoFisher), and 90 ml of buffered peptone water (BPW; Oxoid-ThermoFisher) was poured inside. The bag was blended and macerated by hand for 60 s. From this bag, 1 ml was directly plated on to Petrifilm plates and 1 ml was used to prepare 10-fold dilutions.

**Microbial analysis.** Aerobic counts were analyzed on aerobic Petrifilm plates, and *Enterobacteriaceae* spp. were analyzed on *Enterobacteriaceae* count Petrifilm plates (3M, Copenhagen, Denmark). Plates for aerobic counts were incubated at 30°C for 72 h. Plates for *Enterobacteriaceae* spp. were incubated at 30°C for 24 h.

**Statistical analysis.** All bacterial counts were transformed to log values. The log values were calculated as the number of bacteria recovered from all swabbed areas or rinsed areas ($cm^2$). The mean log values ± standard deviations were determined for repetitive samples. Statistical analysis was performed by using Prism software (version 5, GraphPad Software, Inc., La Jolla, CA). Statistical analysis was performed using a paired $t$ test. For $P$ values of $<0.05$, the data sets compared are considered significantly different within a 95% confidence interval.

## RESULTS AND DISCUSSION

**Steam-ultrasound treatment of transportation boxes and crates.** The optimal steam process is chosen depending on the material applied. Examples are molded polystyrene cups with foil lids that are treated continuously with saturated steam at 165°C and 6 bars at conventional industrial line speeds (within seconds) to achieve complete bacterial reduction (sterilization). The external surface of the cup is cooled to avoid thermal changes *(14, 23)*. Polypropylene cups (higher thermal resistance) can be treated at lower temperatures (140 to 147°C) and longer times of 4 to 6 s. The Martin-Doyle process continuously sterilizes tinplate cans by passage through superheated steams with temperatures of 220 to 256°C at normal pressure for 45 s *(14)*. High temperatures and/or long treatment times are necessary in order to penetrate the boundary sublayer and access the surface *(10, 21)*.

Steam-ultrasound treatment was applied on transportation boxes and crates made from polypropylene and polyethylene. Boxes used to transport red meat and fish products within the plant and chicken transportation crates for use outside the plant were contaminated with levels of 5 to 6 log CFU/24 $cm^2$ (Table 1). With 1 s of steam-ultrasound treatment (2 bars and 30 to 40 kHz) at 95°C, the aerobic counts on the fish transportation boxes were significantly ($P < 0.0001$) reduced, to 2.16 log CFU/24 $cm^2$. The fish residues on these boxes were detached from the surface after treatment exposure. This was observed during sampling after the treatment, when coagulated proteins and residues were easily swabbed off the surface. Fish residues and possible biofilms that had dried on the surface during transportation may have resulted in some entrapped bacteria that were not exposed to the treatment. It was postulated that an increased treatment time of a maximum of 2 s would have penetrated the residues, and consequently, reductions below the detection limit would have been reached. This assumption was considered in the treatment of contaminated chicken crates. The amounts of fecal contaminants found on the surfaces of these crates were greater than the amounts of fish residues that were found on the surface of the fish boxes. These live-chicken crates are often contaminated with *Campylobacter* spp. and are potential vectors for transmission between flocks *(11, 15)*. The initial aerobic counts on the two crates were found to be approximately 6.0 log CFU/24 $cm^2$ on average, and the initial *Enterobacteriaceae* spp. count was approximately 2.0 log CFU/24 $cm^2$ on average. One second of steam-ultrasound treatment was tested on 12 plates that were cut out from the bottom of one crate. Large coagulated contaminants were easily swabbed off the surface, as described above for the fish boxes. Analysis showed that aerobic counts were significantly reduced ($P < 0.0001$), to 1.80 log CFU/24 $cm^2$, while the *Enterobacteriaceae* spp. counts were reduced to below the detection limit. When the treatment was increased to 2 s on the second crate, levels below the detection limit were also achieved for aerobic counts, confirming the earlier hypothesis. The highest degree of food debris was found on the meat transportation box. As observed for the other transportation materials, food debris was detached from the surface and high bacterial levels appeared on the swabs. Two seconds of steam-ultrasound treatment was sufficient to reduce the aerobic counts to below the detection limit. *Enterobacteriaceae* spp. levels were not determined on this food box.

**Steam-ultrasound treatment of conveyor belts.** Modular belts are particularly difficult to disinfect due to the highly structural surfaces, and the areas around the hinge

TABLE 1. *Results of steam-ultrasound treatment (1 and 2 s) of different food contact surface materials (transportation boxes, crates, conveyor belt, and knives) precontaminated at process lines of cheese and meat plants*

| Food contact material and type of microbial count | No. of sample pieces[a] | Length of treatment (s) | Swabbing area ($cm^2$) | Mean log CFU (SD)[b] | |
|---|---|---|---|---|---|
| | | | | Before treatment | After treatment |
| Fish transportation boxes, no. 1 and 2 | 9 | 1 | 24 | | |
| Aerobic count | | | | 6.21 (0.48) | 2.16 (0.44)*** |
| Live chicken transportation crate 1 | 12 | 1 | 24 | | |
| Aerobic count | | | | 5.80 (0.44) | 1.80 (0.43)*** |
| *Enterobacteriaceae* spp. | | | | 1.48 (0.76) | <1 |
| Live chicken transportation crate 2 | 12 | 2 | | | |
| Aerobic count | | | | 5.68 (0.45) | <1 |
| *Enterobacteriaceae* spp. | | | | 2.36 (0.10) | <1 |
| Red meat transportation box | 3 | 2 | 24 | | |
| Aerobic count | | | | 4.71 (0.14) | <1 |
| Conveyor flex belt (cheese transportation) | 8 | 2 | 50[c] | | |
| Aerobic count | | | | 3.16 (0.10) | <1 |
| *Enterobacteriaceae* spp. | | | | 1.90 (0.30) | <1 |
| Conveyor flex belt (meat transportation) | 3 | 2 | 50[c] | | |
| Aerobic count | | | | 5.37 (0.45) | 2.89 (0.32)* |
| *Enterobacteriaceae* spp. | | | | 2.37 (0.10) | <1 |
| Conveyor rubber belt (meat transportation) | 3 | 2 | 24 | | |
| Aerobic count | | | | 4.38 (0.28) | 1.53 (0.52)** |
| *Enterobacteriaceae* spp. | | | | 1.68 (0.24) | <1 |
| Meat knives (handle)[d] | 3 | 1 | 14 | | |
| Aerobic count | | | | 6.09 (0.14) | <1 |
| *Enterobacteriaceae* spp. | | | | 2.53 (0.15) | <1 |
| Meat knives (steel) | 3 | 1 | 5 | | |
| Aerobic count | | | | 5.26 (0.08) | <1 |
| *Enterobacteriaceae* spp. | | | | 2.46 (0.20) | <1 |

[a] Numbers of plates that were cut from the bottom of boxes or conveyor belt pieces or number of knives to which treatment was applied.
[b] Mean initial contamination levels on the surfaces before treatment and reduced levels achieved after the treatment are shown in this table. The detection limit for aerobic counts and *Enterobacteriaceae* spp. is <1 log CFU. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.0001$.
[c] Modular conveyor belts were cut into smaller pieces where each piece was approximately 50 $cm^2$.
[d] Sampling was performed on the handles of knives, near the area where handle and steel are connected.

pin that interconnects the consecutive rows of belt modules get contaminated easily *(7, 15, 20)*. The modular belts received for steam-ultrasound treatment were heavily contaminated with cheese and meat residue. Aerobic counts of 3.16 CFU/50 $cm^2$ and *Enterobacteriaceae* spp. levels of 1.90 log CFU/50 $cm^2$ were analyzed on conveyor belts used for cheese transportation (Table 1). After 2 s of steam-ultrasound treatment, these average initial levels were reduced to below the detection limits. Pieces of flat surface modular belts for meat transportation, visibly contaminated with high loads of meat residues, were handled in the same way. The average initial aerobic counts levels of 5.37 log CFU/50 $cm^2$ were significantly ($P < 0.05$) reduced, to 2.89 log CFU/50 $cm^2$, while the *Enterobacteriaceae* spp. levels (2.37 CFU/50 $cm^2$) were reduced to below the detection limit with 2 s of steam-ultrasound treatment. The rubber conveyor belt was received from the same meat processing plant. It was highly contaminated with meat residues attached below the belt and dragged along the line, judging by the wear tracks that were found on the surface. This part of the rubber belt was contaminated with aerobic counts of 4.38 log CFU/24 $cm^2$ and *Enterobacteriaceae* spp. levels of 1.68 log CFU/24 $cm^2$, on average. Steam-ultrasound treatment achieved significantly ($P < 0.01$) large aerobic count reductions (aerobic counts were reduced to 1.53 log CFU/24 $cm^2$), while the *Enterobacteriaceae* spp. levels were reduced to below the detection limit. Protein residues on the surface seemed to have coagulated and were easily detached from the surface during swabbing.

**Steam-ultrasound treatment of meat knives.** Fast-operating meat processing plants may frequently experience difficulty in disinfecting meat knives with conventional cleaning techniques, where knives are hand washed in water at 20 to 40°C followed by brief immersion (less than 10 s) in 82°C water baths, termed ''sterilizers.'' Immersions implemented in some processing plants have been observed to be approximately 1 s, which did not have significant impact on bacterial reductions *(9, 25, 26)*. On the other hand, longer immersions (10 s) of heavily contaminated knives in hot water baths often result in protein coagulations on the surface of the knives, which entrap surviving bacteria, leading to unsatisfactory bacterial reductions *(26)*. Midgley and Eustace *(19)* suggested using lower temperatures of 60°C and longer immersion times. The authors concluded that their alternative method was as good as the traditional method in regard to microbial reductions, although several benefits were found for using lower temperature, including the reduced risk of operator injury. In this study, three meat knives were investigated using steam-ultrasound. The knives had been used for one whole day at a Danish meat processing plant. Disinfection of knives at the plant was performed using a two-knife disinfection system where each knife was briefly immersed (1 to 5 s) in 82°C water baths. The highest levels of meat debris on commercial meat knives are found where the handle and steel are connected *(2, 4)*. This study also focused on this area, where aerobic counts and initial levels of *Enterobacteriaceae* spp. were found to be 6.09 and 2.53 log CFU, respectively, on only 5 $cm^2$ (Table 1). This was compared with the steel, where aerobic counts and *Enterobacteriaceae* spp. levels of 5.26 and 2.46 log CFU, respectively, were found on a 14-$cm^2$ swabbed area. In all cases, 1 s of steam-ultrasound treatment was sufficient for reducing bacterial levels to below the detection limit. The pressure applied from the treatment was also able to remove high levels of food debris from the surface. However, an extended rinsing step after the steam-ultrasound treatment resulted in more visibly clean surfaces.

In conclusion, steam-ultrasound treatments of 1 to 2 s were shown to be highly efficient in reducing aerobic count and *Enterobacteriaceae* spp. contaminations on food boxes, chicken transportation crates, conveyor belts, and meat knives, naturally contaminated at cheese and meat processing plants. Due to the fast treatment time, the product remains cool and is not affected by the treatment. However, future work should include testing with repeated treatment over a longer period of time, especially on materials with operational temperatures that are below the temperatures reached by the steam-ultrasound treatment. The system should be tested at the processing plant using a large-scale model. The experimental objectives should include tests on pathogenic and biofilm-producing bacteria. The study has also shown that it would be preferable to perform mechanical cleaning of the surface before the steam-ultrasound treatment, in order to avoid contaminants building up on the surface over a longer period of processing time.

This study shows that rapid steam-ultrasound treatment may be a promising way to perform continuous disinfection of different food contact surfaces without the use of chemicals.

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