Surface Pasteurization of Post-Harvest Raw Whole Onions to Eliminate *Listeria* Contamination Prior to Further Processing

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ABSTRACT

Introduction: The 2011-2012 outbreak of *Listeria monocytogenes* associated with cantaloupe created a heightened awareness for potential *L. monocytogenes* contamination of vegetables and produce. Due to easy contamination from various environmental sources and the long term exposure prior to harvest, *L. monocytogenes* may establish biofilms on produce or post-harvest surfaces. Processing methods to eliminate *Listeria* on produce surfaces reduces subsequent contamination of handle/cut/sliced pieces during downstream further processing operations.

Purpose: In-plant validation of a flame oven for eliminating surface contamination of *L. monocytogenes* of raw onions destined for further processing.

Methods: A gas-fired flame oven was used to test for lethality and process effectiveness on native microflora of onions (APC, yeast and mold) and inoculated (nonpathogenic) *Listeria innocua* (i.e., cocktail of antibiotic resistant ATCC 33090, ATCC 33091, and ATCC 51742). Control onions were sampled without heat treatment while heat-treated onions were placed on a conveyer belt for transit through the gas flames (47 sec) and processed through the oven before being sampled. Process evaluation included microbial enumeration of pre- and post-process levels of organisms. For selective enumeration of *L. innocua*, plating was done on TSA containing rifamycin and streptomycin (both at 50µg/ml) while tryptic soy agar (TSA) and potato dextrose agar (PDA), were used for indigenous bacteria and yeasts/molds, respectively. The data obtained was analyzed using one way ANOVA (p< 0.05).

Results: The onions inoculated with *L. innocua* showed approximately 5-log reduction. Approximately 5-log reductions were also obtained for indigenous bacterial contamination and 4-log reduction of yeast and molds when compared to unprocessed onions (p< 0.05). The gas flame burnt off the outer paper-like layer of the onions without damaging the onion 'meaty' layers and hence reduced yield loss.

Significance: The gas flame-fired oven heat treatment could be an effective means of reducing transfer of *Listeria* spp. during subsequent slicing/dicing operations.

INTRODUCTION

Consumption of fresh vegetables and fruits is believed to have numerous health benefits. Moreover, fresh cut produce (trimmed, cut, sliced, diced, peeled, etc.) is perceived as being fresh, tasty, and convenient by consumers. But with the increased trend in produce consumption, there has been a simultaneous and rapid rise in foodborne illnesses associated with fresh produce, as well. Data from Food and Drug Administration (FDA) has shown 14,132 illnesses from 131 produce-related reported outbreaks, 1360 cases of hospitalizations and 27 fatalities between 1996 to 2010 (FDA, 2013). Due to easy transmission from the ground and/or from post-harvest processing contact contamination, there is the presence of different microbes capable of producing biofilms on fresh produce. *Listeria monocytogenes* is one of the major concerns for contamination of vegetables and produce because of the severity of its infection and its ability to grow at refrigerated temperature (Gandhi et al., 2007). In recent US history, with 147 cases across 28 states, 33 deaths and one fetal loss, the 2011 cantaloupe outbreak was the deadliest produce-related listeriosis outbreak (CDC, 2011).

The contaminations of produce by pathogens such as *L. monocytogenes* can occur in the field, at the time of harvesting or post-harvest handling and especially during fresh-cut processing. Soil, water, decaying vegetation, animal manure, sewage effluents, etc. are the major sources of contamination of *Listeria* spp. in the field (Farber, J. and Peterkin, P., 1991). *L. monocytogenes* is a source of recurrent contamination in fresh cut industries due to their ability to form persistent biofilms on food processing equipment and the processing environment. The degree of contamination of fresh-cut produce by *L. monocytogenes* can easily be higher than the whole products from which they are prepared. Commercially prepared fresh-cut fruits and vegetables are subjected to shredding, slicing, dicing, cutting, etc. with or without washing, during which pathogenic or spoilage microorganisms can invade the cut region or be spread to a greater amount of processed product. The availability of nutrients and moisture on the exposed tissue of the cut surface and greater amount of handling time while cutting, encourage pathogenic colonization (Leverentz, B. et al., 2003). Onions are one of the most widely consumed fresh products, used in salads, burgers and sandwiches. The presence of microbial pathogens like *Listeria* on the surface of onions might cause the transfer of the organism to inner cut surfaces when they are cut/diced (Scollon et al., 2016) and present a direct impact on consumers' health. Thus the importance of post harvest management (modifications or processing) of produce such as onions is crucial to ensure that the pathogenic micro-organisms on surfaces are removed prior to further processing and consumption.

The emphasis of this project was the validation of a gas flame fired oven for:

a) **Examining the yield loss after heat treatment of onions,**
b) **Evaluating the surface pasteurization process to eliminate indigenous microflora (bacteria, yeast/mold),**
c) **And providing validation for a commercial (Unitherm Food Systems) gas flame oven to decrease/control *Listeria* contamination.**

SOURCES OF *LISTERIA* CONTAMINATION OF RAW ONIONS

Listeria monocytogenes, and other gastrointestinal foodborne pathogens, are readily found associated with animals (wild, food production, domestic), insects, reptiles, and/or birds, and can be disseminated in their feces. So any food that is subject to environmental contact in the field, can possibly be contaminated with these bacterial pathogens at the time of harvest.

Many of these pathogens, and especially *Listeria*, are known to form tenacious biofilms on food processing equipment, so even if they are not contaminated from the field, they could be contact-contaminated during transit to, or during, further processing in processing facilities.

There have been numerous recalls on processed onion products over the period of last decade. 2012 recall of diced and slivered red and yellow onions from Gill's Onions, was one of the well-known recalls. It triggered grocery stores such as; Whole Foods, Trader Joe's, Wegmans, Weis and Publix to recall all products containing onions from Gill's Farms. Most recently (in 2016), CRF Frozen Foods and the Oregon Potato Co. have recalled their diced onion products leading to secondary recalls from other associated brands too.

Recalls/Outbreaks Attributed to *Listeria monocytogenes* Contamination of Onions

Product	Year	Source/Supplier	Stores Affected
Onions	2016	CRF Frozen Foods, Oregon Potato Co. (Pasco, WA)	Trader Joe's, Safeway, WalMart, CostCo, others.
Dices onions (fresh cut vegetables)	2016	Country Fresh LLC (Conroe, TX)	Publix, WalMart, Winn Dixie, Harris Teeter, Bi-Lo.
Onions	2012	Gills Onions (Oxnard, CA)	Whole Foods, Trader Joe's, Wegmans, Weis, Publix.

One way to reduce the incidence of contamination or outbreaks due to contaminated onions is to introduce an intervention designed to reduce or eliminate the surface contamination that may exist on raw onions thereby minimizing/reducing/eliminating distribution to other surfaces and/or cut products derived from the whole onions.

MATERIALS & METHODS

Onions. Three types of onions (red, white and yellow) were provided by Unitherm Food Systems, Inc (Bristow, OK). They were held at room temperature prior to processing.

Bacterial Cultures. Antibiotic resistant strains of non-pathogenic *Listeria innocua* namely *L. innocua* ATCC 33090-2, *L. innocua* ATCC 33091-2 and *L. innocua* ATCC 51742-2, resistant to Rifamycin (50 µg/ml) and Streptomycin (50 µg/ml), were used to inoculate the onions for processing through commercial ovens at Unitherm Food Systems, Inc. Indigenous bacteria present on the surface of onions were also subjected to enumeration, before and after processing through the flame oven.

Culture media. Frozen *Listeria innocua* strains were revived in Brain Heart Infusion (BHI) media broth. They were sub cultured overnight in BHI before inoculating onions. The samples inoculated with *Listeria innocua* strains were plated on Tryptic Soy Agar (TSA) plates containing Rifamycin (50 µg/ml) and Streptomycin (50 µg/ml) for selective enumeration of our inoculum on non-sterile product (i.e., this precludes the enumeration of other contaminating bacteria). For enumerating the indigenous bacteria and yeasts/molds, non-selective TSA and Potato Dextrose Agar (PDA; acidified with 10% tartaric acid) were used respectively.

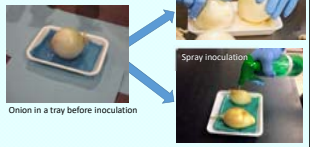
Gas flame-fired process validation on intact onions. A gas-fired flame oven (**Unitherm Flame Peeler and Pasteurizer**) at Unitherm Food Systems' facility (Bristow, OK) was used to test for lethality on native microflora on onions and also onions inoculated with nonpathogenic *Listeria innocua* strains. The evaluation of the process is made by microbial enumeration of pre-process and post-process levels of

A gas-flame grill oven in the test kitchen pilot plant of Unitherm Food Systems Inc. (Bristow, OK).

Process. For pre-process indigenous microflora enumeration, each type of onions (red, yellow and white) were processed two-per-bag. By using nitrile laboratory gloves, an additional set of onions of each type, were placed on the grills and transported through the gas-flame tunnel. A 50-ml rinse (buffered peptone water; BPW, 0.1%) was added to each bag of two onions, and hand massaged for 3-5 minutes. After rinsing, approximately 10-ml sample was removed from each bag to sample tubes that were placed on ice and transported for microbial testing at FAPC. The data obtained was used to determine if the surface gas-flame regimen would be beneficial for its intended processing application. Similarly, two different methods were used to inoculate the onions with *L. innocua cocktail*. For the first method, *L. innocua* 3-strain cocktail (7 ml of each strain) was suspended in 100 ml BPW and poured into a white plastic tray with adsorbent pads for contact contamination; onions were rolled over in the tray containing culture solutions for 1-2 min. Different trays were used for three different types of onions; 12 onions were rolled in each inoculum tray. Similarly, inoculation was also performed with the spray method. 7 ml each of overnight *L. innocua* strains were mixed with 100 ml of 0.1% BPW, containing 5% of glycerol (assumed to help the culture stick onto the onion surface). The inoculated onions were then kept aside in different metal trays to air dry for about 30-40 min. After air drying, the onions were categorized for pre-process and post-process microbial load determination, bagged two-per-bag, and plated after rinse treatment and appropriate dilutions.

Inoculation of Onions. Intact whole red, yellow, and white onions obtained from local suppliers were inoculated with non-pathogenic *Listeria innocua* (3-strain mixture) by 2 methods in our pilot plant trials: one method was a dip inoculation whereby onions were rolled in an inoculum in trays, while another was a manual spray application. Onions were set aside for 30 min to dry after each inoculation method.

Different methods of inoculating onions with bacterial culture.



ONION PROCESSING USING A GAS FLAME OVEN

We thank Unitherm Food Systems (Bristow, OK) for the opportunity to perform validation studies on their "**Unitherm Flame Peeler and Pasteurizer**" located in the test kitchen of their pilot plant facility. We performed these studies in approximately 3 separate occasions using red, yellow, and white onions. Unitherm has been a progressive company in the manufacture of food processing equipment, implementing various unique technologies designed to reduce or eliminate risk from foodborne hazards to achieve food safety benefits for both consumers and manufacturers.

RESULTS

Effect of 'flame peeling' on reducing yield loss. Trimming the surface sheets of onion using manual or cutting technologies often removes substantial amounts of useable onion product, contributing to 'yield loss' approaching 10-15% of initial weight. We weighed onions before, and after, travelling through the flame oven and simulated the 'scrubbing' effect of the tandem process by manual rinse. The post-process onions showed yield losses of < 4.0 % (i.e., 2.9-3.6%) (Fig. 1).

Onion Types	Avg. Yield Loss (%)	Std. Dev.
White Onions	2.92	0.18
Yellow Onions	3.63	0.77
Red Onions	2.86	0.26

Figure 1. Yield loss of white, yellow, and red onions subjected to the flame peeler and pasteurizer oven.

We also examined the effect of surface flame pasteurization on the general microbiota of processed onions. Most onions that are further processed end up sliced or diced, and the contribution of microbial biota could easily reduce the shelf life of the product, even under refrigerated conditions. The slicing/dicing liberates plant juices that might be nutritious to the various components of the microbiota associated with onions (Fig. 2).

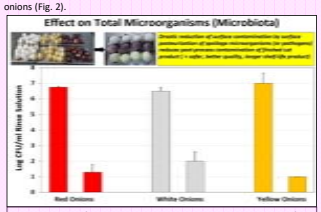


Figure 2. Level of microbiota on red, white, and yellow onions before, and after, processing through the Flame Peeler and Pasteurizer Oven. Data bars represent the means and errors bars represent standard deviation; all trials were done in triplicate replication.

As onions are grown exposed to the outdoor elements for extended periods and because of their proximity to the soil, we examined specifically for yeast and mold counts, before and after processing, as these would also contribute to spoilage during refrigerated storage (Fig. 3). Onions inocuated with *L. innocua* also demonstrated a significant reduction, although not as much as observed with general microbiota.

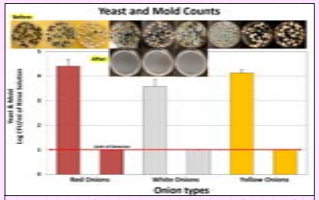


Figure 3. Level of yeast and mold on onions before, and after, processing through the Flame Peeler and Pasteurizer Oven. Data bars represent the means and errors bars represent standard deviation; all trials were done in triplicate replication.

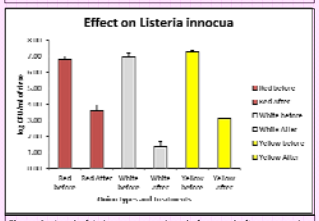


Figure 4. Level of *L. innocua* on onions before, and after, processing through the Flame Peeler and Pasteurizer Oven. Data bars represent the means and errors bars represent standard deviation; all trials were done in triplicate replication.

DISCUSSION and CONCLUSION

The flame grill did an effective job at 'burning off' the outer paper-like layer of the onions without damaging the onion 'meaty' layers. This will very likely reduce waste and increase yield of the process compared to mechanical peelers that remove a considerable amount of the onion 'meat'. The presence of residual contaminants after heating may be due to non-aseptic handling during sampling and due to 'masking effect' of the 'hairy root' region of onion which may have protected the indigenous/inoculated organisms (i.e., the difference between residual indigenous flora and the inoculated Listeria). Thus compared to the untreated onions (pre-process), the heat-treated post-process onions (processed through the gas flame oven for about 47 secs) showed significant reduction in microbial load ($p < 0.05$). Hence the food processing equipment was validated for effectively decreasing the microbial load leading to reduced risk from contamination of further processed onion products.

For produce which are consumed raw, post-harvest processing is crucial to reduce or prevent the presence of pathogenic microorganisms. Heat treatment for all produce might not be feasible or advisable but the necessity of proper pasteurization before consumption is a great alternative to reduce the indigenous bacterial flora, including pathogens and spoilage organisms, to improve safety and reduce spoilage. Proper validation studies of new equipment/techniques must be conducted to examine their effectiveness to combat pathogens and ensure produce food safety.

To control the ever-growing problem of *Listeria* contamination on produce, food manufacturers must adhere to strict sanitation and testing programs. The effectiveness of cleaning and sanitation needs to be analyzed and verified. Several popular testing methods including ATP Bioluminescence, protein and sugar detection swabs, and microbial testing (including rapid DNA detection) can be performed to determine the validity of cleaning and sanitation programs. Onions are found in market as whole onions, sliced, diced, or in salads or frozen vegetable mix. The sliced onions have shelf life of around 2 weeks at refrigeration temperature (4 °C), which is very suitable time and condition for previously low levels of *Listeria* to reach much higher levels (Farber J.et al., 1998). Thus storage of cut/sliced/diced onions is another factor which should be taken care of specially by the consumers. Recently, Scollon et al. (2016), demonstrated that transfer of surface *L. monocytogenes* to cut slices readily occurred even after multiple slicing episodes, indicating the impact that surface contamination can have on the microbial integrity of sliced/diced-processed onions.

In conclusion, every step of onion production, processing and consumption are vital to determine the extent of *Listeria* prevalence. The farmers should implement Good Agricultural Practices (GAPs) while growing onions. Similarly, the processors need to use Good Manufacturing Practices (GMPs) and effective sanitation programs, as set by the respective industry and/or regulatory agencies. This is very critical for minimizing pre-harvest contamination, reducing process cross-contamination and removing the contaminants after processing. In the same way, consumers need to responsible as well for consumption of raw onions and other fresh produce. Proper washing, lessened refrigeration, etc. are few things consumers need to focus on. Hence, effective strategies and actions from farm to fork is necessary to prevent *Listeria* contamination of fresh produce such as onions.

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Unitherm Food System Inc. "**Flame Peeler & Pasteurizer**" tandem equipment process. Top left: flame oven; top right: scrubber; bottom left: onions after flame oven; bottom right: onions after oven and scrubber.