Biocontrol of *Listeria monocytogenes* ATCC 7644 on fresh-cut tomato (*Lycopersicon esculentum*) using nisin combined with organic acids

Adebola Olubukola Oladunjoye*, Suren Singh, and Oluwatosin Ademola Ijabadeniyi

Department of Biotechnology and Food Technology, Durban University of Technology, Durban 4001, South Africa

Received April 14, 2016
Revised June 13, 2016
Accepted June 14, 2016
Published online October 31, 2016

*Corresponding Author
Tel/Fax: +27611277232
E-mail: debbyoladunjoye@yahoo.com

pISSN 1226-7708
eISSN 2092-6456

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Abstract The biocontrol of *Listeria monocytogenes* on fresh-cut tomato using nisin and organic acids was investigated. Fresh-cut samples inoculated with 10^8 CFU/mL of *L. monocytogenes*, treated with nisin (5,000 IU/mL), a combination of nisin and organic acids (acetic and citric acids at 3 and 5% each), with chlorine at 200 ppm as a control, and stored for six days at 4, 10, and 25°C were used to evaluate certain physicochemical qualities (pH, titratable acidity, soluble solid content, vitamin C content, and color). Nisin treatment significantly ($p < 0.05$) reduced bacterial population by 1.91-3.07 log CFU/mL. Nisin-citric acid combination provided 2.65-3.29 log CFU/mL reduction, while nisin-acetic acid combination provided 2.93-4.15 log CFU/mL reduction. The control treatment provided <1-2 fold log reductions. Slight variations in physicochemical properties of fresh-cut tomato were observed. Nisin and organic acids can be used to improve the microbial safety of fresh-cut tomato.

Keywords: *Listeria monocytogenes*, fresh-cut tomato, nisin, organic acids

Introduction

Recently, fresh produce consumption has continued to attract consumers' interest globally because of its perceived health and nutritional benefits (1). Fresh and minimally processed tomato is an important vegetable of interest in the fresh produce industry because of its extensive utilization and composition of health-promoting constituents (2). Meanwhile, because of certain pre-harvest and post-harvest factors in the food chain, this type of fresh produce has become susceptible to microbial contaminations (3). Consequently, an increasing number of cases of diseases and product recalls due to bacterial pathogens such as *Listeria monocytogenes* has been reported (4).

The abovementioned issue has prompted the United States Food and Drug Administration and United States Department of Agriculture Food Safety Inspection Service to enact a "zero tolerance" policy on this pathogen in ready-to-eat foods. *Listeria monocytogenes*, a gram positive, psychrotrophic microorganism, has been reported as an etiologic foodborne pathogen. It has the ability to survive under a wide range of environmental conditions such as temperatures of 4-37°C and high osmotic conditions and in the presence of common sanitizers (5). This recalcitrant pathogen has also been reported as a contaminant in a broad range of fresh and minimally processed produce in the USA and other developed countries with severe

clinical cases and deaths (4,6).

As a control measure, the use of chlorine as a sanitizer has been conventionally adopted to reduce microbial contamination on fresh produce prior to onward processing (7,8). However, its continuous usage has been discouraged in recent times because of toxicity concerns on the nutritional make-up of the food and health of a processor (9). This has elicited the demand for the use of less hazardous and eco-friendly intervention measures such as the use of nisin and organic acids.

Nisin is a bacteriocin that contains cationic peptides produced by some species of lactic acid bacteria, mostly *Lactococcus lactis* subsp. *lactis*. It is known to exhibit a bactericidal effect on Gram-positive bacteria (10). Nisin demonstrates this effect by creating pore channels within bacterial cell membrane via a charged potential difference between the peptide and membrane, thus disrupting the fusion of peptidoglycan cell walls (11).

This low-mass peptide is a biological and natural preservative substance that has attained a generally regarded as safe status by USA Food Drug and Administration, thus enhancing its utilization in the food industry (12). It is also commercially available, and its successful application stems from its broad range of bactericidal action against Gram-positive bacteria (13). On the other hand, organic acids exhibit an antimicrobial effect primarily by reducing the pH of the cellular environment. They also cause a disruption of

material transport, and to a relatively lesser extent, they damage and increase the permeability of cytoplasmic membrane. They are considered as inexpensive natural agents of a control as they are less deleterious to both personnel's health and processing equipment, with enhanced consumer perception (14).

The demand for nisin and other natural biocidal agents such as organic acids had been on the increase in recent times to assuage post-treatment health risks associated with chemical sanitizers (11). However, the efficacy of nisin has been reportedly compromised by the development of certain nisin-resistance mechanisms in Gram-positive bacteria such as *L. monocytogenes*, which includes the modification of a cell wall (15). Nevertheless, the use of nisin and organic acids as antimicrobials against this pathogen has been reported by other authors in certain food commodities. For instance, Samelis *et al.* (16) reported no growth in *L. monocytogenes* population for the first 90 days when 5,000 IU/mL nisin combined with 3–5% acetic acid in sliced pork bologna stored at 4°C in vacuum packages.

Similarly, Bari *et al.* (17) reported 2.20–4.35 log reduction in mung bean, cabbage, and broccoli when 50 µg/mL nisin was combined with small concentrations of organic salts and acids. However, limited information is available on the use of this synergy against *L. monocytogenes* on fresh-cut tomato. It is assumed that the synergistic effect of these antimicrobials in controlling this opportunistic pathogen of public health concern in tomato will enhance its safety as this fresh produce is often consumed and utilized by consumers of various age ranges.

Materials and Methods

Preparation of fresh produce Fresh tomatoes were obtained from a local grocery supermarket (Woolworth supermarket, Durban, South Africa). Prior to experimental studies, produce was washed with distilled water, surface disinfected with 70% ethanol, and allowed to dry at room temperature before cutting into 10 mm-thick slices.

Bacteria and preparation of inoculum The procedure for bacteria preparation and inoculation was performed by using a modified method reported by Oliveira *et al.* (18). *Listeria monocytogenes* serovar 1/2c (ATCC 7644) frozen stock culture (Merck, Kenilworth, NJ, USA) was allowed to thaw in a water bath at 25°C for 3 min. The thawed culture was grown in a 50 mL tryptone Fraser broth base (CM0895; Oxoid Ltd., Basingstoke, UK) with a supplement (5 g/L yeast extract, 1.35 g/L potassium hydrogen phosphate, 12 g/L disodium hydrogen phosphate) and thereafter incubated at 37°C for 20–24 h. Bacteria cells were harvested by centrifugation (5810R; Eppendorf, Hamburg, Germany) at 3,000xg for 15 min at 4°C and resuspended in a sterile saline peptone solution (8.5% NaCl and 1% peptone). A cell suspension of 10⁸ CFU/mL was finally prepared using a McFarland standard solution (0.05 mL of 1.175% BaCl₂·H₂O and 9.95 mL of 1% H₂SO₄).

Preparation of antimicrobials and sample inoculation Procedure for sample inoculation and antimicrobial treatment was performed by modifying the method described by Samelis *et al.* (19). Nisin (Sigma-Aldrich, St. Louis, MO, USA) of 0.5 g/100 mL, which is equal to 5,000 IU/mL, was prepared and stored at refrigeration temperature, while the concentration of organic acids-acetic and citric acid (Merck) was each prepared at 3 and 5 g/100 mL. Chlorine solution with a concentration of 200 mg/L was used as a control. Slices of tomato were placed on an aluminum foil under a biohazard hood, and 0.25 mL of inoculum was deposited on both sides of each slice using a sterile bent glass rod on the surface. Inoculated slices were left to stand separately at 5°C for 15 min to allow attachment and were later dipped into 50 mL in each of the various antimicrobial solutions at room temperature for a minute. Solutions were poured aseptically into pre-sterilized stainless steel pots equipped with strainers to facilitate dipping and draining for 1 min each. Tomato slices were then stored in a perforated stomacher bag (Tuffy Brands Pty., Cape Town, South Africa) at different temperatures (4, 10, and 25°C) at intervals of two days for six days to ascertain the level of reduction.

Bacterial recovery analysis Recovery of *L. monocytogenes* ATCC 7644 populations from the slices was performed after six days at each of the storage temperatures using the method reported by (20). Herein, 9 mL of distilled buffered peptone water was added to tomato slices placed in the perforated stomacher bag and blended in a homogenizer for 120 s at high speed. Aliquots of the mixture were serially diluted in the sterile saline peptone solution, and 100 µL was spread plated on a *Listeria* agar base (Oxoid Ltd.) with a *Listeria* selective supplement (Sigma-Aldrich). Colonies were counted after incubation at 37°C for 48 h using a Doc-it colony counter (Colony Doc-It Imaging station, UVP Analytik, Jena, Germany). All treatments were conducted in triplicate.

Physicochemical quality of fresh-cut produce The physicochemical quality (pH, soluble solid content, titratable acidity, vitamin C content, and color) of fresh-cut produce were determined after treatment. pH was determined using a penetration electrode pH meter (Model Basic 2°; Crison Instrument, Barcelona, Spain).

Total soluble solid (TSS) was determined using a handheld refractometer (Digital Refractometer PR-201α, Atago Coy Ltd., Tokyo, Japan) in a juice extracted from the blended fresh-cut produce.

Total titratable acidity (TTA) was determined by taking 10 mL of a homogenized sample and diluting with 10 mL of distilled water. Diluted sample was titrated with 0.1 N NaOH up to 8. The results were calculated as g of citric acid/L.

For vitamin C content determination, 10 g of fresh-cut sample was blended with about 50 mL of an acetic acid solution and transferred into a 100 mL volumetric flask. The solution was gently shaken to have a homogenous dispersion, which was then filtered. Few drops of bromine water solutions were added to the filtrate to obtain a

colored solution for ascorbic acid oxidation, followed by the addition of few drops of thiourea solutions for the removal of excess bromine. Finally, a 2,4-dinitrophenylhydrazine solution was added together with the standard, and vitamin C content was determined using a UV spectrophotometer (Jenway 7305 Spectrophotometer; Bibby Scientific Ltd., Stone, UK). Color CIE L*, a*, and b* values were determined using a colorimeter (Color flex EZ 0840, Hunterlab, VA, USA). Each experiment was performed three times and results were obtained in triplicates.

Statistical analysis All experiments were replicated three times with each of the treatments. Data obtained were subjected to the analysis of variance and means separated using the Duncan multiple range test ($p < 0.05$). Results were analyzed using the SPSS software package (SPSS Inc., Chicago, IL, USA).

Results and Discussion

Effects of anti-microbial treatments on *Listeria monocytogenes* population The effects of antimicrobial treatments on the growth of *L. monocytogenes* population in fresh-cut tomato stored at 4, 10, and 25°C for six days are shown in Fig. 1. Generally, there was a decrease in the log reduction of the pathogen with increasing storage temperature. This trend may be attributed to the recalcitrant nature of the pathogen. *L. monocytogenes* has been found to survive under a long range of temperature conditions of -2-45°C (5).

All natural antimicrobials resulted in a greater log reduction of the pathogen in the tomato slices compared to the control chlorine treatment. This can be associated with the development of chlorine-resistant mutants by the bacteria on fresh-cut produce (21). The pathogen has an inclined tendency to aggregate together, especially on a solid matrix to form biofilms that resist common sanitizers such as chlorine. However, this resistance has been found to anchor on certain factors such as the type of strain, physicochemical properties of a substrate for attachment, bacterial growth phase, and temperature (22). Similar work reported by Singh *et al.* (23) showed an enhanced resistance of the pathogen's biofilm to a chlorine sanitizer. Furthermore, increased storage temperature and low pH of the internal tissue of fresh produce, which might account for a reduced antimicrobial activity of chlorine, have been previously reported (24).

Among natural antimicrobials, acetic acid seems to enhance the antilisterial capacity of nisin on fresh-cut tomato since it provides slightly higher log reduction (2-4 fold) when combined with nisin at either 3 or 5%. Previous work of this combination on a meat product has been reported, with no significant growth of *L. monocytogenes* population in the first 90 days of storage at 4°C (16). Expectedly, nisin reduced the population growth of this pathogen, which further enhanced the effect of acetic acid on fresh-cut produce. Low pH of food and pKa, which is the acid dissociation constant, has been found to enhance the antimicrobial activity of organic acids (25). Nisin acts

on its target cells via the formation of permeable pore channels on the cytoplasmic membrane, leading to the removal of cellular substances (10). Many countries have reportedly approved the commercial uses of nisin and organic acids as preservatives in food, while organic acids and their salts act as antioxidants and prevent the development of browning in fresh-cut produce (26). However, nisin alone or in combination with citric acid (3 or 5%) resulted in similar log reduction values (2-3 fold). Hoelzer *et al.* (27) reported similar log reduction values of this pathogen in cabbage and strawberry treated with nisin and citric acid. These similar log reduction values suggest that citric acid did not significantly influence the antilisterial capacity of nisin. Using nisin alone or in combination with citric acid, Bari *et al.* (17) reported similar results when working with fresh-cut cabbage and mung bean.

Effects of antimicrobial treatment on some physicochemical parameters of fresh-cut tomato slices

The effects of nisin treatment alone and in combination with organic acids on some physicochemical properties of fresh-cut tomato stored for zero and six days are reported in Table 1. Generally, with increasing storage temperature, slight variations in the physicochemical parameters compared to the control were observed. The period of storage also resulted in slight changes of these parameters. All natural antimicrobials slightly reduced the pH of tomato slices (Table 1). pH slightly increased with storage temperature and period. pH contributes to acidity, which is an important factor contributing to tomato's flavor and microbial safety. The slightly lower pH observed in the treated samples compared to control sample stored at 4°C may explain the higher log reductions of *L. monocytogenes* to other storage temperatures (Fig. 1). Temperature remains an important factor in determining the storage quality of fresh or minimally processed produce as most biochemical reactions that occur are temperature dependent (28). It has been suggested that the storage of tomato at 10-15°C can provide optimum temperature to enhance its shelf stability for five to seven days (28,29). Presumably, fresh cut might need a much lower temperature because of exposed internal tissues (30). However, possibility of chilling injury must also be prevented. Previously, some authors have reported the antimicrobial efficacy of nisin to increase with a decrease in pH to below 5 (31). Therefore, it can be inferred that the intrinsic low-acid condition of fresh-cut produce and decreasing nature of pH by organic acids will enhance the shelf stability of the produce when stored at low temperatures by this antimicrobial combinations. The TSSs of the treated samples were not very different from that of the control but slightly reduced with storage temperature and period (Table 1). Our result is in agreement with those of previous studies on tomato (32). TSS indicates the acid-sugar ratio of fresh produce and inversely correlates to the fruit size. TTA similarly decreased as the storage temperature increases. This result agrees with findings reported by Beckles (33). This decrease can be linked to a loss of organic acid content. TTA represents the degree of acid breakdown to sugar, which indicates the level of

Fig. 1. Population growth of *L. monocytogenes* treated with nisin alone and with a combination of nisin and organic acids on fresh-cut tomato stored at 4°C (A), 10°C (B), and 25°C (C). Ns-nisin, Ca-citric acid, Ac-acetic acid, CL-chlorine control.

sweetness of fresh-cut produce. It also shows the maturity index of fresh produce. However, nisin in combination with other antimicrobials had no significant difference in vitamin C content in tomato slices compared to control samples (Table 1). These vitamin C values decreased with increased storage temperature. This decrease can be associated with temperature sensitivity which has been found to accelerate the breakdown of the vitamin of the exposed tissues. Also, previous studies have reported that vitamin C is more stable under acidic conditions, but its loss can be accelerated by chilling injury (34). Hence, a balance should be ensured between the nature

of preservatives and temperature of storage. Some natural antimicrobials have been reported to increase vitamin C content of fresh produce through a metabolic process (35). The chroma a^* value, which represents the degree of redness, was significantly ($p \leq 0.05$) higher for the treated samples compared with the control. The quality and composition of tomato can change during processing and storage. However, the presence of organic acids and nisin, which requires low pH to function, has been reported to contribute to tomato ripening (36). Furthermore, the authors of (36) suggested that this process can lead to the biosynthesis of lycopene, which

Table 1. Effects of antimicrobial treatment on physicochemical parameters (pH, total soluble solid (TSS, °Brix) content, total titratable acidity (TTA, g citric acid/L), and color (CIE L* a* b*) of fresh-cut tomato stored for six days at 4, 10, and 25°C

Parameter	Day 0							Day 6						
	pH	TSS	TTA	Vit C	L*	a*	b*	pH	TSS	TTA	Vit C	L*	a*	b*
Tomato (4°C)														
Control	4.72 ^{1),b,2)}	5.70 ^b	0.75 ^a	1.18 ^a	44.28 ^d	14.95 ^a	18.92 ^a	4.77 ^b	4.50 ^a	0.69 ^a	1.16 ^a	40.96 ^b	15.31 ^a	20.02 ^a
Nisin	4.63 ^a	5.71 ^a	0.77 ^a	1.17 ^a	34.69 ^a	15.05 ^b	19.27 ^b	4.65 ^a	4.50 ^a	0.73 ^{ab}	1.15 ^a	39.36 ^a	15.59 ^c	23.90 ^b
Ns+Ca3%	4.60 ^a	5.75 ^a	0.79 ^a	1.16 ^a	35.59 ^b	15.07 ^b	19.24 ^b	4.64 ^a	4.60 ^a	0.70 ^a	1.14 ^a	36.89 ^a	15.97 ^b	23.26 ^b
Ns+Ca5%	4.57 ^a	5.79 ^a	0.79 ^a	1.16 ^a	38.20 ^c	15.03 ^b	19.21 ^b	4.59 ^a	4.65 ^a	0.70 ^a	1.14 ^a	36.69 ^a	15.40 ^b	23.65 ^b
Ns+Ac3%	4.61 ^a	5.71 ^a	0.78 ^a	1.18 ^a	38.64 ^c	15.89 ^b	20.42 ^c	4.64 ^a	4.55 ^a	0.67 ^a	1.16 ^a	39.53 ^a	15.54 ^c	24.02 ^c
Ns+Ac5%	4.59 ^a	5.71 ^a	0.79 ^a	1.18 ^a	38.69 ^c	15.56 ^b	20.43 ^c	4.61 ^a	4.55 ^a	0.68 ^a	1.16 ^a	39.56 ^a	15.91 ^c	24.09 ^c
Tomato (10°C)														
Control	4.72 ^b	5.60 ^a	0.72 ^a	1.16 ^a	45.22 ^a	16.33 ^a	19.63 ^a	4.78 ^{ab}	4.40 ^a	0.63 ^a	1.16 ^a	46.58 ^d	16.69 ^a	12.09 ^a
Nisin	4.64 ^a	5.60 ^a	0.74 ^a	1.15 ^a	45.60 ^a	23.25 ^c	32.38 ^e	4.72 ^{ab}	4.60 ^a	0.65 ^a	1.17 ^a	32.98 ^a	23.95 ^c	24.03 ^b
Ns+Ca3%	4.61 ^a	5.65 ^a	0.73 ^a	1.16 ^a	47.53 ^a	20.37 ^b	24.54 ^c	4.67 ^a	4.43 ^a	0.66 ^a	1.18 ^a	36.63 ^c	20.87 ^b	24.26 ^b
Ns+Ca5%	4.59 ^a	5.61 ^a	0.73 ^a	1.16 ^a	47.98 ^a	20.53 ^b	28.25 ^d	4.62 ^a	4.50 ^a	0.66 ^a	1.18 ^a	35.28 ^b	20.55 ^b	24.41 ^b
Ns+Ac3%	4.63 ^a	5.65 ^a	0.74 ^a	1.15 ^a	49.49 ^a	25.75 ^d	22.37 ^b	4.68 ^a	4.60 ^a	0.69 ^a	1.16 ^a	37.73 ^d	25.88 ^c	24.36 ^b
Ns+Ac5%	4.62 ^a	5.60 ^a	0.75 ^a	1.15 ^a	49.51 ^a	25.76 ^d	22.39 ^b	4.69 ^a	4.60 ^a	0.69 ^a	1.16 ^a	37.78 ^d	25.89 ^c	24.39 ^b
Tomato (25°C)														
Control	4.74 ^b	5.50 ^a	0.57 ^a	0.88 ^a	62.13 ^d	13.01 ^a	27.73 ^b	4.74 ^a	4.20 ^a	0.53 ^b	0.89 ^a	13.90 ^d	13.03 ^c	28.58 ^c
Nisin	4.65 ^a	5.55 ^a	0.57 ^a	0.87 ^a	53.53 ^c	14.18 ^b	27.33 ^b	4.68 ^a	4.27 ^a	0.47 ^a	0.93 ^a	14.51 ^c	14.01 ^c	26.43 ^c
Ns+Ca3%	4.61 ^a	5.51 ^a	0.58 ^a	0.88 ^a	42.24 ^b	14.89 ^b	23.58 ^a	4.72 ^a	4.27 ^a	0.49 ^a	0.91 ^a	14.25 ^b	14.77 ^a	23.51 ^a
Ns+Ca5%	4.69 ^a	5.59 ^a	0.57 ^a	0.88 ^a	42.31 ^b	14.12 ^b	23.68 ^a	4.71 ^a	4.23 ^a	0.49 ^a	0.91 ^a	37.15 ^b	14.17 ^a	25.62 ^b
Ns+Ac3%	4.69 ^a	5.50 ^a	0.58 ^a	0.87 ^a	41.27 ^a	14.75 ^b	23.51 ^a	4.79 ^a	4.24 ^a	0.47 ^a	0.89 ^a	36.09 ^a	14.81 ^b	23.21 ^a
Ns+Ac5%	4.68 ^a	5.56 ^a	0.56 ^a	0.87 ^a	41.29 ^a	14.77 ^b	23.57 ^a	4.75 ^a	4.21 ^a	0.45 ^a	0.92 ^a	36.12 ^a	14.79 ^b	26.19 ^c

¹⁾Values are means of three replicate experiments.²⁾Mean values in the same column with the same superscripts are not significantly different ($p \leq 0.05$).

enhances red pigmentation in tomato. Color is an important factor of maturity and quality indices, economic value, and consumer perception of tomato. It reflects the concentration of a pigment called lycopene, an antioxidant that plays health-promoting functions in fresh produce. The treatment of fresh tomato slices using nisin and a combination of nisin and other organic acids resulted in a significant reduction of inoculated *L. monocytogenes*. Nisin-acetic acid combination provided the lowest reduction values for the pathogen at the storage temperatures, while chlorine control was the least effective sanitizer. The results of this study showed the promising potential synergy of nisin with organic acids in controlling *L. monocytogenes* and improving the safety and keeping quality of fresh-cut tomato.

Disclosure The authors declare no conflict of interest.

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