Food Control 80 (2017) 131–142

Contents lists available at ScienceDirect

Food Control

journal homepage: www.elsevier.com/locate/foodcont

Growth and biofilm formation by *Listeria monocytogenes* in cantaloupe flesh and peel extracts on four food-contact surfaces at 22 °C and 10 °C

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ARTICLE INFO

Article history:
Received 24 January 2017
Received in revised form
7 April 2017
Accepted 28 April 2017
Available online 30 April 2017

Keywords:
Listeria monocytogenes
Growth
Biofilm
Cantaloupe

ABSTRACT

The nationwide listeriosis outbreak that occurred in the United States during 2011 highlighted the importance of preventing cantaloupe contamination with *Listeria monocytogenes* (*Lm*) within farm and processing environments. The objectives of this study were to determine the effects of strain and temperature on growth and biofilm formation of *Lm* in cantaloupe flesh and peel extracts on different food-contact surfaces. Growth of *Lm* strains was markedly greater at high concentration of cantaloupe extracts and temperature in comparison to low concentration and temperature. For 50 mg/ml of cantaloupe extract inoculated with 3 log CFU/ml, the growth of *Lm* was 8.5 log CFU/ml in 32 h at 22 °C and 6–7 log CFU/ml in 72 h at 10 °C. For 2 mg/ml of cantaloupe extract that was inoculated with *Lm*, the growth was 7–7.5 log CFU/ml in 72 h at 22 °C and 3.5 log CFU/ml in 72 h at 10 °C. There were no differences ($P > 0.05$) among *Lm* strains for biofilm formation in cantaloupe extracts, but biofilm formation was greater at high temperature and high concentration. For 50 mg/ml cantaloupe extracts inoculated with 3 log CFU/ml, the biofilm formation of *Lm* on stainless steel surface was approximately 7 log CFU/coupon at 22 °C in 4–7 days and 5–6 log CFU/coupon at 10 °C in 7 days. For 2 mg/ml cantaloupe extracts, the biofilm formation of *Lm* on the stainless-steel surface was approximately 5–6 log CFU/coupon at 22 °C and 4–4.5 log CFU/coupon at 10 °C in 7 days. The biofilm formation by cantaloupe outbreak strain *Lm* 2011L-2625 in cantaloupe extracts was least on buna-n rubber when compared to stainless steel, polyethylene and polyurethane surfaces ($P < 0.05$). These findings show that a very low concentration of nutrients from cantaloupe flesh or peel can induce *Lm* growth and subsequent biofilm formation on different food-contact processing surfaces.

1. Introduction

Listeria monocytogenes is a Gram-positive rod shape, non-spore forming, facultative anaerobic, bacterium which is responsible for listeriosis in humans and animals (Gandhi & Chikindas, 2007). Among all the foodborne pathogens, *L. monocytogenes* is the most dangerous due to its high mortality rate of 20–25% (Todd & Notermans, 2011). One of the deadliest listeriosis outbreaks (33 deaths and one miscarriage) reported in the world history which occurred in the USA in 2011 was associated with whole cantaloupe (McCollum et al., 2013). Among the 13 known serotypes of *L. monocytogenes*, strains that belong to serotypes 1/2a, 1/2b and 4b are mostly associated with foodborne listeriosis outbreaks (Borucki, Peppin, White, Loge, & Call, 2003; Nelson et al., 2004).

L. monocytogenes is commonly found in soil, sewage and fecal matter (Locatelli, Spor, Jolivet, Piveteau, & Hartmann, 2013). Cantaloupe can often be contaminated with pathogens that are associated with soil and animals since it is grown close to the ground. The outer rind of cantaloupe fruit provides an excellent surface for pathogenic microorganisms to attach and hide (Webb, Erickson, Davey, & Doyle, 2015a, 2015b). *L. monocytogenes* cells that are attached to the rind can contaminate cantaloupe-

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http://dx.doi.org/10.1016/j.foodcont.2017.04.043
0956-7135/

processing environments and promote growth and subsequent biofilm formation on food-contact and non-food contact surfaces when appropriate environmental conditions exist. The investigations conducted by U.S. Food and Drug Administration during the multi-state cantaloupe outbreak in 2011 have found the presence of *L. monocytogenes* on the food-contact and non-food contact surfaces of the cantaloupe processing facility of Jensen farm, Colorado (McCollum et al., 2013).

A biofilm is a collection of microbial cell communities that are attached to a surface and embedded in a self-produced matrix that consists of polysaccharides (Vestby, Møretrø, Langsrud, Heir, & Nesse, 2009; Whitehead & Verran, 2015). Biofilms are the most predominant form of bacteria in food processing environments since microbial cells have extra protection from desiccation, UV light, antimicrobials and sanitizing agents (Bernbom, Vogel, & Gram, 2011; Vestby, M, ø, retr, ø, Langsrud, Heir, & Nesse, 2009). Biofilms formed on the food-contact surfaces can cause cross-contamination when improperly cleaned, thus creating a serious food safety issue (Wilks, Michels, & Keevil, 2006).

L. monocytogenes is capable of adhering to various types of food-contact surfaces that are found in the food processing environments (Di Bonaventura et al., 2008). Attachment and biofilm formation of *L. monocytogenes* is influenced by the physiochemical properties of environment (nutrients, temperature, pH, salt concentration) as well as the cell surface (hydrophobicity, flagellation and motility) (Di Bonaventura et al., 2008; Kalmokoff et al., 2001). Survival, growth and biofilm formation in standard microbiology growth media may not reflect the actual behavior of *L. monocytogenes* in food processing environments. This is because the media contain optimum levels of all the nutrients that are necessary for microbial growth. For example, Beuchat, Brackett, Hao, and Conner (1986) studied the growth of *L. monocytogenes* in cabbage juice at 5 °C and observed that *L. monocytogenes* was capable of growing in sterile cabbage juice containing <5% NaCl. In contrast, *L. monocytogenes* growth was observed when ≤10% NaCl was present in tryptic phosphate broth. Kim, Ryu, and Beuchat (2006) studied the biofilm formation of *Enterobacter sakazakii* in different growth media at 25 °C and found that *E. sakazakii* did not form biofilms on the stainless steel containing lettuce juice broth but produced biofilms on the same surfaces in infant formula broth. These findings suggest that the growth and biofilm formation of foodborne bacterial pathogens can drastically differ depending on the growth media.

In cantaloupe processing environments, *L. monocytogenes* can be exposed to nutrients that are leaked from cantaloupe fruits. Both cantaloupe flesh and peel contain nutrients such as sugar, proteins, carotenoids, vitamin C and various minerals that are essential for microbial growth (Koubala, Bassang'na, Yapo, & Raihanatou, 2016). There are no published data on the growth and biofilm formation of *L. monocytogenes* in cantaloupe residue that persists on the food processing surfaces. Penteado and Leitao (2004a) studied the growth of *L. monocytogenes* in low-acid fruit pulps (melon, watermelon and papaya) at 10 °C, 20 °C and 30 °C. According to their study, the growth of *L. monocytogenes* varied depending on the fruit type.

Even though food-soils are removed from the cantaloupe processing environments routinely, residues may persist on the food-contact surfaces when they are improperly cleaned (Fryer & Asteriadou, 2009). There is a need for critical understanding of the survival, growth and biofilm formation that reflects the actual behavior of *L. monocytogenes* in cantaloupe residue when it is present on food processing surfaces. Therefore, the objectives of the present study were to determine the effects of strain, temperature, nutrient level, and food-contact surface on the growth and biofilm formation of *L. monocytogenes* in cantaloupe flesh and peel extracts.

2. Materials and methods

2.1. Bacterial strains and culture conditions

L. monocytogenes strains that were used in this study are described in Table 1. The working stock cultures of these strains were prepared by inoculating a colony grown on PALCAM agar onto tryptic soy agar that contained 0.6% yeast extract (TSAYE) slants and incubated at 37 °C for 24 h. These working stocks were stored at 4 °C for 4–5 weeks. The overnight culture of each strain was prepared by inoculation of 10 ml of tryptic soy broth that contained 0.6% yeast extract (TSBYE) from the working stocks and incubated at 37 °C for 18–20 h with shaking at 150 rpm (Imperial III incubator, Lab-Line Instrument Inc., IL, USA).

2.2. Food-contact surface preparation

Four types of food-contact surfaces that are commonly used in the cantaloupe processing industry were obtained (Table 2) and were cut into 1 cm × 1.5 cm size coupons. Only stainless steel coupons were reused during experiments. These coupons were soaked in an alkali detergent for 1 h and washed thoroughly with deionized water. Washed stainless steel coupons were autoclaved at 121 °C for 15 min. Buna-n rubber, ultra-high molecular weight polyethylene and thermoplastic polyurethane coupons were surface sterilized by submerging in 100% alcohol for 15 min prior to washing with sterile water to eliminate alcohol residue. Sterile coupons were surface dried inside a biosafety cabinet prior to use. Sterility of these coupons was confirmed by incubating coupons in fresh TSBYE at 37 °C for 48 h.

2.3. Preparation of cantaloupe extracts

Cantaloupe (*Cucumis melo* L. var. reticulatus) fruits were obtained from a local supermarket and washed under running tap water for 1 min. The peel was then removed and the flesh was deseeded. To prepare cantaloupe flesh and peel extracts, 100 g of cut cantaloupe flesh or peel was blended with 400 ml of deionized water for 30 s using a juice extractor (Hamilton beach, Model: 53514). The blended flesh and peel extracts were then filtered using a filter bag (BA6141/STR) and centrifuged (2500g) (Beckman, Model TJ-6 centrifuge) for 5 min to eliminate coarse particles. Cantaloupe extracts were autoclaved at 121 °C for 15 min and stored at room temperature until needed. These cantaloupe extracts (200 mg/ml) were further diluted in physiological saline to obtain 50 mg/ml and 2 mg/ml of concentrations, respectively, based on the fresh weight of the cantaloupe.

2.4. Determination of physical/chemical properties of cantaloupe extracts

The pH of sterile cantaloupe flesh and peel extracts was determined using a calibrated pH meter (Accumet Basic, AB15 pH meter, Fisher Scientific). Brix values were determined using a refractometer (RHB-18ATC, GxPro). In order to determine the dry weight of cantaloupe flesh or peel extract, 25 ml of each extract was transferred to a pre-weighed 50 ml centrifuge tube. These extracts were freeze dried until a constant weight was obtained. The dry weight was calculated using the following equation.

$$\text{Dry weight (mg/ml)} = \frac{(\text{Post dry weight of the tube} - \text{Weight of the empty tube}) \text{ mg}}{\text{Volume of extract}}$$

2.5. Evaluation of growth of L. monocytogenes in cantaloupe extracts at different temperatures

Growth of *L. monocytogenes* EGD (Bug600), 2011–2624 and G1091 in 50 and 2 mg/ml of cantaloupe flesh and peel extracts at 22 °C and 10 °C was studied. The overnight cultures of these strains were prepared as described previously. One ml volumes of overnight cultures were centrifuged for 5 min (9000g, MARATHON 21000R, Fisher Scientific). The resulting cell pellets were resuspended in 1 ml of physiological saline to obtain 9 log CFU/ml of stationary phase cells.

Cantaloupe flesh or peel extracts (50 and 2 mg/ml) were inoculated with the *L. monocytogenes* stationary phase cells to yield an initial cell concentration of 3 log CFU/ml. The inoculated cantaloupe extracts were incubated at 10 °C (Low temperature, VWR, Scientific Products, Sheldon manufacturing, INC., OR, 97113) and at room temperature (22 °C). Cell numbers were enumerated for 72 h at 8 h intervals by serially diluting an aliquot from the cell suspensions in physiological saline and plating on tryptic soy agar that was enriched with 0.6% yeast extract, Escullin and Ferric ammonium citrate (TSA-YEEF). These plates were incubated at 37 °C (Imperial III Incubator, Lab line instrument Inc., IL, USA) for 48 h to obtain colony forming unit (CFU) counts.

2.6. Calculation of generation time

The generation time (g) was calculated from the slope of the line of the semi logarithmic plot of exponential growth using the mean of 4 replications. The equation of g = 0.301/slope of the semi logarithmic plot of exponential growth was used to calculate the generation time as described previously (Penteado & Leitao, 2004a, 2004b).

2.7. Evaluation of biofilm formation by different strains of L. monocytogenes in cantaloupe extracts at different temperatures

One ml from each overnight culture (Table 1) was centrifuged, and the resulting cell pellets were resuspended in 1 ml of physiological saline. Cantaloupe flesh or peel extracts (50 and 2 mg/ml) were inoculated with stationary phase cells to obtain a 3 log CFU/ml initial cell concentration. Sterile stainless steel coupons were placed in polystyrene 24-well plates with one coupon per well. Each well was filled with 2 ml of cell suspension that was prepared in either cantaloupe flesh or peel extract. These coupons were incubated at 22 °C or 10 °C for 1, 4 or 7 days.

2.8. Enumeration of attached cells in the biofilm

To enumerate attached cells in biofilms at the end of 1, 4 or 7 days, used growth media was removed from the wells and coupons were washed twice by adding 2.75 ml of physiological saline to remove loosely bound cells. Washed coupons were transferred into plastic tubes (15 ml-Tronado™) each containing 5 ml of 0.1% peptone water with 0.02% Tween 80 and five sterile glass beads. Tubes were vortexed for 1 min (Vortex mixer, Labnet International, Inc, Edison, NJ, USA) and aliquots of detached cell suspensions were serially diluted in physiological saline. Each dilution was plated on TSA-YEEF and incubated at 37 °C for 48 h.

2.9. Effect of the nature of the food-contact surface on biofilm formation by L. monocytogenes 2011–2625 in cantaloupe extracts at different temperatures

Stainless steel, buna-n rubber, ultra-high molecular weight polyethylene and thermoplastic polyurethane coupons were placed in 24-well plates with one coupon per well. *L. monocytogenes* 2011L-2625 cell suspension of 2 ml at 3 log CFU/ml was prepared in cantaloupe flesh or peel extract (50 and 2 mg/ml) and added to each well. These coupons were incubated at 22 °C and 10 °C for 1, 4 or 7 days. At the end of each incubation period, biofilm formation on the four different surfaces was enumerated as described previously.

Table 1
L. monocytogenes strains tested in this study.

L. monocytogenes	Serotype	Isolation source	Obtained from
2011L-2625	1/2a	Cantaloupe outbreak 2011	UGA
EGD (Bug600)	1/2a	Clinical	Institute Pasteur, France
2011–2624	1/2b	Cantaloupe outbreak 2011	UGA
F8385	1/2b	Carrot	UGA
ScottA	4b	Clinical	FDA
G1091	4b	Coleslaw	UGA

UGA: University of Georgia.
FDA: Food and Drug Administration, USA.

Table 2
Description of food-contact surfaces tested in this study.

Material	Thickness (inch)	Manufacturer	Used for
Stainless steel (304 #4)	0.018	Thyssen Krup	Table tops, Utensil & equipment surface
Nitrile buna rubber	0.031	Warco Nitrite	Conveyor belt
Thermoplastic polyurethane	0.01	Habasit	Conveyor belt
Ultrahigh molecular weight polyethylene	0.125	Sibe Automation	Cutting boards, Conveyor belt, Table tops

2.10. Statistical analysis

A completely randomized design with a 2-way factorial structure and 4 replications was used. Mean values (log CFU/ml or log CFU/coupon) were calculated using four values obtained and analysis of variance with Tukey's honestly significant difference test ($P < 0.05$) was performed to separate means where significant differences occur. For the comparison study of biofilm formation among 6 strains, the two factors included strain and temperature. For the comparison of average biofilm formation of *L. monocytogenes* in 1, 4 and 7 days, the days were compared with combination of concentration and temperature combination. For the comparison of biofilm formation of *L. monocytogenes* 2011–2625 on different food contact surfaces, the two factors included surfaces and temperature ($P < 0.05$).

3. Results

3.1. Physical and chemical properties of cantaloupe extract

The pH of cantaloupe flesh and peel extracts was around 6.5. Cantaloupe flesh extract had around 1.6°B of total soluble solids while cantaloupe peel extract showed a 0.7 °B of total soluble solids. The fresh weight for both cantaloupe flesh and peel extracts was 200 mg/ml. The dry weight of cantaloupe flesh extract was 1.7 mg/ml and peel extract was 0.7 mg/ml.

3.2. Growth curves of L. monocytogenes in cantaloupe extracts

The growth rate of *L. monocytogenes* in cantaloupe flesh and peel extracts was greater at higher cantaloupe extract concentration and higher temperature. The growth curves of *L. monocytogenes* in cantaloupe flesh at 50 and 2 mg/ml at 22 °C and 10 °C are shown in Fig. 1. In 50 mg/ml cantaloupe flesh, the cell numbers of *L. monocytogenes* Bug600, 2011L-2625 and G1091 increased by 5.5 log CFU/ml in 32–40 h at 22 °C and remained stable at that level afterwards, while *L. monocytogenes* increased by 3.5 log CFU/ml in 72 h at 10 °C (Fig. 1A,C,E). In 2 mg/ml cantaloupe flesh extract, the cell numbers of *L. monocytogenes* strains Bug600, 2011L-2625 and G1091 were increased by 4.5 log CFU/ml in 56 h at 22 °C and 0.5 log CFU/ml in 72 h at 10 °C (Fig. 1B,D,F). In 50 and 2 mg/ml cantaloupe flesh extract, the cell number of *L. monocytogenes* from 8 to 72 h was greater ($P < 0.05$) at 22 °C when compared to 10 °C.

A similar pattern was also observed for the growth of *L. monocytogenes* in cantaloupe peel extract at 50 and 2 mg/ml concentrations at 22 °C and 10 °C (Fig. 2). In 50 mg/ml cantaloupe peel extract, the cell numbers of *L. monocytogenes* Bug600, 2011L-2625 and G1091 were increased by 5.5 log CFU/ml at 22 °C in 24–40 h and remained stable at that level afterwards, while *L. monocytogenes* strains were increased by 3–4 log CFU/ml at 10 °C in 72 h (Fig. 2A,C,E). In 2 mg/ml cantaloupe peel extract, the cell numbers of *L. monocytogenes* strains Bug600, 2011L-2625 and G1091 were increased by 4–4.5 log CFU/ml in 48–56 h at 22 °C and 0.5 log CFU/ml at 10 °C in 72 h (Fig. 2B,D,F). For 50 and 2 mg/ml cantaloupe peel extract, the cell number of *L. monocytogenes* from 8 to 72 h was greater ($P < 0.05$) at 22 °C when compared to 10 °C.

The lag phase of *L. monocytogenes* strains was less than 8 h in 50 and 2 mg/ml cantaloupe flesh or peel extracts at 22 °C while it was extended to 16–18 h in 50 mg/ml or 72 h in 2 mg/ml cantaloupe extracts at 10 °C.

In 50 mg/ml cantaloupe extracts, the generation time was markedly increased by 2–4 h by shift of temperature from 22 °C to 10 °C for all *L. monocytogenes* strains tested (Table 3). In 2 mg/ml cantaloupe extract, the generation time of *L. monocytogenes* extended by about 2–4 h beyond the corresponding generation time in 50 mg/ml cantaloupe extract at 22 °C. Since no exponential growth was observed during the duration of 72 h, the generation time was not calculated for *L. monocytogenes* in 2 mg/ml at 10 °C.

3.3. Influence of strain and temperature on biofilm formation by L. monocytogenes on stainless steel surface containing cantaloupe extracts

B Biofilm formation by *L. monocytogenes* strains was greater in 50 mg/ml cantaloupe extract at 22 °C ($P < 0.05$) as compared to 2 mg/ml at 10 °C. In 50 mg/ml cantaloupe flesh extract, biofilm formation of *L. monocytogenes* strains was 5–6 log CFU/coupon in 1 d and 7 log CFU/coupon in 4 or 7 d at 22 °C. At 10 °C, biofilm formation was 1.5–3.5 logs less compared to 22 °C ($P < 0.05$) (Fig. 3A,C,E). In 2 mg/ml cantaloupe flesh extract, biofilm formation by *L. monocytogenes* was 4.5 log CFU/coupon in 1 d or 5–6 log CFU/coupon in 4 or 7 d at 22 °C. At 10 °C biofilm formation was 1–2.5 logs less ($P < 0.05$) than that at 22 °C (Fig. 3B,D,F). With one exception, there were no differences ($P < 0.05$) in biofilm formation among the six strains of *L. monocytogenes* in cantaloupe flesh extract under all environmental condition tested. For 50 mg/ml cantaloupe flesh extract, *L. monocytogenes* Bug600 had 1 log less biofilm formation in 1 day as compared to other strains at 22 °C.

A similar pattern was also observed for the biofilm formation by six strains of *L. monocytogenes* in cantaloupe peel extract at 50 and 2 mg/ml concentrations at 22 °C and 10 °C (Fig. 4). In 50 mg/ml cantaloupe peel extract, biofilm formation of *L. monocytogenes* strains was approximately 6 log CFU/coupon in 1 d and 7 log CFU/coupon in 4 or 7 d at 22 °C. At 10 °C biofilm formation was 1.5–4 logs less ($P < 0.05$) than that at 22 °C (Fig. 4A,C,E). In 2 mg/ml cantaloupe peel extract, biofilm formation by *L. monocytogenes* strains were approximately 4–5 log CFU/coupon in 1 d or 5–6 log CFU/coupon in 4 or 7 d at 22 °C while their corresponding biofilm formation at 10 °C was 1.5–3 logs less ($P < 0.05$) than that observed at 22 °C (Fig. 4B,D,F). No significant differences in biofilm formation were observed among the six strains of *L. monocytogenes* in cantaloupe peel extract under all environmental condition tested with the exception of 50 mg/ml cantaloupe flesh extract in 1 day at 10 °C, where *L. monocytogenes* G1091 had 1 log greater biofilm formation as compared to other strains.

In 50 mg/ml cantaloupe flesh or peel extract, there was no increase ($P < 0.05$) in average biofilm formation by *L. monocytogenes* observed from 4 to 7 d at 22 °C, but there was 1.5–2.0 log increase observed at 10 °C (Table 4). In 2 mg/ml cantaloupe flesh or peel extract, there was no increase ($P < 0.05$) in average biofilm formation by *L. monocytogenes* strains observed from 4 to 7 d at both 22 °C and 10 °C.

3.4. Biofilm formation by L. monocytogenes 2011L-2625 on different food-contact surfaces containing cantaloupe extracts

In 50 mg/ml cantaloupe flesh extract inoculated with 3 log CFU/ml, *L. monocytogenes* 2011L-2625 biofilm formation was approximately 6.5 log CFU/coupon in 1 d or 7 log CFU/coupon in 4 or 7 d on stainless steel, polyethylene and polyurethane surfaces 22 °C. The corresponding biofilm formation on buna-n rubber was 2.0–3.5 logs less ($P < 0.05$) than that observed on the other three surfaces at 22 °C (Fig. 5A,C,E). In 50 mg/ml cantaloupe flesh extract, *L. monocytogenes* 2011L-2625 formed 1–2 log CFU/coupon biofilm on all the four surfaces in 1 d at 10 °C. However, *L. monocytogenes* 2011L-2625 formed 5–6 log CFU/coupon biofilms on stainless steel, polyethylene and polyurethane surfaces in 4 or 7 d at 10 °C while its corresponding biofilm formation on buna-n rubber was 2 logs less ($P < 0.05$) than that observed on other surfaces at 10 °C.

In 2 mg/ml cantaloupe flesh extract, *L. monocytogenes* 2011L-

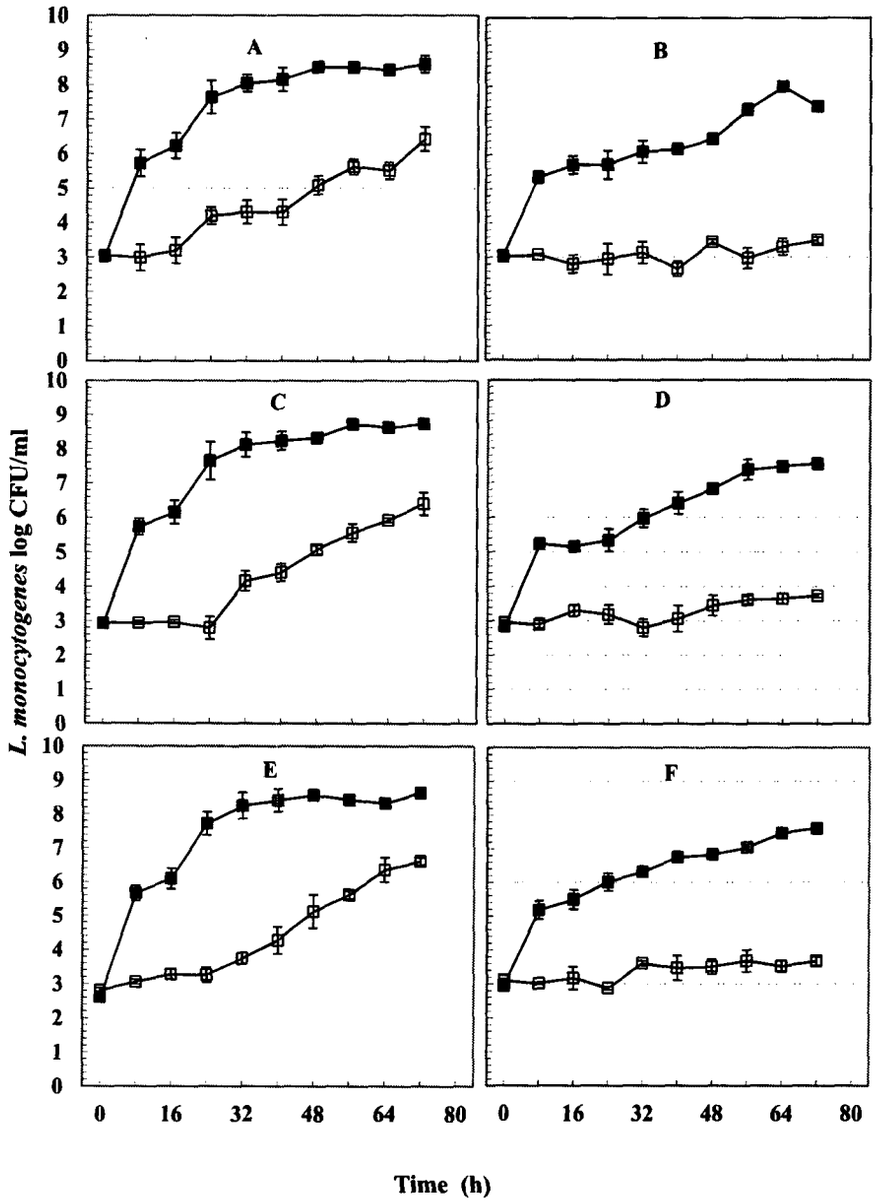

Fig. 1. Growth of *L. monocytogenes* Bug600 (A, B), 2011–2625 (C, D) and G1091 (E, F) in 50 mg/ml (A, C, E) and 2 mg/ml (B, D, F) of cantaloupe flesh extract at 10 °C (□) and 22 °C (■). Error bars indicate standard error.

2625 biofilm formation was approximately 4 log CFU/coupon in 1 d or 6 log CFU/coupon in 4 or 7 d on the stainless steel, polyethylene and polyurethane surfaces. The corresponding biofilm formation on buna-n rubber was approximately 1.5 logs less ($P < 0.05$) than that observed on the other three surfaces at 22 °C (Fig. 5B,D,F). In 2 mg/ml cantaloupe flesh extract, biofilm formation by *L. monocytogenes* 2011L-2625 was approximately 2 log CFU/coupon on all the four surfaces in 1 d at 10 °C. However, *L. monocytogenes* 2011L-2625 formed 5.5–6 log CFU/coupon of biofilm on the stainless steel, polyethylene and polyurethane surfaces in 4 or 7 d while its corresponding biofilm formation on buna-n rubber was 1–1.5 logs less ($P < 0.05$) than that observed on the other surfaces at 10 °C.

In 50 mg/ml cantaloupe peel extract, *L. monocytogenes* 2011L-2625 biofilm formation was approximately 6.5–7.0 log CFU/coupon in 1, 4 or 7 d on stainless steel, polyethylene and polyurethane surfaces while its corresponding biofilm formation on buna-n rubber was 2.5–3.5 logs less ($P < 0.05$) than that observed on the other three surfaces at 22 °C (Fig. 6A,C,E). In 50 mg/ml cantaloupe peel extract, *L. monocytogenes* 2011L-2625 formed 1–2 log CFU/coupon biofilm on all four surfaces in 1 d at 10 °C. However, *L. monocytogenes* 2011L-2625 formed 4.5–6.0 log CFU/coupon biofilms on stainless steel, polyethylene and polyurethane surfaces in 4 or 7 d while its corresponding biofilm formation on buna-n rubber was 1–2 logs less ($P < 0.05$) than that observed on other surfaces at 10 °C.

In 2 mg/ml cantaloupe peel extract, *L. monocytogenes* 2011L-2625 biofilm formation was 4–5 log CFU/coupon in 1 d or 6 log CFU/coupon in 4 or 7 d on stainless steel, polyethylene and polyurethane surfaces. The corresponding biofilm formation on buna-n rubber was approximately 1–2 logs less ($P < 0.05$) than that observed on the other surfaces at 22 °C (Fig. 6B,D,F). In 2 mg/ml cantaloupe flesh extract, biofilm formation by *L. monocytogenes* 2011L-2625 was approximately 2 log CFU/coupon on all the four

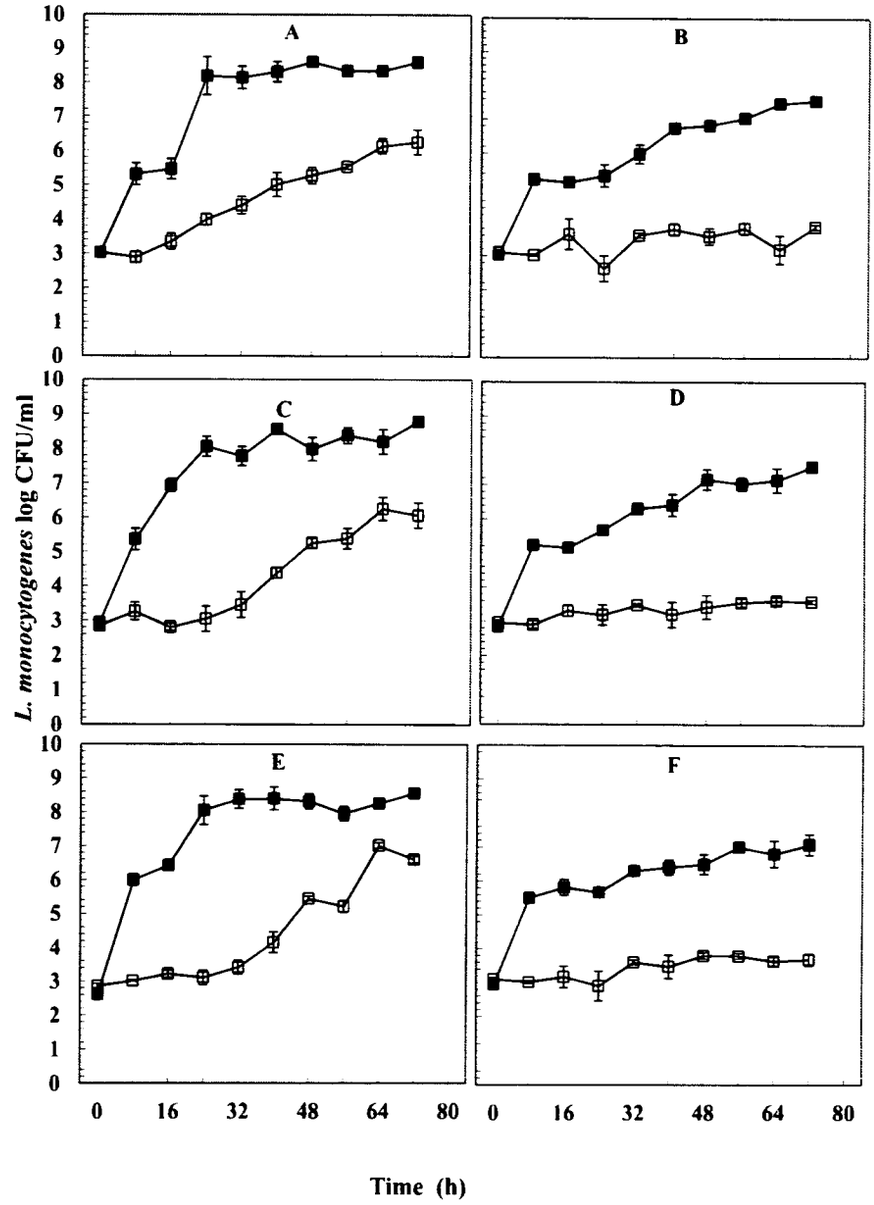

Fig. 2. Growth of L. *monocytogenes* Bug600 (A, B), 2011–2625 (C, D) and G1091 (E, F) in 50 mg/ml (A, C, E) and 2 mg/ml (B, D, F) of cantaloupe peel extract at 10 °C (□) and 22 °C (■). Error bars indicate standard error.

Table 3
Generation time of *L. monocytogenes* in cantaloupe extracts at 10 °C and 22 °C.

L. monocytogenes strain	Cantaloupe extract	Generation time of *L. monocytogenes* (h)			
		50 mg/ml		2 mg/ml	
		10 °C	22 °C	10 °C	22 °C
EGD (Bug600)	Flesh	5.9 (0.93)	2.0 (0.95)	NA	5.4 (0.83)
2011L-2624	Flesh	4.4 (0.95)	2.0 (0.91)	NA	4.6 (0.86)
G1091	Flesh	4.1 (0.89)	2.2 (0.99)	NA	5.2 (0.83)
EGD (Bug600)	Peel	5.6 (0.98)	1.5 (0.91)	NA	5.2 (0.87)
2011L-2624	Peel	4.3 (0.94)	2.3 (0.85)	NA	4.1 (0.84)
G1091	Peel	3.2 (0.92)	1.8 (0.88)	NA	5.7 (0.70)

R^2 values are shown in brackets.
NA: Not available.

surfaces in 1 d at 10 °C. However, *L. monocytogenes* 2011L-2625 formed 6 log CFU/coupon biofilms on stainless steel, polyethylene and polyurethane surfaces in 4 or 7 d while the corresponding biofilm formation on buna-n rubber was 1–2 logs less ($P < 0.05$)

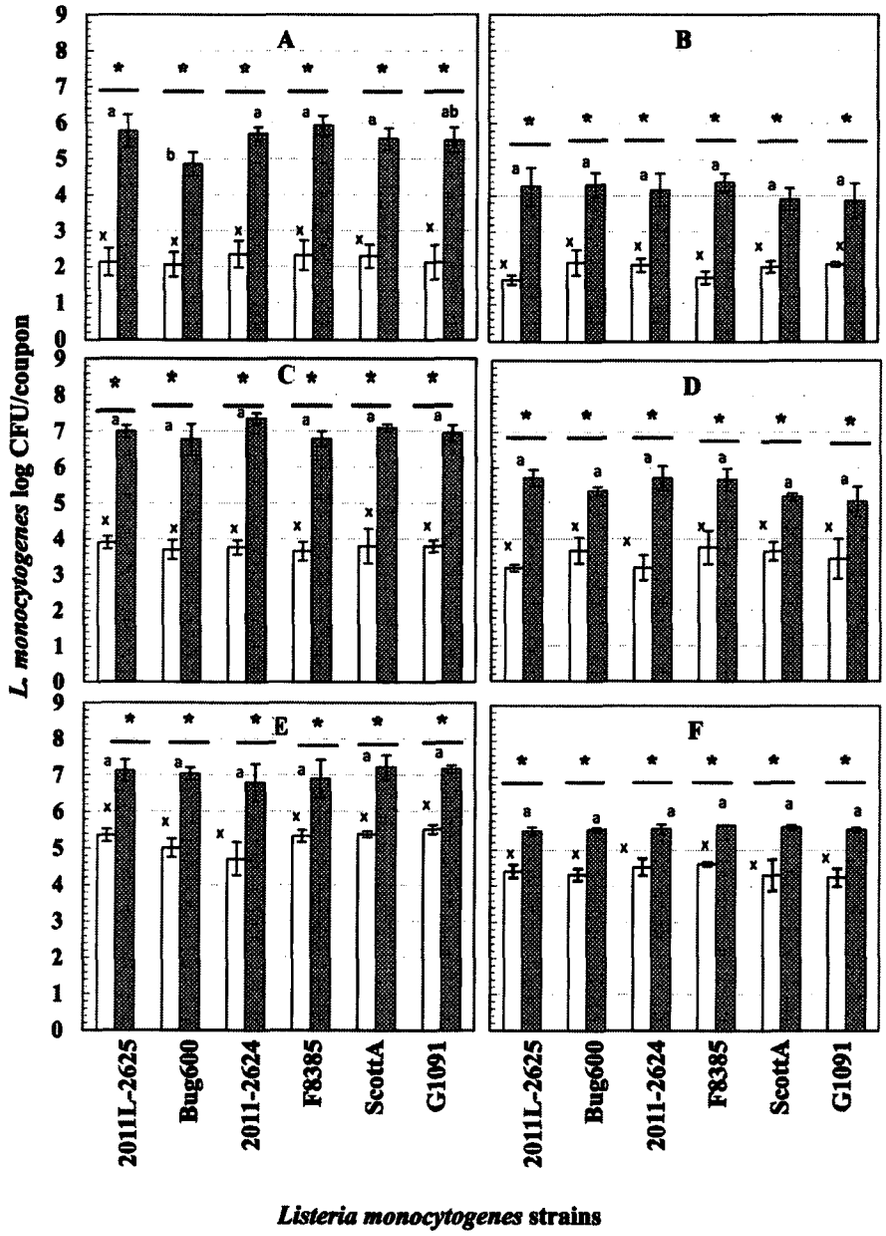

Fig. 3. Biofilm formation by six strains of *L. monocytogenes* on the stainless steel surface in 50 mg/ml (A, C, E) and 2 mg/ml (B, D, F) of cantaloupe flesh extract at 10 °C (□) and 22 °C (■) in 1 day (A, B),4 days (C, D) and 7 days (E, F). Note: Bars with different lowercase letters indicate significant differences between biofilms formed at the same temperature and * indicate significant difference between biofilms belong to the same strain formed at different temperature based on Tukey's Honestly Significant Difference test ANOVA test ($P < 0.05$). Error bars indicate standard error.

than that observed on other surfaces at 10 °C.

Overall, biofilm formation of *L. monocytogenes* 2011L-2625 on buna-n rubber was significantly lower than the biofilm formation on stainless steel, polyethylene and polyurethane surfaces in 50 and 2 mg/ml cantaloupe flesh and peel extract concentrations at 22 °C and 10 °C.

4. Discussion

In the cantaloupe processing environments, nutrients from cantaloupe fruits can leak and accumulate on food contact surfaces when improperly cleaned. Such residues may promote the growth and subsequent biofilm formation by *L. monocytogenes* on the food processing surfaces. This study focused on the critical understanding of the survival, growth and biofilm formation that reflected the behavior of *L. monocytogenes* in cantaloupe residue if present at two concentrations on different processing surfaces. Cantaloupe extracts were autoclaved at 121 °C for 15 min to eliminate any interferences from background microflora and spores against *L. monocytogenes*. Autoclaved food extracts were frequently used for studying the behavior of *L. monocytogenes*, *Escherichia* coli, *Salmonella* Enteritidis in fruit or vegetable juices. For example, Raybaudi-Massilia, Mosqueda-Melgar, and Martín-Belloso (2009) studied the antimicrobial activity of malic acid against *Listeria monocytogenes*, *Salmonella* Enteritidis and *Escherichia coli* O157:H7 in apple, pear and melon juices using autoclaved fruit juices at 121 °C for 15 min. Mosqueda-Melgar, Raybaudi-Massilia, and Martín-Belloso (2007) sterilized melon and water melon juice at 121 °C

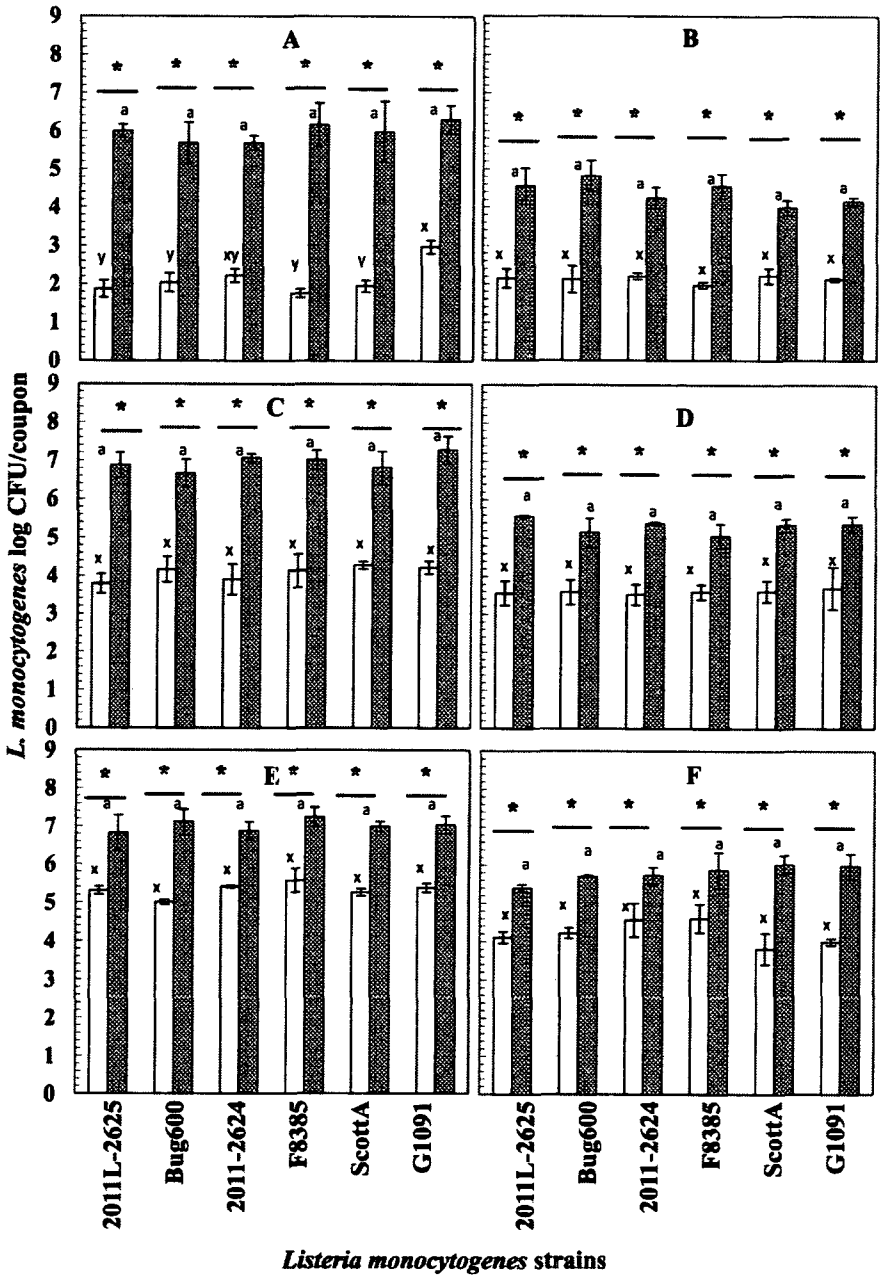

Fig. 4. Biofilm formation by six strains of *L. monocytogenes* on the stainless steel surface in 50 mg/ml (A, C, E) and 2 mg/ml (B, D, F) of cantaloupe peel extract at 10 °C (□) and 22 °C (■) in 1 day (A, B),4 days (C, D) and 7 days (E, F).: Bars with different lowercase letters indicate significant differences between biofilms formed at the same temperature and * indicate significant difference between biofilms belong to the same strain formed at different temperature based on Tukey's Honestly Significant Difference test ANOVA test ($P < 0.05$). Error bars indicate standard error.

Table 4
Average biofilm formation of six strains of *L. monocytogenes* in cantaloupe extracts under different conditions.

Age of biofilm (days)	Cantaloupe extract	Average biofilm formation of *L. monocytogenes* (log CFU/coupon)			
		50 mg/ml		2 mg/ml	
		10 °C	22 °C	10 °C	22 °C
1	Flesh	2.2 ± 0.1^{aA}	5.5 ± 0.2^{aC}	2.0 ± 0.1^{aA}	4.1 ± 0.1^{aB}
4	Flesh	3.8 ± 0.1^{bA}	7.0 ± 0.1^{bC}	3.5 ± 0.1^{bA}	5.5 ± 0.2^{bB}
7	Flesh	5.2 ± 0.1^{cB}	7.0 ± 0.1^{bC}	4.0 ± 0.2^{bA}	5.6 ± 0.1^{bB}
1	Peel	2.1 ± 0.18^{aA}	6.0 ± 0.10^{aC}	2.1 ± 0.04^{aA}	4.3 ± 0.12^{aB}
4	Peel	4.1 ± 0.07^{bA}	6.9 ± 0.09^{bC}	3.5 ± 0.02^{bA}	5.3 ± 0.07^{bB}
7	Peel	5.7 ± 0.08^{cB}	7.1 ± 0.06^{bC}	4.2 ± 0.13^{bA}	5.3 ± 0.09^{bB}

Numbers in the same column of the same extract sharing different lowercase letters or numbers in the same raw sharing different upper case letters are significantly different.

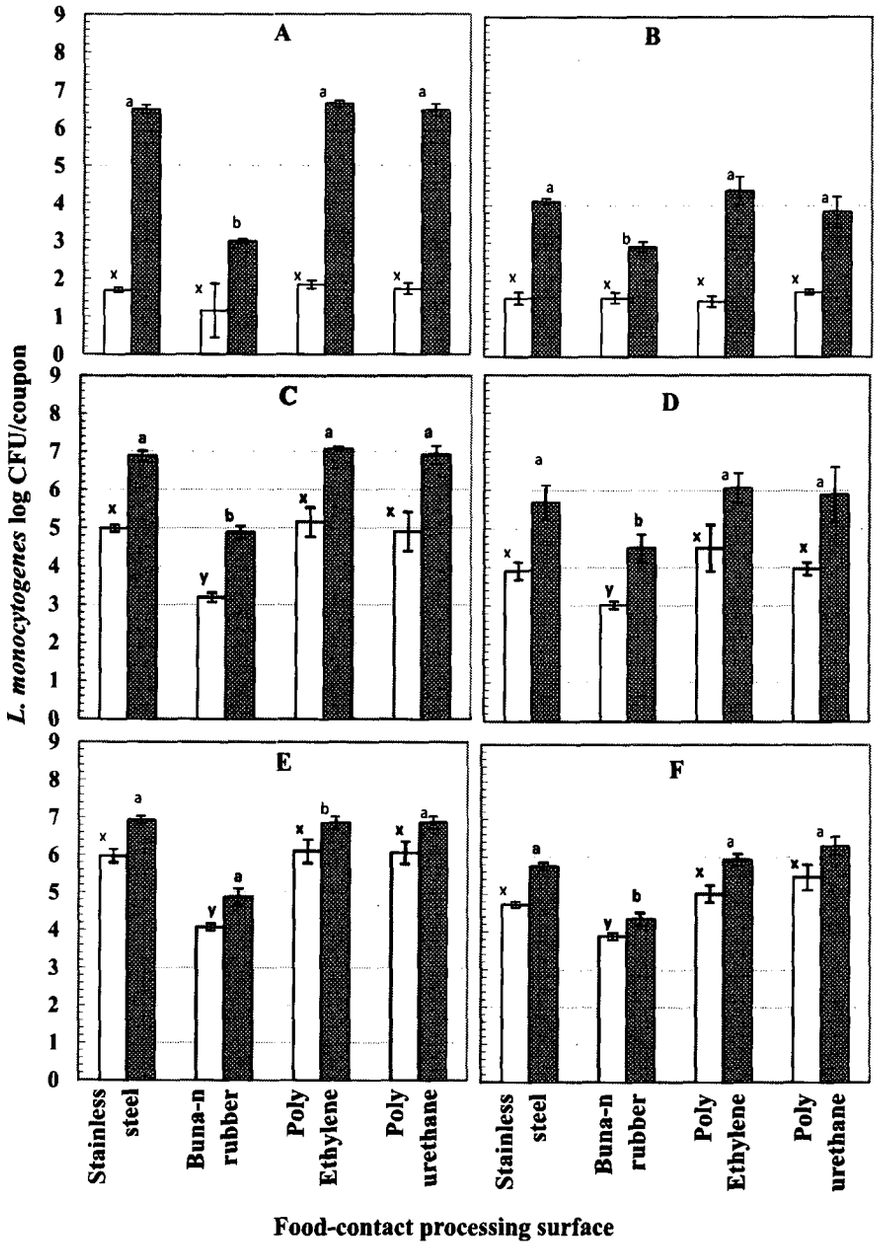

Fig. 5. Biofilm formation by *L. monocytogenes* 2011L-2625 on four food-contact processing surfaces in 50 mg/ml (A, C, E) and 2 mg/ml (B, D, F) of cantaloupe flesh extract at 10 °C (□) and 22 °C (■) in 1 day (A, B),4 days (C, D) and 7 days (E, F). Bars with different lowercase letters indicate significant differences between biofilms formed at the same temperature.

for 15 min to study the combined effects of high-intensity pulsed electric fields and natural antimicrobials to inactivate pathogenic microorganisms in fruit juices. Oliveira et al. (2014) sterilized fruit juices at 115 °C for 10 min to study the effectiveness of a bacteriophage in reducing *Listeria monocytogenes* in apple, pear and melon juices. Beuchat et al. (1986) studied the growth of *L. monocytogenes* in cabbage juice after autoclaving at 121 °C for 15 min. Preliminary studies revealed that growth of *L. monocytogenes* was similar in autoclaved or filter sterilized cantaloupe flesh or peel extracts with respect to the length of the lag phase or maximum growth in cell population. Autoclaving did not change the pH or the brix value of cantaloupe flesh and peel extracts significantly. The pH of both cantaloupe flesh and peel extracts were around 6.4–6.5 and was optimum for *L. monocytogenes* growth. The brix value indicated that cantaloupe flesh extract contained more soluble solids than the peel extract which was also reflected in the dry weight that was calculated for each extract. This was probably due to the high sugar content in cantaloupe flesh when compared to peel.

This study was conducted at two temperature conditions. The 22 °C condition was chosen as cantaloupe processing operations are mostly done at room temperature. The 10 °C condition was chosen to study how lowering temperature can affect the growth and biofilm production of *L. monocytogenes*. While whole or cut cantaloupe products are recommended to be stored at 2–5 °C, in real world, temperature fluctuations can happen during cold storage environments and in transportation, where temperature may raise up to 10 °C (Bett-Garber, Greene, Lamikanra, Ingram, & Watson, 2011; Koseki & Isobe, 2005). Therefore, observations taken at 10 °C will be useful to understand growth and biofilm formation of *L. monocytogenes* at low temperature conditions in the

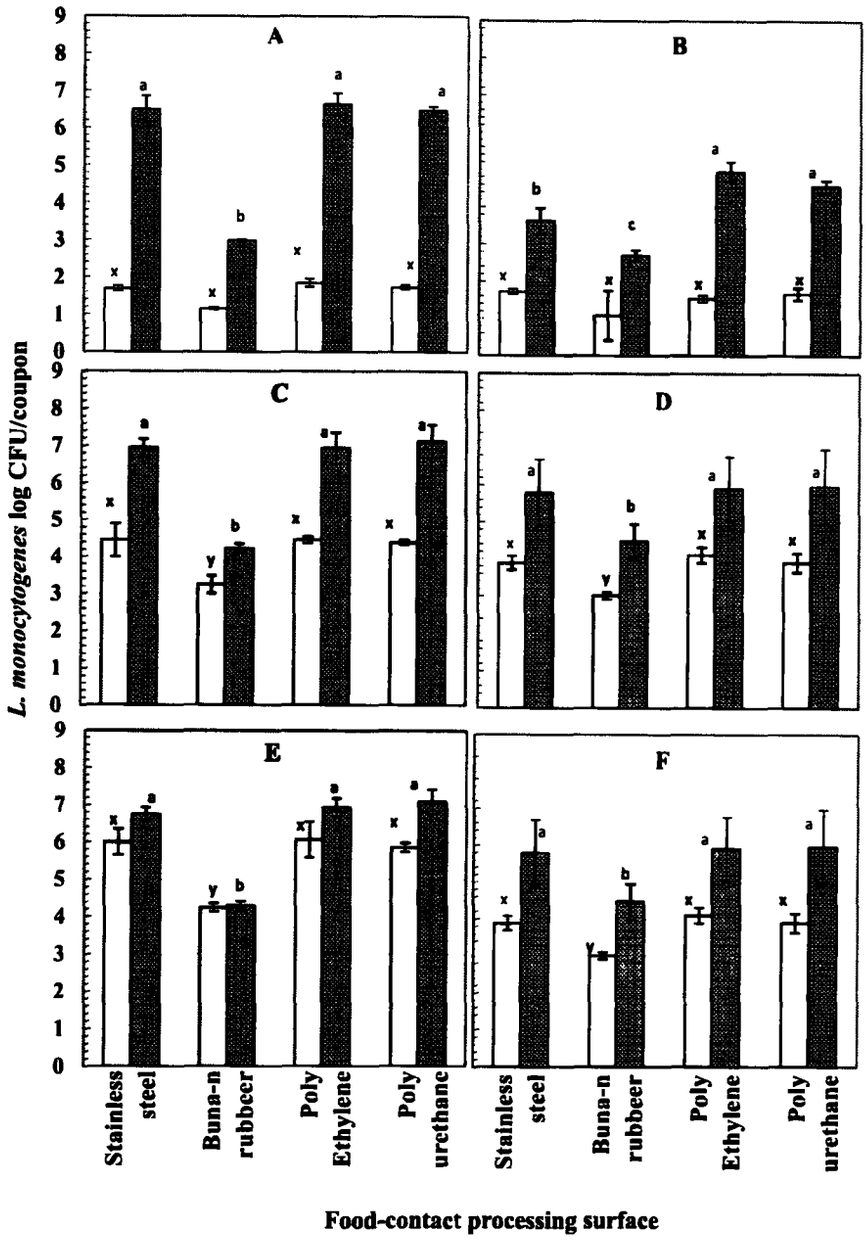

Fig. 6. Biofilm formation by *L. monocytogenes* 2011L-2625 on four food-contact processing surfaces in 50 mg/ml (A, C, E) and 2 mg/ml (B, D, F) of cantaloupe peel extract at 10 °C (□) and 22 °C (■) in 1 day (A, B),4 days (C, D) and 7 days (E, F). Bars with different lowercase letters indicate significant differences between biofilms formed at the same temperature.

presence of cantaloupe residue.

Results indicate that concentration as low as 2 mg/ml of cantaloupe flesh and peel extract is an adequate substrate for *L. monocytogenes* to induce growth. The influence of temperature on the growth rate of *L. monocytogenes* was evident in 50 mg/ml cantaloupe flesh extract where all *L. monocytogenes* strains yielded a doubling time of approximately 2 h at 22 °C and 4–6 h at 10 °C. Similarly, Penteado and Leitao (2004a) and De Modelos (2007) observed that the generation time of *L. monocytogenes* ScottA in cantaloupe pulp increased from 0.84 h to 1.74 h or from 1.74 h to 7.12 h, respectively, when the growth temperature was decreased from 30 °C to 22 °C or from 22 °C to 10 °C.

The concentration of cantaloupe extract in the growth media also affected the generation time of *L. monocytogenes*. For example, for 50 mg/ml cantaloupe extracts, *L. monocytogenes* strains yielded a generation time of approximately 2 h as compared to 4.5–5.5 h for 2 mg/ml cantaloupe extract at 22 °C. Overall, the lesser temperature and decreasing nutrient concentration in the growth media, there was an increase in the doubling time that inversely decreased the growth rate of *L. monocytogenes*.

Results from the current study indicate that low temperature (10 °C) is not a barrier for *L. monocytogenes* growth in cantaloupe processing environments. For example, all *L. monocytogenes* strains showed rapid growth at 10 °C in 50 mg/ml cantaloupe extracts. However, there was no significant growth until 72 h in 2 mg/ml cantaloupe extracts suggesting that the growth of *L. monocytogenes* was inhibited at 10 °C when the available nutrients were limited.

A stainless steel surface was used for the comparison study of biofilm formation by *L. monocytogenes* strains belonging to serotypes 1/2a, 1/2b and 4b since it is the most commonly found surface

in food processing environments (Hilbert, Bagge-Ravn, Kold, & Gram, 2003). With few exceptions, there were no differences ($P < 0.05$) in biofilm formation among the six *L. monocytogenes* strains that were tested in cantaloupe flesh or peel extract under different environmental conditions. Previously, it was reported that *L. monocytogenes* strains belonging to serotype 1/2a formed biofilms with greater bacterial counts than 1/2b and 4b (Borucki et al., 2003). Di Bonaventura et al. (2008) also reported that *L. monocytogenes* strains belonging to serotype 1/2c formed biofilms with greater bacterial counts on stainless steel and glass when compared to strains belonging to serotype 1/2a, 1/2b or 4b at 37 °C. However, it was recently reported that the ability of *L. monocytogenes* to form biofilms depends on the individual strains while the correlation between biofilm formation and *L. monocytogenes* serotype remained inconclusive (Doijad et al., 2015).

L. monocytogenes biofilm formation was greater ($P < 0.05$) at 22 °C when compared to 10 °C in 1, 4 or 7 days in both 50 and 2 mg/ml cantaloupe flesh concentrations. Similarly, Di Bonaventura et al. (2008) reported that *L. monocytogenes* biofilm formation was greater at 37 °C or 22 °C when compared to 12 °C or 4 °C. This may be due to the increase in cell hydrophobicity with increasing temperature, which enhances the subsequent cell attachment to hard surfaces (Chavant, Martinie, Meylheuc, Bellon-Fontaine, & Hebraud, 2002; Di Bonaventura et al., 2008). Flagella induced cell motility is required for cell attachment and the initiation of biofilm formation (Todhanakasem & Young, 2008; Vatanyoopaisarn, Nazli, Dodd, Rees, & Waites, 2000). Di Bonaventura et al. (2008) found that the motility of *L. monocytogenes* was drastically reduced at lower temperature, where among 44 *L. monocytogenes* strains, 30 were motile at 22 °C while only 4 strains were motile at 12 °C. This suggests that at 10 °C, the decreased motility of *L. monocytogenes* could result in lower cell attachment and subsequently less biofilm formation on stainless steel as compared to 22 °C in cantaloupe extract. Ochiai et al. (2014) reported that the persistent *L. monocytogenes* strains that were obtained from a chicken processing plant had enhanced ability to alter biofilm formation in response to changing the temperature from 30 °C to 37 °C when compared to non-persistent *L. monocytogenes* strains. These findings suggest that regulation of biofilm formation through temperature control in *L. monocytogenes* may be significant for its survival and growth in food processing environments.

Biofilm formation of foodborne pathogens under very low concentrations of nutrients has been studied by many researchers (Solomon, Niemira, Sapers, & Annous, 2005; Stepanović, Ćirković, & Ranin, 2004; Yang, Khoo, Zheng, Chung, & Yuk, 2014). Foodborne pathogens may have access to different levels of nutrients depending on where they persist in food processing facilities. In the present study, it was observed that the decreasing cantaloupe extract concentration from 50 to 2 mg/ml decreased *L. monocytogenes* biofilm formation at 22 °C and 10 °C in 7 days. Stanley and Lazazzera (2004) reported that starvation could induce more biofilm formation in *Bacillus subtilis*. The starvation condition of *Bacillus subtilis* promoted higher activation of transcription factors such as RpoS and Spo0A that are needed for biofilm formation. Yang et al. (2014) reported that *S. Enteridis* produced more cellulose in the extracellular polymeric substances matrix of biofilm when grown in 1/20 TSB as compared to an un diluted TSB. However, the effects of nutrient density of the growth medium on biofilm formation may depend on the nature of the microorganism. Stepanović et al. (2004) observed that *L. monocytogenes* produced a greater amount of biofilm in TSB when compared to 1/20 TSB, while *Salmonella* spp. showed the opposite behavior. Similarly, Solomon et al. (2005) reported that *Salmonella* spp. from clinical samples promoted greater biofilm formation in 1/20 TSB when compared to TSB while the meat isolate *Salmonella* spp. from meat samples did not differ in biofilm formation as the nutrient density in the growth media varied.

Various materials such as stainless steel, glass, polyurethane, buna-n rubber and polyethylene are used in the food processing and retail environments as food-contact surfaces. *L. monocytogenes* cells adhere to a wide variety of surfaces found in the food processing and retail environments (Beresford, Andrew, & Shama, 2001). It was observed that *L. monocytogenes* formed biofilms on stainless steel, buna-n rubber, polyethylene, polyurethane surfaces in cantaloupe extracts at 50 and 2 mg/ml concentrations at both 22 °C or 10 °C. However, regardless of the growth temperature or the concentration of cantaloupe extract, *L. monocytogenes* 2011L-2526 formed 1–3.5 logs less biofilm on buna-n rubber as compared to stainless steel, polyethylene or polyurethane. Previously, Ronner and Wong (1993) also reported that *L. monocytogenes* and *Salmonella* formed less biofilm on buna-n rubber than stainless steel surface. Surfaces such as glass and stainless steel are hydrophilic while rubber and plastic surfaces are more hydrophobic (Stepanovi, ć, Ć, irkovi, ć, & Ranin, 2004). Bonsaglia et al. (2014) reported that *L. monocytogenes* formed more biofilm on hydrophilic materials as compared to hydrophobic materials regardless of the growth temperature while Chavant et al. (2002) reported that *L. monocytogenes* biofilm formation was more rapid on hydrophilic surfaces as compared to hydrophobic materials. Even though polyethylene and polyurethane are hydrophobic, biofilm formation on those surfaces was as great as stainless steel in this study.

In summary, results indicate that *L. monocytogenes* can induce significant growth and biofilm formation on different food-contact surfaces in very low concentrations of cantaloupe flesh and peel extracts. The growth and biofilm formation of *L. monocytogenes* was greater in 50 mg/ml cantaloupe flesh or peel extract concentration at 22 °C as compared to 2 mg/ml cantaloupe flesh or peel extract at 10 °C. No major differences were found among the *L. monocytogenes* strains tested for biofilm formation in cantaloupe extracts. *L. monocytogenes* formed less biofilm on buna-n rubber than stainless steel, polyurethane and polyethylene containing cantaloupe extract. These findings illustrate the critical importance of appropriate cleaning and sanitizing of cantaloupe processing surfaces because even the presence of small sediments of cantaloupe residue on the food-contact surfaces can promote growth and subsequent biofilm formation in the processing environments, which creates serious food safety risks.

Acknowledgements

This research was supported in part by the Strategic Research Initiative and Food Safety Initiative awards to RN by the Mississippi Agricultural and Forestry Experiment Station (MAFES) under project MIS-401160. The authors would like to thank Dr. Cathy C. Webb, Center for Food Safety, Department of Food Science and Technology, University of Georgia for providing *L. monocytogenes* outbreak strains used in this study.

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