Antimicrobial Peptides from *Lactobacillus plantarum* UTNGt2 Prevent Harmful Bacteria Growth on Fresh Tomatoes

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In a previous study, the antimicrobial peptides extracted from *Lactobacillus plantarum* UTNGt2 of wild-type fruits of *Theobroma grandiflorum* (Amazon) were characterized. This study aimed to investigate the antimicrobial mechanisms of peptides in vitro and its protective effect on fresh tomatoes. The addition of partially purified Gt2 peptides to the *E. coli* suspension cells at the exponential ($OD_{605} = 0.7$) growth phase resulted in a decrease with 1.67 (log10) order of magnitude compared to the control without peptide. A marginal event (< 1 log10 difference) was recorded against *Salmonella*, while no effect was observed when combined with EDTA, suggesting that the presence of a chelating agent interfered with the antimicrobial activity. The Gt2 peptides disrupted the membrane of *E. coli*, causing the release of β -galactosidase and leakage of DNA/RNA molecules followed by cell death, revealing a bacteriolytic mode of action. The tomatoes fruits coated with Gt2 peptides showed growth inhibition of the artificially inoculated *Salmonella* cocktail, demonstrating their preservative potential.

Keywords: Antimicrobial peptides, plantaricin W, preservation, tomatoes, bacteriolytic

Introduction

Foodborne diseases are a growing public health problem worldwide, particularly for infants, children and the elderly. Despite advances in food safety, each year more than 9 million people develop illnesses caused by food contamination [1]. A recent report indicated that salmonellosis and campylobacteriosis are the most commonly foodborne diseases within the European region [2]. In Ecuador (2001), the Ministry of Public Health reported 311,401 cases of infections with *Salmonella* and *Shigella*, while the number doubled in 2017 [3]. At the local facilities, artisanal fresh and minimally processed food products are stored in defective conditions or incorrectly manipulated, therefore, the risk of contamination is significant [4]. It might be a cultural phenomenon as no improvement of selling sites has been undertaken. Consequently, the attention of policymakers was assumed to increase control, prevention and consumer protection by improving communication regarding food safety among producers, packers, processors and distributors and by facilitating

relevant research on food preservation. A recent investigation in Brazil suggested the need for continuous training of vendors [5]. Commonly, chemical and physical preservation protocols are used to control the presence of pathogenic or spoilage bacteria in food, nonetheless, the application of natural methods for preservation remains underdeveloped.

Lactic acid bacteria (LAB) have attracted significant attention to the food industry due to their GRAS (Generally Regarded as Safe) status [6]. Antimicrobial substances (bacteriocins and organic acids) produced by several LAB strains have been proven to be effective in controlling growth of microorganisms in several food products [7–10]. Thus, their incorporation in food has become an area of dynamic research in the last decade [9, 11, 12]. A large arsenal of bacteriocins with different properties, target species, and producer organisms has been characterized [13]. The majority of bacteriocins belong to class I and II, are active in the nanomolar range, and cause membrane permeabilization leading to the dissipation of membrane potential, the leakage of ions, ATP, as well as DNA/RNA

molecules from the target bacteria [14].

Recently, we reported a *Lactobacillus plantarum* Gt2 strain producing peptides having similarity with plantaricin W alpha and beta precursors showing stable inhibitory activity at a high temperature range (60–121°C), wide pH range (2–10), and positively activated by several ionic and anionic compounds [4]. Nonetheless, the actual mechanism by which partially purified Gt2 peptides target Gram-negative bacteria remains unknown. The aim of this research was to determine how these peptides act against some commensal ubiquitous food microorganisms (*Salmonella* and *E. coli*) and to evaluate their protective effect on postharvest fresh tomatoes for further use as natural preservatives.

Materials and Methods

Bacterial Strains

Lactobacillus plantarum UTNGt2 (GenBank Accession No. KY041688.1) was isolated from tropical wild-type fruits of *Theobroma grandiflorum* (cupoasu or white coffee) collected from the Sucumbíos Province, in the Amazon region of Ecuador. A bacteriocin producer strain *Lactobacillus plantarum* ATCC 8014 (LP) was used as a standard [15]. The indicator strains were: *E. coli* ATCC 25922, *Salmonella enterica* subsp. *enterica* ATCC 51741.

Partially Purified Peptide Preparation

The partially purified peptides from both Gt2 and LP strains were obtained as previously described [4]. Briefly, the Gt2 and LP strains grown in MRS broth at 37°C for 24 h were used to extract cell free supernatant (CFS) by centrifugation at 13,000 ×g for 30 min (4°C). The CFS was filtered using a 0.22 µm porosity syringe filter (# STF020025H, ChemLab Group, USA). Then, 60% ammonium sulfate was added to CFS, incubated overnight with refrigeration without stirring and centrifuged at 8,000 ×g for 30 min at 4°C. The peptides were recovered in 25 mM ammonium acetate (pH 6.5), desalted by using a Midi Dialysis Kit (Cat. # PURD10005-1KT, Sigma-Aldrich, USA) pre-equilibrated with phosphate buffer (pH 7.0) and stored at –20°C before use. Titer estimated as $\text{au} \cdot \text{ml}^{-1}$ is defined as the highest dilution that inhibited the growth of the indicator strain. Antimicrobial activity was analyzed using the agar-well diffusion method [4].

Effect of Gt2 Peptides on Indicator Cell Viability

The indicator bacteria *E. coli* ATCC 25922 and *Salmonella enterica* ATCC 51741 were grown independently in tubes containing LB (Luria Bertani, Difco) and nutrient broth medium (Difco). The peptides at the final concentration 1 MIC [16] were added to the indicator strain culture (100 ml) at the exponential growth phase ($\text{OD}_{605} = 0.7$) followed by incubation at 37°C for 6 h using the plate-agar method to determine the viable cell counts at given incubation time intervals. In independent experiments, the effect

of EDTA on Gt2 activity was evaluated [16]. The results were presented as log₁₀ reductions, calculated as the difference between log₁₀ (CFU) of the untreated cells (no bacteriocin, no EDTA) and the treated cells (bacteriocin added, EDTA or a combination thereof). A log₁₀ reduction of < 1 was considered insignificant. The experiments were repeated three times and untreated indicator strain cultures were used as controls.

Cell Membrane Integrity Assay

If the bacterial membrane of the target cells is compromised, the cellular components (DNA/RNA molecules) can be released and detected by measuring the absorbance at 260 nm [17]. The indicator bacterial suspensions (*E. coli* and *Salmonella*) were grown overnight in appropriate broth culture media, harvested by centrifugation and washed twice with 1 × PBS (phosphate-buffered saline, pH 7.5). The bacterial cells were treated independently with peptides Gt2 (1 MIC), peptides LP (1 MIC) and nisin from *Lactococcus lactis* 2.5% (# N5764, Sigma) (50 µg/ml) and incubated at 30°C. One flask was maintained as control (no peptides). Cell cultures were centrifuged after one hour incubation, the supernatant filtered, while optical density was measured using a spectrophotometer (Nova60, Millipore, Merck). Subsequently, the supernatant was precipitated with isopropanol and ammonium acetate (3M), washed with 75% ethanol, followed by electrophoresis in 1% agarose gel with ethidium bromide, running in 1 × TBE (Tris-borate EDTA, pH 8.0) buffer (Sigma) to detect released DNA/RNA molecules.

Cytoplasmic Membrane Permeabilization

The *E. coli* cytoplasmic membrane permeabilization by peptides of Gt2, LP (1 MIC final concentration) and nisin (50 µg/ml) were investigated using ONPG (o-nitro-phenyl-L-D-galactoside, # N1127, Sigma, USA) as substrate [17]. Briefly, bacteria cells grown to logarithmic phase in LB (Luria Bertani, Difco) medium containing 2% lactose were collected and washed twice with 10 mM sodium phosphate buffer (pH 7.5) and 100 mM NaCl. The Gt2 and LP peptides and nisin were added to the bacterial suspension at optical density 0.6, incubated 5 min at 30°C, then ONPG with a final concentration of 30 mM was added to each cell suspension. The hydrolysis of ONPG to O-nitrophenol (ONP) over time (120 min) was monitored at 420 nm. To distinguish between the cytoplasmic enzyme release and peptide uptake to the cells, the bacteria were removed by centrifugation for 120 min and the supernatant was used to measure the β-galactosidase release. The release was quantified as production of o-nitrophenol (ONP) at ml/min and calculated as described: $[\text{A}_{415} \times 1,000 / \text{sample volume } (\mu\text{l})] / \text{reaction time (min)} \times 4.86$; where A₄₁₅ was the absorbance at 415 nm and 4.86 was the coefficient of extinction ($\text{mM}^{-1} \text{cm}^{-1}$) of ONP respectively [17].

Effect of Gt2 Peptides on Whole Cell Protein Profile of Target *E. coli* Cells

The whole cell protein profile of *E. coli* after the treatment with

Gt2 peptides was analyzed using SDS-PAGE method [18]. Overnight cultures of *E. coli* were centrifuged at 3,000 rpm, washed twice with $1 \times$ PBS buffer (pH 7.4); the cells' pellet was suspended in $1 \times$ SDS-PAGE loading buffer, boiled for 5 min at 100°C and centrifuged at 300 rpm. The supernatants of treated and non-treated *E. coli* were used in SDS-PAGE electrophoresis. The tricine-SDS-PAGE method using pre-casted acrylamide gels (12%) and a Thermo Fisher OWL (10×10) vertical electrophoresis system was used. The gel was stained with Takara CBB Safe Stain (Cat. # T9320A, Takara, Bio Company, Japan) for 4 h and destained with a solution of 30% methanol (v/v) and glacial acetic acid, 10% (v/v) until the bands became clear.

Inhibitory Effect of Gt2 Peptides on Tomatoes during Storage

Tomatoes chosen at stage four according to the color chart of tomato ripening (USDA standards) were purchased from a local vender, washed with 5% bleach solution for 5 min, then twice with distilled water and left to dry in a laminar flow cabinet before inoculation with the pathogen cocktail. The inoculation was performed with a mixture of *Salmonella enterica* subsp. *enterica* ATCC 51741 and *Salmonella* UTNSm2 (lab collection isolated from contaminated local food). Tomatoes (3 per treatment) were submerged in 200 ml of bacterial inoculum cocktail (10^8 CFU/ml) and rotated by stirring with a glove-covered hand for 10 min to ensure that bacteria penetrated the fruits; followed by air drying for 1 day in a biosafety cabinet. The tomatoes were washed as follows: a) the fruits were individually immersed in peptide Gt2 (1 MIC) solution; b) immersed in peptide LP (1 MIC) solution; c) nisin (50 $\mu\text{g}/\text{ml}$); in a total volume of 200 ml, manually rotating each fruit for 5 min to assure complete coverage and contact of surfaces with the wash solution, and then left to dry overnight under the biosafety cabinet. The fruits were packed in trays covered with plastic alimentary film then stored for 14 days with refrigeration. The tomatoes washed with sterile water, no peptides added, no *Salmonella* (control 1) and tomatoes inoculated with bacterial cocktail but not peptides (control 2) were used as control. At different time intervals (1, 7, and 14 days) the viability of inoculated bacteria was evaluated using the agar plate method [16]. The effect of peptides on hampering the rottenness and fungi forming on tomatoes was evaluated after the treatment with Gt2, LP peptides and nisin, without inoculating with *Salmonella* during storage (23 days). Tomato samples (3 samples per treatment) without any treatment were used as control. Treated and non-treated samples were stored at either room temperature (18°C) or were refrigerated (5°C). The experiment was repeated three times using tomatoes monthly purchased from the same distributor.

Statistical Analysis

All experiments were performed in triplicate; three independent experiments repeated and the results were expressed as mean $\pm$ standard deviation. Analysis of variance was applied with Tukey to determine the significant differences between the means (SPSS version 15.0).

Fig. 1. Effect of the G2 Strain with and without EDTA on *E. coli* ATCC 25922 cell density.

Bars represent log₁₀ reduction calculated as difference between cells with and without bacteriocin; log₁₀ reductions <1 were considered insignificant.

Results and Discussion

Peptides from Gt2 Strain Diminished Target Cell Viability

The initial cell density of *E. coli* was $6.05 \log_{10}$ CFU/ml at the inoculation time with the peptides. The addition of Gt2 peptides resulted in a significant ($p < 0.05$) decrease of cell viability with $1.62 \log_{10}$ relative to the control in the absence of the peptide (Fig. 1). Similarly, the addition of LP peptides resulted in a $1.47 \log_{10}$ reduction. Early studies have demonstrated that some bacteriocins are inactive against Gram-negative bacteria if not combined with chemical or physical treatments that disrupt the outer membrane [19]. For example, the *L. monocytogenes* cell viability reduction was recorded when nisin was combined with EDTA or nisin-sodium-diacetate-EDTA [20, 21]. In the present study, about the same level of cell reduction ($1.67 \log_{10}$ CFU/ml) was achieved when Gt2 peptides were co-treated with EDTA, suggesting that the presence of a chemical agent did interfere with its antimicrobial activity. A marginal decrease ($0.86 \log_{10}$ reduction) of *Salmonella* cell viability was recorded when Gt2 peptides were added only or in combination with EDTA at 6 h of incubation while no clear change (0.23 – $0.27 \log_{10}$) in cell density was registered upon treatment with LP peptides only or combined with EDTA (data not shown). Previously, we showed that EDTA enhanced the inhibitory activity towards *Salmonella* when combined with peptides produced by another LAB strain [16]. Thus, we suggest that

the bactericidal effect might depend on the effectiveness of the producer strain and the level of inhibition varies with the target bacteria. In the present study, the Gt2 peptides showed bacteriolytic effect against pathogens without combining them with an extra destabilizing membrane factor, indicating that the inhibitory activity profile does not follow a general trend.

Peptides Produced by the Gt2 Strain Alter the Target Cell Membrane Integrity

The release of cellular components with strong UV absorption at 260 nm arises when cells are exposed to drugs (e.g. antibiotics, antimicrobial peptides) as indicators of target membrane damage [17]. When *E. coli* suspension cells were treated with Gt2 and LP peptides, the optical density increased rapidly after one hour of incubation (data not shown). Moreover, DNA/RNA molecules were detected in agarose gel electrophoresis, indicating that the target bacterial membrane became compromised (Fig. 2). This result was in agreement with the bactericidal mode of action of Gt2 peptides towards *E. coli*. Earlier studies indicated that some bacteriocins are able to interact with the target bacterial membrane, forming ion channels, leading to the increase of cytoplasmic membrane permeability and hence to death [12, 14, 22, 23]. Similar results were obtained when the peptides were added to *Salmonella* suspension cells, while nisin was underwhelmingly inactive against both *E. coli* and *Salmonella*. Previous research indicated that

Fig. 2. Detection of DNA/RNA molecules released when *Salmonella* was treated with the peptides.

Legend: gDNA- genomic DNA from *Salmonella enterica* subsp. *enterica* ATCC 51741. (-) negative control (no DNA/RNA molecules); LP, Gt2: treatment with peptides (1 h); Nis-nisin (Sigma).

nisin, a Class I lantibiotic bacteriocin, has marginal inhibitory activity towards Gram-negative bacteria and its effect on membrane permeability is enhanced when it is combined with a chelating agent [24–26]. The Gt2 amino acid sequence deduced from the gene nucleotide sequencing concedes to two-component bacteriocin (two-peptide lantibiotics), plw α and plw β of plantaricin W [4], post-transcriptionally modified peptides that work together in one functional inhibitory unit [27]. Although bacteriocins from lactic acid bacteria are smaller peptides, we were in agreement with other authors indicating that the superior inhibitory effect against Gram-negative bacteria might be related with the presence of more than one secreted peptide (e.g. SDS-PAGE analysis showed more than one band corresponding to larger peptides), thus further study is needed to identify the protein fraction(s) responsible for the antimicrobial activity as well as its amino acid composition. The results indicated that Gt2 peptides altered the cellular membrane of target bacteria, leading to the leaching of cytoplasmic molecules followed by cellular death.

Gt2 Peptides Induced Inner Membrane Permeabilization in *E. coli*

Generally, cytoplasmic β -galactosidase cannot pass through the integral bacteria cell membrane unless the inner membrane is compromised. In addition to inhibitory activity, bacteriocins constitute an anti-resistance compound to classic antibiotics as they interact with bacterial membranes, create ion permeable channels leading to increased cytoplasmic permeability, thus resulting in bacterial cell death [28]. The ability of Gt2 peptides to permeate the membrane of *E. coli* was evaluated as a function of cytoplasmic β -galactosidase release, with bacteria grown in lactose-containing medium. When cells were treated with Gt2 or LP, there was a lag time of 30 min followed by a progressive release of β -galactosidase for up to 120 min until reaching a steady state (Fig. 3A). On the other hand, the nisin displayed a marginal effect. No β -galactosidase activity was detected in culture medium of bacterial cells without peptides. Similarly, the peptide F1 produced by *L. paracasei* increased the membrane permeability of *Staphylococcus aureus* [14]. Moreover, the results show that the Gt2 peptides caused considerable release of the enzyme into the medium within the incubation time (Fig. 3B). The fact that the enzyme units in cell-free medium were lower than the determined units suggests that Gt2 raised cytoplasmic membrane permeability to ONPG as well as the outward release of the

Fig. 3. The cytoplasmic membrane permeation of *E. coli* ATCC 25922.

(A) Beta-galactosidase activity (measured from the absorbance at 420 nm) in the culture medium of *E. coli* cells treated with Gt2, LP peptides and nisin. Data were expressed as mean $\pm$ standard deviation; (B) beta-galactosidase release. Bacteria (after 120 min incubation) were removed by centrifugation and enzyme release was assayed in the cell-free supernatant. Legend: LP: peptides produced by *L. plantarum* ATCC 8014; Gt2: peptides produced by *L. plantarum* Gt2; Nis: nisin (Sigma); control: *E. coli* non-treated cells.

enzyme. This result is in concordance with the study of Ibrahim et al. [29]. Additionally, a slightly different protein profile of *E. coli* untreated and treated with Gt2 peptides was observed in SDS-PAGE analysis (Fig. 4). Although the proteins were not identified, the changes in the expressed one were related with the damaging of the proteins from the cells treated with Gt2 peptides compared to untreated control. Previous investigations of the total protein profile of *E. coli* treated with the synthetic bactericidal agent zinc oxide nanoparticle (ZNP) indicated cytoplasmic membrane damage of the target cells [17].

Protective Effect of Gt2 Peptide-Based Suspension on Tomato Fruits

Tomato (*Lycopersicon esculentum* Mill.) is one of the most

Fig. 4. SDS-PAGE study of *E. coli* ATCC 25922 whole cell proteins after peptide treatment; Legend: 1: *E. coli* cells untreated; 2, 3-*E. coli* cells treated with Gt2 and LP peptides; M: molecular marker (Takara, Japan); arrows indicated different bands.

popular vegetables in Ecuador. The common “tomato riñón” is daily consumed in fresh salads or as garnish for various foods, and is a rich source of vitamin A and other nutrients. Tomatoes are usually consumed at their maximum organoleptic quality which takes place when they reach the full red color stage but before excessive softening. As the fruit contain a large amount of water it is prone to contamination by various microorganisms; recently several cases of illnesses were reported when Roma tomato fruits contaminated with *Salmonella* ser. Branderup were consumed in a restaurant in Delhi [30]. In this study, the potential of Gt2 peptides to delay or protect the postharvest deterioration of tomato fruits was evaluated. The tomatoes were purchased at the fourth ripening stage according to the USDA standard index [31], which is considered a suitable stage for the treatment, as at stage five or six they rapidly become soft. The initial density of the total inoculated *Salmonella* cocktail was 5.78 log₁₀ CFU/g at day 0 before treatment with both partial purified peptides and nisin. A statistically significant decrease of *Salmonella* population ($p < 0.05$) was registered when tomatoes were treated with either Gt2 or LP peptides (Table 1), while in the control tomatoes (2) the cell count

Table 1. Survival of *Salmonella* cells from tomatoes treated with the peptides after storage.

Treatments ^a	<i>Salmonella</i> population ^b (log10 CFU/g)			
	Day 0	Day 1	Day 7	Day 14
Control 1: Tomato + water	(-)	(-)	(-)	(+)
Control 2: Tomato + <i>Salmonella</i> cocktail	5.78 ± 0.20 ^A	5.45 ± 0.18 ^A	5.42 ± 0.12 ^A	5.30 ± 0.11 ^A
Tomato + <i>Salmonella</i> cocktail +Gt2 peptides	5.78 ± 0.20 ^A	5.43 ± 0.13 ^A	4.76 ± 0.26 ^C	2.97 ± 0.22 ^D
Tomato + <i>Salmonella</i> cocktail +LP peptides	5.78 ± 0.20 ^A	5.36 ± 0.10 ^A	4.86 ± 0.16 ^C	3.63 ± 0.17 ^C
Tomato + <i>Salmonella</i> cocktail + nisin	5.78 ± 0.20 ^A	5.39 ± 0.12 ^A	5.21 ± 0.10 ^B	4.50 ± 0.18 ^B

^aConcentrations of antimicrobial agents were 50 mg/ml nisin and 1 MIC of Gt2 and LP peptides. (-): no bacteria; (+): detection of *Salmonella* in untreated tomatoes at 14 days of storage.

^bValues are means ± standard deviations of three trial experiments. Means in the same column followed by a different capital letter are significantly ($p < 0.05$) different.

was maintained at the same level, indicating that the *Salmonella* cells were fitting to the fruits' complex composition. Although the *Salmonella* population did not diminish completely, the log10 reductions were 2.33 and 1.67 units for Gt2 and LP peptides respectively, at 14 days of storage, suggesting that the peptide/protein-solution might penetrate the tomato surface forming a barrier between the fruit and the environment, improving its safety and consequently its shelf life. These findings, correlated with the in vitro study of mode of action, as the *Salmonella* cell culture was not completely eliminated upon direct interaction with the peptides. No such reduction was achieved when tomatoes were treated with nisin-solution (0.80 log10 unit). Unpredictably, at 14 days, the un-treated tomatoes (control 1) showed contamination with *Salmonella* (Table 1). This might be due to the internal fruit contamination before harvesting. Thus, our results demonstrated that a Gt2 peptide-based suspension was

effective against the *Salmonella* cocktail, and might be a solution to protect the fruits from harmful bacteria during storage. A recent study on fresh whole cantaloupe washed with nisin-EDTA indicated its efficacy to decrease the *Salmonella* population on day 7 of storage that could lead to both increasing shelf life and reducing the risk of foodborne illness from contamination with *Salmonella* [32]. If in the cantaloupe study the peptide-solution was used as washing solution, at different intervals, in our experimental study the solution was applied one time only to avoid the deterioration of the fruit during manipulation, considering that tomatoes are more perishable than cantaloupe. It is known that tomatoes are susceptible to spoilage by fungi which can produce mycotoxins that are detrimental to human health [33]. In this study, the first indication of rottenness was observed on day 7 of storage at room temperature in the untreated tomatoes, while the deterioration was delayed by 10 and 7 days in the tomatoes

Fig. 5. Protective effect of coated tomatoes with antimicrobial peptide-based suspension at room temperature. Control: tomato washed with sterile water, no peptides added; Gt2, LP and Nisin: tomatoes inoculated with antimicrobial substances; Gt2, LP, peptides and Nisin, respectively; panel i) day 1; ii) day 17.

treated with the peptides and nisin, respectively. Fig. 5 shows the tomato fruits on day 1 (panel i) and 17 (panel ii) of storage with the visible signs of spoilage in the control tomatoes. No signs of spoilage were detected in the peptide-treated tomatoes, while the sample treated with nisin showed skin deterioration. Although we do not identify the microorganisms that were responsible with the deterioration of control tomato (untreated with peptides), the filamentous fungi were visible observed, suggesting the possible anti-fungal activity of the Gt2 peptides; further analysis are need it to demonstrate this statement. Samples stored in refrigeration were preserved up to 23 days, when the fruits became softer and showed superficial skin wrinkles, leading to a dry and dull appearance. Early research of tomatoes covered with a food packaging biofilm containing the bacteriocin sonorensin indicated the protective effect of bacteriocin up to day 7 [12]. In the current study the fruits were dipped in the peptide solution, dried for 24 h to ensure that the fruit membrane was penetrated and covered with the polymer film; thus, we suggest that the retardation of spoilage development was related to the presence of the active components on fruit membranes. Covering or coating the fruit with active peptide-containing washing suspension seems to be a promising approach for active protection, but at this point we don't know the exact concentration of active compound that was adsorbed by the fruit.

Taken together, the Gt2 peptide suspension effectively inhibits both *E. coli* and *Salmonella* at the exponential growth phase, demonstrating its bacteriolytic mode of action. The Gt2 peptides are perforating the cytoplasmic membrane disturbing the cell integrity, leading to the release of intracellular molecules and finally to cellular death. Coating the tomato fruits with Gt2 peptide-based suspension proves to be a promising approach to control the spoilage in fresh tomatoes, thus enhancing the safety of food products.

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Conflict of Interest

The authors have no financial conflicts of interest to declare.

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