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Impact of food-relevant conditions and food matrix on the efficacy of prenylated isoflavonoids glabridin and 6,8-diprenylgenistein as potential natural preservatives against *Listeria monocytogenes*

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ABSTRACT

Prenylated isoflavonoids can be extracted from plants of the Leguminosae/Fabaceae family and have shown remarkable antimicrobial activity against Gram-positive food-borne pathogens, such as *Listeria monocytogenes*. Promising candidates from this class of compounds are glabridin and 6,8-diprenylgenistein. This research aimed to investigate the potential of glabridin and 6,8-diprenylgenistein as food preservatives against *L. monocytogenes*. Their antimicrobial activity was tested *in vitro* at various conditions relevant for food application, such as different temperatures (from 10 °C to 37 °C), pH (5 and 7.2), and in the presence or absence of oxygen. The minimum inhibitory concentrations of glabridin and 6,8-diprenylgenistein *in vitro* were between 0.8 and 12.5 μg/mL in all tested conditions. Growth inhibitory activities were similar at 10 °C compared to higher temperatures, although bactericidal activities decreased when the temperature decreased. Notably, lower pH (pH 5) increased the growth inhibitory and bactericidal activity of the compounds, especially for 6,8-diprenylgenistein. Furthermore, similar antimicrobial efficacies were shown anaerobically compared to aerobically at the tested conditions. Glabridin showed a more stable inhibitory and bactericidal activity when the temperature decreased compared to 6,8-diprenylgenistein. Therefore, we further determined the antimicrobial efficacy of glabridin against *L. monocytogenes* growth on fresh-cut cantaloupe at 10 °C. In these conditions, concentrations of glabridin of 50, 100 and 250 μg/g significantly reduced the growth of *L. monocytogenes* compared to the control, resulting on average in >1 Log CFU/g difference after 4 days compared to the control. Our results further underscored the importance of considering the food matrix when assessing the activity of novel antimicrobials. Overall, this study highlights the potential of prenylated isoflavonoids as naturally derived food preservatives.

1. Introduction

Listeria monocytogenes is a robust pathogen and the causative agent of listeriosis, one of the most severe food-borne diseases. Listeriosis primarily affects immunocompromised people, the elderly, pregnant women and infants and, although reported with low incidence, is characterised by high case fatality. A total of 1876 confirmed invasive human cases were reported in the EU in 2020, with a case fatality of 13.0 % (EFSA and ECDC, 2021). *L. monocytogenes* is a highly adaptable pathogen; it can grow and survive in a wide range of environmental conditions and thus persist in the food chain (NicAogáin and O'Byrne, 2016). Its ability to grow at low temperatures, low pH, high salt concentration and in the presence or absence of oxygen highlights the challenges of controlling this pathogen in food and food-related environments (Bucur et al., 2018). Due to these aspects, *L. monocytogenes* can be found in a wide variety of raw and processed food.

A food category particularly relevant for *L. monocytogenes* is ready-to-eat (RTE) products, such as deli meat, cold-smoked salmon, cheese and fresh-cut fruit and vegetables. The possible presence of *L. monocytogenes* in this food category has caused food safety concerns for these products (Forauer et al., 2021; Zhang et al., 2020). Although these products are usually stored at refrigerated temperatures, *L. monocytogenes* can grow on various RTE products at low temperatures, and its growth is enhanced when exposed to temperature abuse (Kroft

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et al., 2022; Luo et al., 2015). A well-known example of a product associated with the presence of *L. monocytogenes* is cantaloupe. *L. monocytogenes* can be present on cantaloupe's surfaces and, after processing, it can grow on fresh-cut cantaloupe also at refrigerated temperatures (Danyluk et al., 2014; Salazar et al., 2017). Contaminated cantaloupes with *L. monocytogenes* have been associated in 2011 with a multistate outbreak in the United States (McCollum et al., 2013), highlighting the importance of controlling the growth of this pathogen in cantaloupe.

Lowering the incidence and the growth of *L. monocytogenes* in RTE foods is an important goal to ensure food safety, and strict microbiological criteria are applied to verify control measures (European Commission, 2005). To reduce the growth of *L. monocytogenes,* the use of commercially available preservatives (*e.g.* sorbates, benzoates and nitrites) is one valid option. However, the growing trend of consumers' demand for natural antimicrobials and the increased resistance to commonly used antimicrobials have motivated researchers to find alternatives (Quinto et al., 2019). In this prospect, plants can be a source of novel bioactive compounds, and their antimicrobial activity has been well explored. During stress, plants have been shown to produce secondary metabolites that have remarkable potential as food preservatives. Among the vast diversity of plant secondary metabolites with antibacterial properties, prenylated flavonoids and isoflavonoids (collectively called (iso)flavonoids) are receiving more attention due to their high activity against Gram-positive food-borne pathogens (Araya-Cloutier et al., 2018b; Kalli et al., 2021; Wu et al., 2019).

Prenylated (iso)flavonoids are characterised by the substitution with a prenyl group (5-carbon isoprenoid), and one of the primary sources are plants of the Leguminosae/Fabaceae family (Chang et al., 2021). Our previous studies (Araya-Cloutier et al., 2017; Kalli et al., 2020), in line with others (Ahuja et al., 2012; Yang et al., 2015), showed that prenylated (iso)flavonoids are produced when plants are exposed to stresses and have higher bioactivity than their unprenylated analogues. This aspect highlights the importance of the prenyl substitution for their biological functions. It has been proposed that the antimicrobial properties of prenylated (iso)flavonoids are mainly due to the interaction with the bacterial cytoplasmic membrane (Araya-Cloutier et al., 2018a; Wu et al., 2019). It has been suggested that the prenyl groups, by increasing the hydrophobicity of the compound, improve the interaction and affinity of (iso)flavonoids to bacterial cytoplasmic membranes and thus their antimicrobial effect (Chen et al., 2014). Hydrophobicity of prenylated (iso)flavonoids may vary based on the pH of the matrix due to the presence of dissociable groups (*e.g.* hydroxyl groups). Therefore, the hydrophobicity is commonly represented using the distribution coefficient (LogD), which increases as the hydrophobicity of the compounds increases. Antimicrobial candidates from this class of compounds are glabridin (monoprenylated isoflavan) and 6,8-diprenylgenistein (diprenylated isoflavone) (Fig. 1). Although knowledge about the safety of prenylated (iso)flavonoids is limited, Glavonoid, a novel food ingredient containing 3 % of glabridin and other prenylated (iso) flavonoids in smaller amounts, was approved by the European Food Safety Authority (EFSA). Glavonoid is considered safe up to 120 mg/day for the general adult population (EFSA Panel on Dietetic Products, Nutrition and Allergies, 2011), suggesting promising prospective for the safety of these compounds. Previously, we showed that glabridin and 6,8-diprenylgenistein have shown potent antimicrobial activity against *L. monocytogenes* with minimum inhibitory concentrations (MICs) between 6.3 and 15 μg/mL (Araya-Cloutier et al., 2018a).

Although glabridin and 6,8-diprenylgenistein have been previously studied, their activity has mainly been tested in standard laboratory settings, and the impact of food-relevant conditions on their antimicrobial activity has not been investigated. Standard methods to measure antimicrobial activity (*e.g.* broth microdilution assay) are usually carried out in laboratory settings (*e.g.* 30 °C and neutral pH). Different studies have shown how the activities of antimicrobial compounds can be affected by changes in temperature and pH of the matrix (Klančnik et al., 2011; Parichanon et al., 2022; Shen et al., 2015). This is particularly relevant for *L. monocytogenes* due to its ability to adapt to various environmental conditions. One of the adaptation strategies of *L. monocytogenes* is the change in cytoplasmic membrane composition in response to external stresses, such as low temperature and low pH (Diakogiannis et al., 2013; Gandhi and Chikindas, 2007). The bacterial cytoplasmic membrane is the cell's first barrier and the target of various compounds. Therefore, membrane composition alteration can significantly influence antimicrobial activities (Lee et al., 2019). Furthermore, different studies reported lower activity of antimicrobials food products compared to *in vitro* results, probably due to food matrix influences (Ma et al., 2020; Oulahal and Degraeve, 2021; Yuan et al., 2017). These findings underline the importance of testing novel antimicrobials in conditions relevant to food and on food products to assess their potential use in the food industry.

This study aims to assess the effect of temperature, pH, and oxygen on the activity of glabridin and 6,8-diprenylgenistein against *L. monocytogenes*. Based on these findings, the most promising candidate

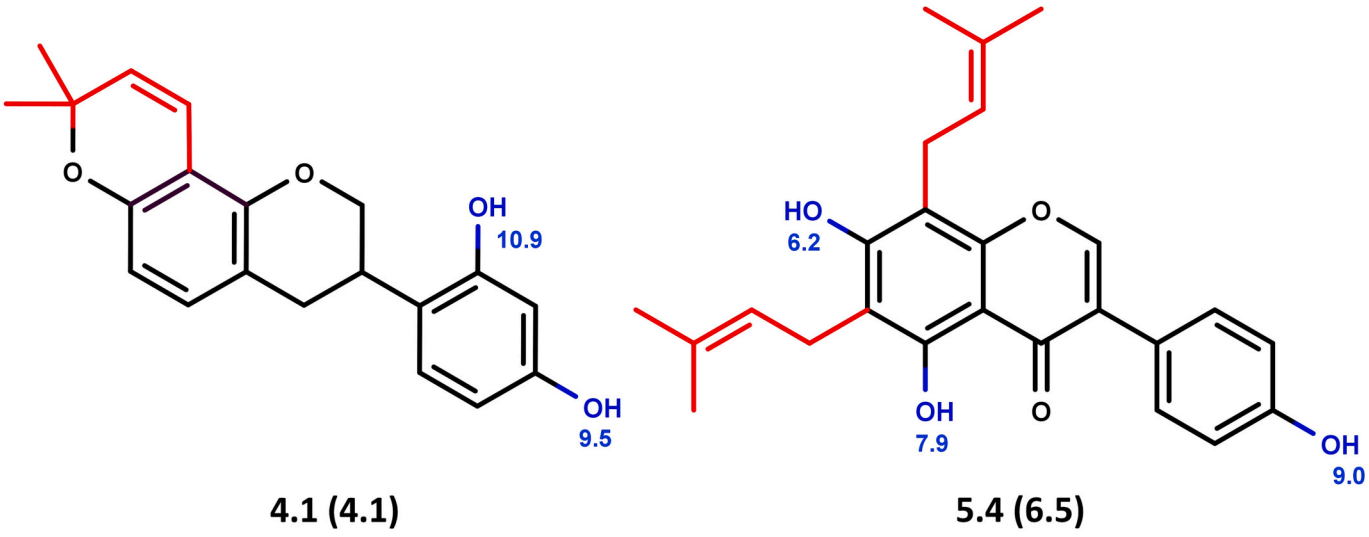

Fig. 1. Structures of glabridin (left) and 6,8-diprenylgenistein (right). The prenyl groups are highlighted in red. Hydroxyl groups with the corresponding pKa are highlighted in blue. Numbers below the structures represent the distribution coefficient (LogD) measured at pH 7.2 and pH 5 (between brackets; a higher value indicates higher lipophilicity). pKa and LogD values were calculated using MarvinSketch 21.8.

was further tested in fresh-cut cantaloupe melon at 10 °C to investigate the applicability as naturally derived food preservatives in RTE products.

2. Materials and methods

2.1. Chemicals and media

Glabridin was purchased from Fujifilm Wako Pure Chemical corporation (Osaka, Japan), and 6,8-diprenylgenistein was purchased from Plantech UK (Reading, UK), both with a purity ≥95 %. Dimethyl sulfoxide (DMSO) and ethanol absolute (EtOH, ≥99.5 %) were purchased from Merck KGaA (Darmstadt, Germany). Tryptone Soya Broth (TSB) and agar bacteriological were purchased from Oxoid Ltd. (Basingstoke, UK). Brain Heart Infusion (BHI) was purchased from Becton Dickinson (Le Pont-de-Claix, France). Agar Listeria according to Ottaviani and Agosti (ALOA) was purchased from bioMérieux (Marcy-l'Étoile, France). Peptone Physiological salt solution (PPS) was prepared with 0.1 % (*w/v*) neutralised bacteriological peptone (Oxoid Ltd., Basingstoke, UK) and 0.85 % (w/v) of sodium chloride (Sigma-Aldrich, St. Louis, MO, USA) dissolved in demineralised water. The pH of TSB is 7.2, and this is referred to later as "neutral pH". When needed, the pH of TSB was adjusted to 5.0 with 5 M HCl (VWR, Fontenay-sous-Bois, France), later referred to as "low pH".

2.2. Bacterial strain and culture condition

All experiments were carried out with *L. monocytogenes* EGDe. Cells were streaked from −80 °C glycerol stock on a BHI agar plate and incubated for 24 h at 37 °C. One colony was transferred to 10 mL of TSB and incubated for 18 h at 30 °C in a shaking incubator at 160 rpm to obtain an overnight culture (ON). The ON was then used to inoculate (1:1000 *v/v*) 20 mL of fresh TSB to preculture cells at different conditions. Aerobic precultures were incubated in 100 mL Erlenmeyer flasks at the desired temperature at 160 rpm. Anaerobic precultures were incubated at the desired temperature in 50 mL centrifuge tubes in an anaerobic jar flushed with a gas mix (10 % CO_2, 10 % H_2 and 80 % N_2) with the Anoxomat (Mart Microbiology, Lichtenvoorde, The Netherlands). Depending on the assay, cultures were incubated at 10, 20, 30 or 37 °C. TSB was adjusted to pH 5.0 and used to preculture cells at low pH for acidic-adapted cells. In all conditions, cells were cultured until the stationary phase to obtain standardised working cultures (Table S1), and this was checked by measuring the optical density at 600 nm (OD_{600}) and enumerating viable cells by plating appropriate dilutions on BHI plates.

2.3. Broth microdilution assay

Antibacterial activity was determined using the broth microdilution assay described by Araya-Cloutier et al. (2018a) with some modifications. Antibacterial activity was tested at different temperatures, pH values and aerobically or anaerobically. The working culture was obtained under the same conditions of the assay to adapt cells to the test environment. The working culture was diluted in TSB to reach a final inoculum concentration of 4.9 ± 0.2 Log CFU/mL. The stock solution of antimicrobial compounds was prepared in DMSO (10 mg/mL), which was then diluted in TSB to reach the desired concentration. Next, equal volumes of inoculum and compound solution (100 μL) were mixed in a honeycomb plate. For aerobic assays, plates were incubated in a Bioscreen C (Labsystems Oy, Helsinki, Finland) with continuous shaking. For anaerobic assays, the honeycomb plate was placed in an anaerobic jar, and the anoxomat was used to achieve the anaerobic environment, as described in Section 2.2. The jar was incubated in a shaking incubator at 160 rpm. The final concentrations of compounds varied from 0.40 μg/mL to 200 μg/mL. The maximum final concentration of DMSO was 2 % (*v/v*). Negative controls (inoculum with TSB), solvent control (inoculum with 2 % (*v/v*) of DMSO) and blanks (compound solution and TSB without bacteria) were included in every experiment.

Plates were incubated at the desired temperature for different incubation times based on the assay condition (Table S1). For aerobic assays, OD_{600} was periodically checked during the entire incubation time. For anaerobic assays, OD_{600} was checked before and after incubation. The incubation time of the experiment was set to allow the negative control to reach the stationary phase. Inhibition of growth was first checked by the OD_{600} measurements, and cell viability was checked when turbidity did not increase. The replicates with the same concentration of antimicrobials were pooled together, and appropriate dilutions were plated on BHI plates. Plates were incubated at 37 °C for at least 3 days to fully recover damaged cells.

The minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC) were defined based on cell count. MIC is the lowest compound concentration that inhibits growth compared to the inoculum (with a maximum increase in cell count of 0.5 Log CFU/mL), and MBC is the lowest concentration at which at least 3 Log CFU/mL reductions were obtained (Balouiri et al., 2016). All conditions were tested in at least two independent biological replicates performed on different days, and each replicate was performed in triplicate.

2.4. Time-kill curves

Working cultures and antimicrobial solutions were prepared as described in Sections 2.2 and 2.3. An equal volume (2.5 mL) of the inoculum and the antimicrobial solution were mixed, aiming for an initial concentration of 4.9 ± 0.1 Log CFU/mL and incubated at 160 rpm at 10, 20, 30 or 37 °C for different incubation times (Table S1). At different time points, appropriate dilutions of samples were plated on BHI plates; plates were incubated at 37 °C for at least 3 days. Significant differences between counts at different times and temperatures with the same concentration of antimicrobials were tested with a *t*-test in R (R Core Team, 2020) with stats package. Time kill curves were tested with two independent biological replicates, and each replicate was performed in duplicate.

2.5. Antimicrobial activity in fresh-cut cantaloupe

The antimicrobial activity of glabridin was further evaluated in fresh-cut cantaloupe. Cantaloupe was purchased from a local supermarket and stored at 4 °C for a maximum of 24 h. Before sample preparation, the whole cantaloupe was washed carefully with sterilised demineralised water. All instruments used were sterile or cleaned periodically with 70 % EtOH. The whole fruit was cut in half with a knife, and the seeds were removed. Next, the halves were sliced (0.5 cm), and the rind was removed. Squares of 3 g (approximately 2 cm × 2 cm) were obtained from the slices and transferred to petri dishes.

L. monocytogenes was aerobically cultured at 10 °C as described in Section 2.2. The working culture was then diluted in PPS to reach a final inoculum of 2.3 ± 0.1 CFU/g. The stock solution of glabridin was prepared in absolute EtOH (15 mg/mL), which was then diluted in absolute EtOH to reach the desired concentration. Subsequently, 50 μL of glabridin solution were spread on the surface of the cantaloupe with a sterile spreader, and petri dishes were left open for 15 min inside a laminar airflow cabinet to allow evaporation of EtOH. Afterwards, 50 μL of inoculum were spread on the surface of the cantaloupe and petri dishes were left open for 15 min to allow microbial attachment. The final concentrations of glabridin ranged from 25 μg/g to 250 μg/g. The maximum EtOH concentration was 0.7 % *v/w* (prior to evaporation). Negative control (untreated samples with the inoculum) and solvent control (50 μL of EtOH (0.7 % *v/w*) and inoculum) were prepared as the samples and included in every experiment. The petri dishes were covered with parafilm and stored at 10 °C, to test the activity of glabridin under temperature abuse conditions. Immediately after sample preparation and after 0.5, 1, 2, 3 and 4 days, samples were opened.

Cantaloupe pieces were transferred into stomacher bags, diluted with PPS and homogenised first manually and then with a Stomacher 400 (Seward, Worthing, UK) for 1 min at 260 rpm. Appropriate decimal dilutions were then plated on ALOA plates and incubated at 37 °C for 48 h. The lower detection limit was 1.7 Log CFU/g. For each replicate, non-inoculated samples were prepared as previously described and used to check the absence of *L. monocytogenes*. Furthermore, 40 g of cantaloupe were homogenised with Stomacher 400 for 1 min at 260 rpm, and the juice was used to measure the pH of the cantaloupe. The antimicrobial activity in fresh-cut cantaloupe was tested with three independent biological replicates performed on different days.

The biphasic model without the stationary phase was used to estimate the lag time λ (days) and the growth rate μ (Log CFU/g day) of *L. monocytogenes* in fresh-cut cantaloupe with different treatments. The model is described in Eqs. (1)–(2):

$$\text{Lag phase}: \quad \text{for } t \leq \lambda, \text{Log } N_t = \text{Log } N_0 \tag{1}$$

$$\text{Exponential growth phase}: \quad \text{for } t > \lambda, \text{Log } N_t = \text{Log } N_0 + \mu\,(t - \lambda) \tag{2}$$

where N_t is the population density at time t (CFU/g), N_0 is the initial population density (CFU/g), and t is the elapsed time. The model was fitted using an adapted version of the biogrowth package in R (Garre et al., 2022), accessed from https://github.com/xchuam/biogrowth/tree/two_phase_model. First, the model was fitted to the biological replicates data together, and the significance of λ was checked. If λ was not significant for a specific condition, the linear model (Eq. (2), without the λ parameter) was used to estimate μ. The μ and the λ were estimated by fitting the selected model to each biological replicate, and the average and standard deviation of the growth parameters were calculated for each condition. Significant differences between treatments were tested with *t*-test in R (R Core Team, 2020) with stats package.

2.6. Chemical structures and prediction of properties

MarvinSketch (version 21.8.0, Chemaxon, https://www.chemaxon.com) was used to draw chemical structures and predict LogD values and undissociated percentages at pH 7.2 and 5.

3. Results

The inhibitory and bactericidal activity of glabridin and 6,8-diprenylgenistein against *L. monocytogenes* were measured based on the OD_{600} and viable counts. Fig. 2 shows the activity *in vitro* of glabridin and 6,8-diprenylgenistein when tested aerobically at neutral pH and 30 °C. Glabridin delayed the growth at a concentration of 6.3 μg/mL and completely inhibited the growth at a concentration of 12.5 μg/mL (Fig. 2A). Based on cell count, >3 Log reductions were achieved with a concentration of 25 μg/mL of glabridin (Fig. 2B). Therefore, 12.5 μg/mL and 25 μg/mL were defined as MIC and MBC, respectively. 6,8-Diprenylgenistein completely inhibited growth at 3.1 μg/mL and caused a 3 Log reduction at 6.3 μg/mL (Fig. 2C, D). Compared to the control, 2 % (*v/v*) DMSO did not affect the growth of *L. monocytogenes* (Fig. 2A, C and Table S2).

The activity of antimicrobials can be influenced by various environmental conditions relevant to foods, such as low temperature, pH, and the presence or absence of oxygen. Therefore, their influence was evaluated for glabridin and 6,8-diprenylgenistein. MIC and MBC values of glabridin and 6,8-diprenylgenistein were aerobically measured against *L. monocytogenes* at various combinations of pH and temperatures (Table 1). Importantly, MIC values of prenylated isoflavonoids at pH 7.2 were rather similar at different temperatures (maximum two-fold change), especially for glabridin. To highlight this stable inhibition by glabridin, Fig. 3 shows the differences in viable counts between the end

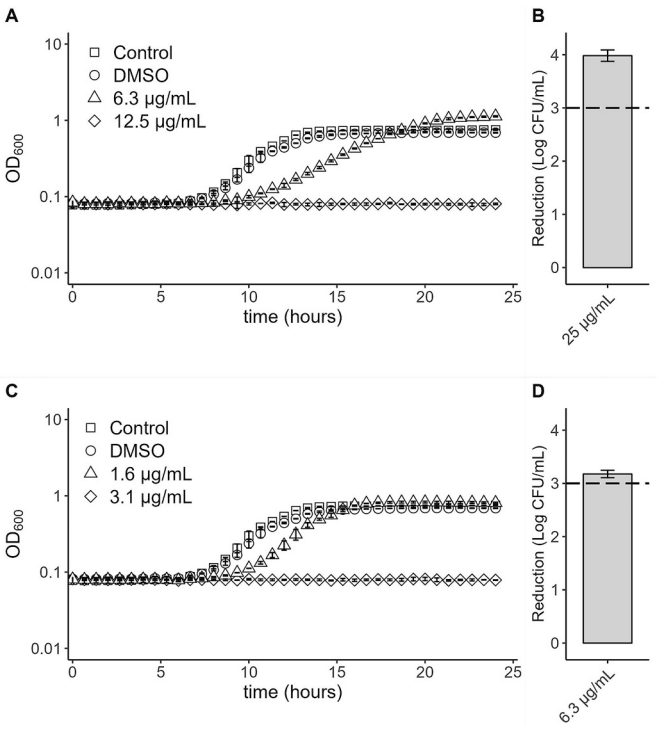

Fig. 2. Broth microdilution assay at pH 7.2 and 30 °C of glabridin (A and B) and 6,8-diprenylgenistein (C and D) against *L. monocytogenes*. Growth of *L. monocytogenes* at 30 °C in the absence and the presence of glabridin (A) and 6,8-diprenylgenistein (C). Diamonds and triangles represent growth in the presence of prenylated isoflavonoids with concentrations equal to MICs and ½ MICs, respectively. Squares and circles represent growth of untreated cells (control) and with 2 % of DMSO (DMSO), respectively. Reduction of viable cell counts after 24 h incubation with glabridin (B) and 6,8-diprenylgenistein (D) with concentrations equal to MBCs. Dashed lines indicate 3 Log reduction, the threshold for the definition of MBC in this study. Data are expressed as averages and error bars represent standard deviations of biologically independent replicates ($n = 2$).

of the broth microdilution assay. No growth was measured with 12.5 μg/mL of glabridin at all temperatures, while the controls increased >4 Log CFU/mL in all conditions. Compared to MICs, MBCs were more affected by the tested temperature because higher MBC values were measured for the two prenylated isoflavonoids at lower temperatures (Table 1). For glabridin tested at pH 7.2, the MBC at 37 °C was 17.5 μg/mL, while the MBC increased to 50 μg/mL at 10 °C. The bactericidal activity of 6,8-diprenylgenistein was even more affected by temperature because the MBC at neutral pH increased from 6.3 μg/mL at 37 °C to >200 μg/mL at 10 °C.

Notably, glabridin and 6,8-diprenylgenistein showed higher antimicrobial efficacy at pH 5 than at pH 7.2 (Table 1). The effect of the temperature on MIC values measured at neutral pH was also shown at pH 5: MIC values were similar at the tested temperature and ranged from 3.1 to 12.5 μg/mL and from 0.8 to 1.6 μg/mL for glabridin and 6,8-diprenylgenistein, respectively. Consistently to neutral pH, MBC values for both compounds increased as the temperature decreased. For glabridin tested at low pH, the MBC was 12.5 μg/mL at 37 °C and between 12.5 and 25 μg/mL at 10 °C. The MBC of 6,8-diprenylgenistein at low pH increased from 1.6 μg/mL at 37 °C to 12.5–50 μg/mL at 10 °C. MBC values were notably lower at pH 5 compared to pH 7.2, especially for 6,8-diprenylgenistein, resulting in a remarkable improvement of its bactericidal activity at low pH.

These results show that tested temperature minimally affected the inhibitory activity of glabridin and 6,8-diprenylgenistein against *L. monocytogenes* and inhibition was still present at 10 °C. Lowering the temperature decreased both tested compounds' bactericidal activity

Table 1
Antibacterial activity of glabridin and 6,8-diprenylgenistein against *L. monocytogenes* at different temperatures and pHs. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) (in μg/mL) were determined aerobically.

Compound	pH	MIC				MBC			
		10 °C	20 °C	30 °C	37 °C	10 °C	20 °C	30 °C	37 °C
Glabridin	7.2	6.3–12.5[a]	12.5	12.5	12.5	50	25	25	17.5
	5.0	3.1–6.3	6.3	6.3–12.5	6.3–12.5	12.5–25	17.5	17.5	12.5
6,8-Diprenylgenistein	7.2	6.3	3.1–6.3	3.1	3.1	>200[b]	25	6.3	6.3
	5.0	0.8–3.1	3.1	1.6–3.1	0.8–1.6	12.5–50	6.3–12.5	3.1–6.3	1.6

[a] Ranges of values derive from differences between biological replicates ($n = 2$).
[b] The highest tested concentration was 200 μg/mL.

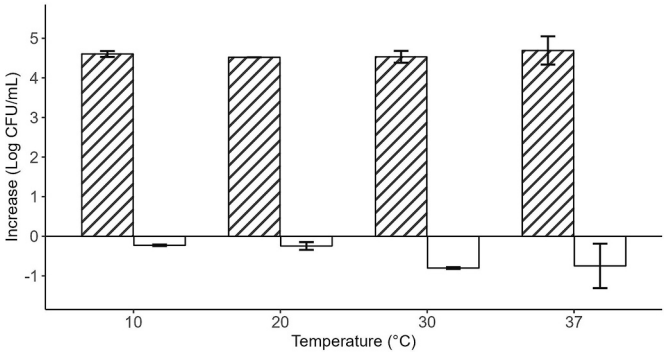

Fig. 3. Growth of *L. monocytogenes* in the presence (empty bars) or absence (striped bars, control) of glabridin (12.5 μg/mL) at different temperatures at pH 7.2. Data represent differences in viable counts between the end of broth microdilution assay and the initial inoculum. Data are expressed as averages and error bars represent standard deviations of biologically independent replicates (n = 2).

while lowering the pH improved their inhibitory and bactericidal activity, 6,8-diprenylgenistein being notably more affected than glabridin.

Table 2 shows the antibacterial activity of glabridin and 6,8-diprenylgenistein tested in anaerobic conditions at the two limits of the temperature range (10 °C and 37 °C) and neutral pH. The MIC and MBC values assessment showed similar growth inhibition and bactericidal efficacy of tested compounds at pH 7.2 in anaerobic conditions compared to aerobic conditions.

In the broth microdilution assay, the cell viability of *L. monocytogenes* was evaluated at the end of the essay. Therefore, we further investigated the antimicrobial efficacy by measuring the reduction over time in the presence of tested antimicrobial compounds at different temperatures. Fig. 4 shows the reduction tested at pH 7.2 at different temperatures in the presence of glabridin and 6,8-diprenylgenistein at their highest MIC value measured. The highest reduction was achieved for both compounds at 37 °C and the lowest at 10 °C, confirming the lower bactericidal activities for glabridin and 6,8-diprenylgenistein when temperature decreases. In the presence of 12.5 μg/mL of glabridin, *L. monocytogenes* was reduced by >3 Log CFU/mL after 24 h at 37 °C, while the reduction at 30 °C, 20 °C and 10 °C was <1 Log CFU/mL (Fig. 4A). Unexpectedly, 12.5 μg/mL was defined as MIC based on the broth microdilution assay; slight differences between experiments (*e.g.* volume) could explain this discrepancy. With a concentration of 6.3 μg/mL of 6,8-diprenylgenistein, *L. monocytogenes* was reduced by >3 Log CFU/mL after 24 h of treatment at 37 °C and 30 °C. However, less than one Log reduction was achieved after 24 h of treatment at 20 °C and 10 °C (Fig. 4B). Importantly, no growth was measured with these concentrations at 10 °C up to 6 days of incubation, confirming the inhibitory activity of glabridin and 6,8-diprenylgenistein at low temperature.

Table 2
Antibacterial activity of glabridin and 6,8-diprenylgenistein against *L. monocytogenes* based on temperature. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) (in μg/mL) were determined anaerobically at pH 7.2.

Compound	MIC		MBC	
	10 °C	37 °C	10 °C	37 °C
Glabridin	6.3	6.3–12.5[a]	25–50	12.5–17.5
6,8-Diprenylgenistein	3.1–6.3	3.1	>200[b]	6.3

[a] Ranges of values derive from differences between biological replicates (n = 2).
[b] The highest tested concentration was 200 μg/mL.

One of the most critical aspects of *L. monocytogenes* is the ability to grow at low temperatures. Due to the higher effect of low temperature (10 °C) on the inhibitory and especially bactericidal activity of 6,8-diprenylgenistein compared to glabridin (Table 1), we decided to further test glabridin in fresh-cut cantaloupe at 10 °C. Fig. 5 shows the growth of *L. monocytogenes* at 10 °C for four days on fresh-cut cantaloupe in the presence and absence of glabridin. The pH of cantaloupe was 6.5 ± 0.3. Cantaloupe supported well the growth of *L. monocytogenes* at 10 °C, and viable counts increased from 2.2 ± 0.1 Log CFU/g to 5.6 ± 0.1 Log CFU/g on day four for the untreated samples. No significant difference ($p > 0.05$) was found between untreated and EtOH control for all sampling points, confirming the suitability of this solvent to test the food matrix effect of cantaloupe on the activity of glabridin. Considering the possible influence of the food matrix and the different method of application (surface treatment), we decided to test glabridin starting from a concentration of 25 μg/g. However, glabridin had a minor influence on the growth of *L. monocytogenes* at this concentration (Fig. S1). Adding glabridin to concentrations equal to 50, 100, and 250 μg/g significantly affected the growth of *L. monocytogenes* on cantaloupe. For all sampling times, treated samples had a lower cell count than the ethanol control and untreated samples. Within the first 24 h, a delay in growth in the treated samples can be observed. Cantaloupe treated with 50 μg/g of glabridin showed a slight increase of *L. monocytogenes* after one day (0.2 Log CFU/g) compared to the initial concentration. No significant growth occurred in samples treated with 100 μg/g and 250 μg/g within one day. Notably, *L. monocytogenes* increased by 0.9 Log CFU/g after one day in the untreated sample. Glabridin increased the lag time of *L. monocytogenes* on cantaloupe, while no significant difference was measured in the growth rate with or without glabridin (Table S3). On day two, growth also occurred in samples with 50, 100, and 250 μg/g of glabridin. Throughout the storage time, cantaloupe treated with 250 μg/g of glabridin resulted, on average, in >1 Log CFU/g difference compared with the control. The standard deviation of treated samples was generally higher than untreated samples and EtOH control; this may be explained by the stress caused by the presence of glabridin. Together these results provide important insights into the application of glabridin to delay the growth of *L. monocytogenes* in fresh-cut cantaloupe.

4. Discussion

This study aimed to investigate the antimicrobial efficacy of glabridin and 6,8-diprenylgenistein against *L. monocytogenes* in food-relevant conditions. Therefore, we assessed the effect of temperature, pH and

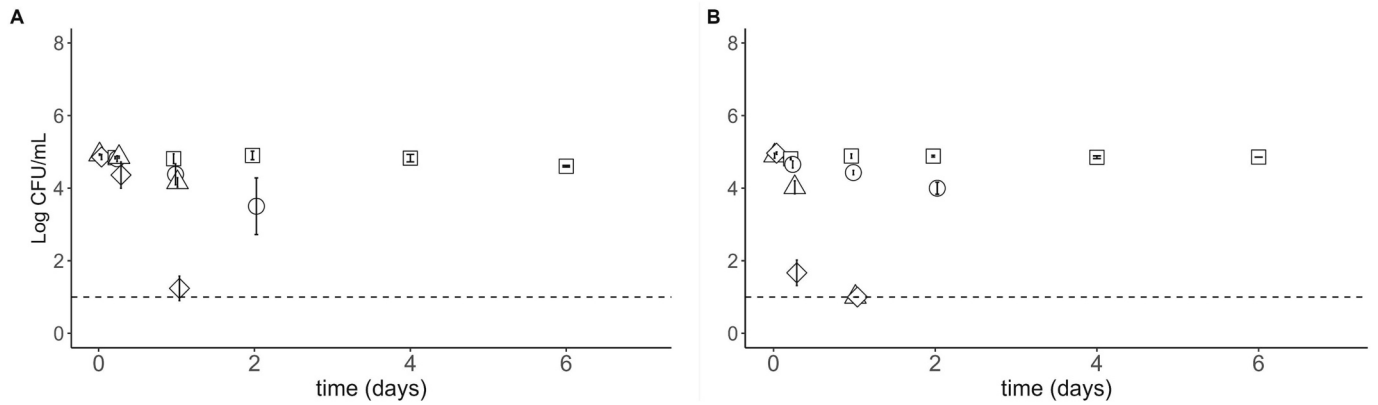

Fig. 4. Reduction of *L. monocytogenes* in the presence of glabridin (12.5 μg/mL, A) and 6,8-diprenylgenistein (6.3 μg/mL, B) tested at pH 7.2 at different temperatures. Tested temperatures are equal to 37 °C (diamonds), 30 °C (triangles), 20 °C (circles) and 10 °C (squares). The initial concentration was 4.9 ± 0.1 Log CFU/mL. Dashed lines represent the detection limit (1 Log CFU/mL). Data are expressed as averages and error bars represent standard deviations of biological replicates (n = 2).

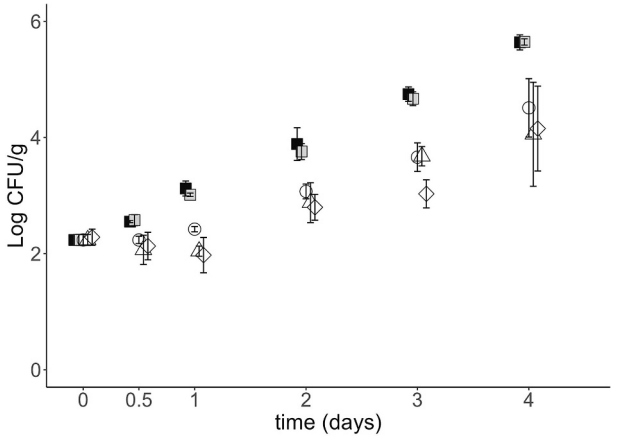

Fig. 5. The effect of glabridin on the growth of *L. monocytogenes* in fresh-cut cantaloupe at 10 °C. Open symbols represent samples treated with glabridin with concentrations equal to 50 μg/g (circles), 100 μg/g (triangles) and 250 μg/g (diamonds). Filled symbols represent untreated samples (control, black squares) and ethanol control (0.7 % *v/w*, grey squares). Data are expressed as averages, and error bars represent standard deviations of biological replicates (n = 3). Data points were jittered to improve visualization; samples were collected after sample preparation and exactly after 0.5, 1, 2, 3, and 4 days.

oxygen on their antimicrobial activity and evaluated the activity of glabridin in fresh-cut cantaloupe. The MICs were between 0.8 μg/mL and 12.5 μg/mL in all tested conditions in TSB, resulting in good inhibitory activity compared to commonly used antimicrobials in food. For example, sodium benzoate, a commercially used antimicrobial, has a MIC value between 100 μg/mL and 10 mg/mL against *L. monocytogenes* in TSB, depending on the pH (Chen et al., 2022; Chen and Zhong, 2018; Lues and Theron, 2012). Moreover, the inhibitory activity of glabridin and 6,8-diprenylgenistein is comparable to other natural antimicrobials, such as thymol, a well-studied compound present in essential oils with a MIC of 187.5–250 μg/mL against *L. monocytogenes* in TSB (Chen and Zhong, 2017; Ma et al., 2013). Notably, the growth inhibitory activity of glabridin and 6,8-diprenylgenistein in food-related conditions, such as low temperature (10 °C), was similar to the laboratory setting (30 °C). Low pH increased their inhibitory activity at all tested temperatures, including low temperature, which is particularly relevant as many food products are slightly acidic.

Culture condition and growth phase are important factors that need to be considered when assessing the activity of antimicrobials against food-borne pathogens (Chen et al., 2020; Harrand et al., 2019). In this study, we precultured *L. monocytogenes* at the corresponding tested condition, *i.e.*, at the selected temperature and pH, until cells reached the stationary phase. These pre-adapted cells were subsequently used to test the activity of the tested compounds *in vitro* (TSB) and on fresh-cut cantaloupe. In this way, we could investigate the effect of specific conditions and the food matrix on the antimicrobial activity considering cell adaptation.

Our results prove that low temperature decreases the bactericidal activity of glabridin and 6,8-diprenylgenistein. Consistently, we observed increased resistance of *L. monocytogenes* as the temperature decreased from 37 °C to 10 °C, both at neutral and low pH and in the presence and absence of oxygen. The antimicrobial mechanism of action for prenylated (iso)flavonoids is not fully elucidated yet. Based on our previous research, we expect the cytoplasmic bacterial membrane to be the main target of these hydrophobic compounds with some possible intracellular action (Araya-Cloutier et al., 2018a; Kalli et al., 2021). One possible explanation for the increased resistance at low temperatures could be the altered cytoplasmic membrane composition in the low-temperature pre-adapted cells used in our experiments. At low temperatures, *L. monocytogenes* can alter its fatty acid composition to maintain membrane fluidity (Chan and Wiedmann, 2009). Previous studies have reported that as temperature decreases, the length of fatty acids decreases, and the branching forms change from iso to anteiso (Annous et al., 1997; Saldivar et al., 2018). Another aspect of the adaptation of *L. monocytogenes* at low temperatures is that the overall cell surface hydrophobicity decreases (Di Bonaventura et al., 2008). Glabridin and 6,8-diprenylgenistein are hydrophobic compounds, and we previously showed that the hydrophobicity of prenylated (iso)flavonoids is a critical property of their antimicrobial activity and affinity to membranes (Araya-Cloutier et al., 2018a; Kalli et al., 2021). It is worth noting that at low temperatures, the bactericidal activity of 6,8-diprenylgenistein decreased more than that of glabridin. Indeed, 6,8-diprenylgenistein is more hydrophobic and the affinity towards bacterial membrane is expected to be higher than glabridin (Araya-Cloutier et al., 2018a). This might explain why greater effects of low temperatures were measured on the bactericidal activity of the most hydrophobic compound, 6,8-diprenylgenistein. Overall, changes in the membrane's composition and cell surface hydrophobicity in *L. monocytogenes* at low temperatures may contribute to the compounds' reduced bactericidal activities at low temperatures.

Low pH enhanced both growth inhibitory and bactericidal activity of glabridin and 6,8-diprenylgenistein. *L. monocytogenes* adapts to low pH with different mechanisms, including alteration of the cytoplasmic membrane composition and fluidity. The ratio of straight-chain/branched-chain fatty acids and the length of the fatty acid carbon

chain increase, and the cytoplasmic membrane fluidity of cells adapted to low pH decreases (Diakogiannis et al., 2013; Mastronicolis et al., 2010). Furthermore, it has been shown that cell surface hydrophobicity increases at low pH in *Listeria innocua*, a closely related microorganism (Moorman et al., 2008). Notably, the effect of low temperature and low pH are opposite regarding cytoplasmic membrane adaptation; this is in line with the opposite effect of low temperature and low pH on the antimicrobial activity of the tested compounds (decrease and increase of activity, respectively). Our previous results highlighted a different effect of pH on the activity of similar prenylated isoflavonoids against *Zygosaccharomyces parabailii* (Kalli et al., 2022). However, the lower activity reported at pH 4 and 3 may be explained by the differences in microorganism tested and preculture conditions.

The tested compounds exist in an equilibrium of dissociated and undissociated forms, and their proportion depends on the pH and respective pKa values. At pH 5, 6,8-diprenylgenistein is mainly present in an undissociated form (94 %); therefore, the hydrophobicity of 6,8-diprenylgenistein is higher at pH 5 than at pH 7.2 (LogD 6.5 and 5.4, respectively, Fig. 1). This increased hydrophobicity may also explain why the growth inhibition and bactericidal activity of 6,8-diprenylgenistein are higher at low pH compared to neutral pH. For glabridin, the range of tested pH does not influence its hydrophobicity (LogD 4.1, Fig. 1), in line with the more similar growth inhibition and bactericidal activity at pH 7.2 and 5. These findings further support the importance of the hydrophobicity of these compounds for their antimicrobial activity. Overall, the data on the effect of temperature and pH suggest that bacterial membrane composition may influence the activity of the tested compounds. Further research is required to fully understand the relation between membrane composition and the activity of glabridin and 6,8-diprenylgenistein, especially with *L. monocytogenes* grown under combined low temperature and low pH stress conditions.

To investigate the effect of the food matrix, we then tested the activity of glabridin against *L. monocytogenes* on cantaloupe, a possible vehicle for this pathogen (Carstens et al., 2019; Marik et al., 2020). Fresh-cut cantaloupe is commonly sold without preservatives, and refrigerated temperature is the primary strategy to control microbial growth. Our data further confirmed that fresh-cut cantaloupe supports the growth of *L. monocytogenes,* and our results are consistent with previous studies that showed an increase of 2–3 Log CFU/g in fresh-cut cantaloupe under similar conditions (Cai et al., 2019; Kroft et al., 2022). *L. monocytogenes* adapted to 10 °C showed immediate onset of growth on fresh-cut cantaloupe, conceivably supported by the relatively high pH of cantaloupe compared to other fruits. In this study, the pH of cantaloupe was 6.5 ± 0.3, which is in line with the one reported by Salazar et al. (2017). Glabridin significantly reduced the growth of *L. monocytogenes* on cantaloupe when applied with a concentration of 50, 100 and 250 μg/g, highlighting its potential as a food preservative in this food matrix. However, the antimicrobial activity was reduced in the food product compared to the TSB medium. At 10 °C and neutral pH, 12.5 μg/mL of glabridin inhibited the growth of *L. monocytogenes in vitro* for up to 6 days; however, 25 μg/g of glabridin had a minor effect on the growth of *L. monocytogenes* on cantaloupe. Furthermore, total inhibition did not occur in the range of tested concentrations.

The interaction with the food matrix is expected to reduce the activity of antimicrobials in food products, which may explain the lower activity in fresh-cut cantaloupe compared to TSB. Lower activity on cantaloupe of other antimicrobials was already reported. Yemmireddy et al. (2020) showed the antimicrobial activity of pecan shell extract against *L. monocytogenes in vitro* (MIC: 1.25–5 mg/mL); however, a minimal effect of the extract was reported on fresh-cut cantaloupe. Moreover, other studies reported lower activity of antimicrobials against *L. monocytogenes* in other food matrixes compared to *in vitro* studies such as in fish products (Desai et al., 2012; Yuan et al., 2017), dairy products (da Silva Dannenberg et al., 2016) and fresh-cut fruits (Belgacem et al., 2020). In a preliminary experiment, we also tested the activity of glabridin in a prepared cold-smoked salmon medium at 10 °C anaerobically (Fig. S2). In this food-based medium, a concentration of 100 μg/mL of glabridin inhibited the growth of *L. monocytogenes* for up to 14 days. Furthermore, we also tested the antimicrobial effect on cold-smoked salmon slices, but due to the solvent effect (ethanol), the efficacy of glabridin in this food product type could not be determined. Despite the promising results obtained in fresh-cut cantaloupe, the effect of the food matrix of other RTE food needs to be further studied. We propose that glabridin could have a similar antimicrobial effect on other RTE fresh-cut fruit, including fruit with lower pH, since an acidic environment enhanced its antimicrobial activity *in vitro*. Moreover, the impact of strain variability and the activity against other microorganisms needs to be further elucidated. Previous *in vitro* studies suggest that their activity will mainly be restricted to Gram-positive bacteria (Araya-Cloutier et al., 2018a). Lastly, important aspects such as safety of these compounds at the concentrations that showed antimicrobial activities, impact on the sensorial characteristics, and cost-benefit analysis for the food industry need to be investigated in future research.

To our knowledge, this is the first study to assess the influence of various food-related conditions and the food matrix on the antimicrobial activity of prenylated isoflavonoids glabridin and 6,8-diprenylgenistein. Overall, these findings confirmed the importance of considering food-related conditions and their application in the food matrix when testing potential novel antimicrobials against *L. monocytogenes*. The *in vitro* study highlighted the potential of the tested prenylated isoflavonoids to control (an)aerobic growth of *L. monocytogenes* at refrigeration temperature and/or at mildly acidic conditions relevant for food. The antimicrobial activity of glabridin in fresh-cut cantaloupe confirmed its potential as a food preservative in selected RTE products. Lastly, this study provides a better understanding of the possible application of prenylated isoflavonoids in food and food-related environments.

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Declaration of competing interest

The authors have no affiliation with any organization with a direct or indirect financial interest in the subject matter discussed in the manuscript.

Data availability

Data will be made available on request.

References

Ahuja, I., Kissen, R., Bones, A.M., 2012. Phytoalexins in defense against pathogens. Trends Plant Sci. 17, 73–90.

Annous, B.A., Becker, L.A., Bayles, D.O., Labeda, D.P., Wilkinson, B.J., 1997. Critical role of anteiso-C15:0 fatty acid in the growth of *Listeria monocytogenes* at low temperatures. Appl. Environ. Microbiol. 63, 3887–3894.

Araya-Cloutier, C., den Besten, H.M.W., Aisyah, S., Gruppen, H., Vincken, J.P., 2017. The position of prenylation of isoflavonoids and stilbenoids from legumes (Fabaceae) modulates the antimicrobial activity against Gram positive pathogens. Food Chem. 226, 193–201.

Araya-Cloutier, C., Vincken, J.P., van de Schans, M.G.M., Hageman, J., Schaftenaar, G., den Besten, H.M.W., Gruppen, H., 2018a. QSAR-based molecular signatures of prenylated (iso)flavonoids underlying antimicrobial potency against and membrane-disruption in Gram positive and Gram negative bacteria. Sci. Rep. 8, 9267.

Araya-Cloutier, C., Vincken, J.P., van Ederen, R., den Besten, H.M.W., Gruppen, H., 2018b. Rapid membrane permeabilisation of *Listeria monocytogenes* and *Escherichia coli* induced by antibacterial prenylated phenolic compounds from legumes. Food Chem. 240, 147–155.

Balouiri, M., Sadiki, M., Ibnsouda, S.K., 2016. Methods for *in vitro* evaluating antimicrobial activity: a review. J. Pharm. Anal. 6, 71–79.

Belgacem, I., Schena, L., Teixidó, N., Romeo, F.V., Ballistreri, G., Abadias, M., 2020. Effectiveness of a pomegranate peel extract (PGE) in reducing *Listeria monocytogenes*

in vitro and on fresh-cut pear, apple and melon. Eur. Food Res. Technol. 246, 1765–1772.
Bucur, F.I., Grigore-Gurgu, L., Crauwels, P., Riedel, C.U., Nicolau, A.I., 2018. Resistance of *Listeria monocytogenes* to stress conditions encountered in food and food processing environments. Front. Microbiol. 9, 2700.
Cai, S., Worobo, R.W., Snyder, A.B., 2019. Combined effect of storage condition, surface integrity, and length of shelf life on the growth of *Listeria monocytogenes* and spoilage microbiota on refrigerated ready-to-eat products. J. Food Prot. 82, 1423–1432.
Carstens, C.K., Salazar, J.K., Darkoh, C., 2019. Multistate outbreaks of foodborne illness in the United States associated with fresh produce from 2010 to 2017. Front. Microbiol. 10, 2667.
Chan, Y.C., Wiedmann, M., 2009. Physiology and genetics of *Listeria monocytogenes* survival and growth at cold temperatures. Crit. Rev. Food Sci. Nutr. 49, 237–253.
Chang, S.K., Jiang, Y., Yang, B., 2021. An update of prenylated phenolics: food sources, chemistry and health benefits. Trends Food Sci. Technol. 108, 197–213.
Chen, H., Zhong, Q., 2017. Lactobionic acid enhances the synergistic effect of nisin and thymol against *Listeria monocytogenes* Scott a in tryptic soy broth and milk. Int. J. Food Microbiol. 260, 36–41.
Chen, H., Zhong, Q., 2018. Antibacterial activity of acidified sodium benzoate against *Escherichia coli* O157:H7, Salmonella enterica, and *Listeria monocytogenes* in tryptic soy broth and on cherry tomatoes. Int. J. Food Microbiol. 274, 38–44.
Chen, X., Mukwaya, E., Wong, M.S., Zhang, Y., 2014. A systematic review on biological activities of prenylated flavonoids. Pharm. Biol. 52, 655–660.
Chen, R., Skeens, J., Orsi, R.H., Wiedmann, M., Guariglia-Oropeza, V., 2020. Pre-growth conditions and strain diversity affect nisin treatment efficacy against *Listeria monocytogenes* on cold-smoked salmon. Int. J. Food Microbiol. 333, 108793.
Chen, H., Brashears, M.M., Zhong, Q., 2022. Sodium benzoate and sodium bisulfate as preservatives in apple juice and alternative sanitisers for washing cherry tomatoes. Int. J. Food Microbiol. 372, 109697.
European Food Safety Authority, European Centre for Disease Prevention and Control, 2021. The European Union One Health 2020 zoonoses report. EFSA J. 19.
Danyluk, M.D., Friedrich, L.M., Schaffner, D.W., 2014. Modeling the growth of *Listeria monocytogenes* on cut cantaloupe, honeydew and watermelon. Food Microbiol. 38, 52–55.
Desai, M.A., Soni, K.A., Nannapaneni, R., Schilling, M.W., Silva, J.L., 2012. Reduction of *Listeria monocytogenes* in raw catfish fillets by essential oils and phenolic constituent carvacrol. J. Food Sci. 77, M516–M522.
Di Bonaventura, G., Piccolomini, R., Paludi, D., D'Orio, V., Vergara, A., Conter, M., Ianieri, A., 2008. Influence of temperature on biofilm formation by *Listeria monocytogenes* on various food-contact surfaces: relationship with motility and cell surface hydrophobicity. J. Appl. Microbiol. 104, 1552–1561.
Diakogiannis, I., Berberi, A., Siapi, E., Arkoudi-Vafea, A., Giannopoulou, L., Mastronicolis, S.K., 2013. Growth and membrane fluidity of food-borne pathogen *Listeria monocytogenes* in the presence of weak acid preservatives and hydrochloric acid. Front. Microbiol. 4, 152.
European Commission, 2005. Commission Regulation (EC) No 2073/2005 of 15 November 2005 on microbiological criteria for foodstuffs.
Forauer, E., Wu, S.T., Etter, A.J., 2021. *Listeria monocytogenes* in the retail deli environment: a review. Food Control 119.
Gandhi, M., Chikindas, M.L., 2007. *Listeria*: a foodborne pathogen that knows how to survive. Int. J. Food Microbiol. 113, 1–15.
Garre, A., Koomen, J., den Besten, H.M.W., Zwietering, M.H., 2022. biogrowth: Modelling of Population Growth. R package version 1.0.0-1. https://CRAN.R-project.org/package=biogrowth.
Harrand, A.S., Kovac, J., Carroll, L.M., Guariglia-Oropeza, V., Kent, D.J., Wiedmann, M., 2019. Assembly and characterisation of a pathogen strain collection for produce safety applications: pre-growth conditions have a larger effect on peroxyacetic acid tolerance than strain diversity. Front. Microbiol. 10, 1223.
Kalli, S., Araya-Cloutier, C., Lin, Y., de Bruijn, W.J.C., Chapman, J., Vincken, J.P., 2020. Enhanced biosynthesis of the natural antimicrobial glyceollins in soybean seedlings by priming and elicitation. Food Chem. 317, 126389.
Kalli, S., Araya-Cloutier, C., Hageman, J., Vincken, J.P., 2021. Insights into the molecular properties underlying antibacterial activity of prenylated (iso)flavonoids against MRSA. Sci. Rep. 11, 14180.
Kalli, S., Araya-Cloutier, C., Chapman, J., Sanders, J.-W., Vincken, J.-P., 2022. Prenylated (iso)flavonoids as antifungal agents against the food spoiler *Zygosaccharomyces parabailii*. Food Control 132.
Klančnik, A., Piskernik, S., Možina, S.S., Gašperlin, L., Jeršek, B., 2011. Investigation of some factors affecting the antibacterial activity of rosemary extracts in food models by a food microdilution method. Int. J. Food Sci. Technol. 46, 413–420.
Kroft, B., Gu, G., Bolten, S., Micallef, S.A., Luo, Y., Millner, P., Nou, X., 2022. Effects of temperature abuse on the growth and survival of *Listeria monocytogenes* on a wide variety of whole and fresh-cut fruits and vegetables during storage. Food Control 137.
Lee, T.H., Hofferek, V., Separovic, F., Reid, G.E., Aguilar, M.I., 2019. The role of bacterial lipid diversity and membrane properties in modulating antimicrobial peptide activity and drug resistance. Curr. Opin. Chem. Biol. 52, 85–92.
Lues, J.F., Theron, M.M., 2012. Comparing organic acids and salt derivatives as antimicrobials against selected poultry-borne *Listeria monocytogenes* strains *in vitro*. Foodborne Pathog. Dis. 9, 1126–1129.
Luo, K., Hong, S.S., Oh, D.H., 2015. Modeling the effect of storage temperatures on the growth of *Listeria monocytogenes* on ready-to-eat ham and sausage. J. Food Prot. 78, 1675–1681.
Ma, Q., Davidson, P.M., Zhong, Q., 2013. Antimicrobial properties of lauric arginate alone or in combination with essential oils in tryptic soy broth and 2% reduced fat milk. Int. J. Food Microbiol. 166, 77–84.
Ma, Q., Davidson, P.M., Zhong, Q., 2020. Properties and potential food applications of lauric arginate as a cationic antimicrobial. Int. J. Food Microbiol. 315, 108417.
Marik, C.M., Zuchel, J., Schaffner, D.W., Strawn, L.K., 2020. Growth and survival of *Listeria monocytogenes* on intact fruit and vegetable surfaces during postharvest handling: a systematic literature review. J. Food Prot. 83, 108–128.
Mastronicolis, S.K., Berberi, A., Diakogiannis, I., Petrova, E., Kiaki, I., Baltzi, T., Xenikakis, P., 2010. Alteration of the phospho- or neutral lipid content and fatty acid composition in *Listeria monocytogenes* due to acid adaptation mechanisms for hydrochloric, acetic and lactic acids at pH 5.5 or benzoic acid at neutral pH. Antonie Van Leeuwenhoek 98, 307–316.
McCollum, J.T., Cronquist, A.B., Silk, B.J., Jackson, K.A., O'Connor, K.A., Cosgrove, S., Gossack, J.P., Parachini, S.S., Jain, N.S., Ettestad, P., Ibraheem, M., Cantu, V., Joshi, M., DuVernoy, T., Fogg Jr., N.W., Gorny, J.R., Mogen, K.M., Spires, C., Teitell, P., Joseph, L.A., Tarr, C.L., Imanishi, M., Neil, K.P., Tauxe, R.V., Mahon, B.E., 2013. Multistate outbreak of listeriosis associated with cantaloupe. N. Engl. J. Med. 369, 944–953.
Moorman, M.A., Thelemann, C.A., Zhou, S., Pestka, J.J., Linz, J.E., Ryser, E.T., 2008. Altered hydrophobicity and membrane composition in stress-adapted *Listeria innocua*. J. Food Prot. 71, 182–185.
NicAogáin, K., O'Byrne, C.P., 2016. The role of stress and stress adaptations in determining the fate of the bacterial pathogen *Listeria monocytogenes* in the food chain. Front. Microbiol. 7, 1865.
Oulahal, N., Degraeve, P., 2021. Phenolic-rich plant extracts with antimicrobial activity: an alternative to food preservatives and biocides? Front. Microbiol. 12, 753518.
Parichanon, P., Sattayakhom, A., Matan, N., Matan, N., 2022. Antimicrobial activity of lime oil in the vapour phase against *Listeria monocytogenes* on ready-to-eat salad during cold storage and its possible mode of action. Food Control 132.
Quinto, E.J., Caro, I., Villalobos-Delgado, L.H., Mateo, J., De-Mateo-Silleras, B., Redondo-Del-Rio, M.P., 2019. Food safety through natural antimicrobials. Antibiotics (Basel) 8.
R Core Team, 2020. R: a language and environment for statistical computing. URL. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/.
Salazar, J.K., Sahu, S.N., Hildebrandt, I.M., Zhang, L., Qi, Y., Liggans, G., Datta, A.R., Tortorello, M.L., 2017. Growth kinetics of *Listeria monocytogenes* in cut produce. J. Food Prot. 80, 1328–1336.
Saldivar, J.C., Davis, M.L., Johnson, M.G., Ricke, S.C., 2018. *Listeria monocytogenes* adaptation and growth at low temperatures. Food Feed Saf. Syst. Anal. 227–248.
Shen, Q., Soni, K.A., Nannapaneni, R., 2015. Stability of sublethal acid stress adaptation and induced cross protection against lauric arginate in *Listeria monocytogenes*. Int. J. Food Microbiol. 203, 49–54.
da Silva Dannenberg, G., Funck, G.D., Mattei, F.J., da Silva, W.P., Fiorentini, Â.M., 2016. Antimicrobial and antioxidant activity of essential oil from pink pepper tree (Schinus terebinthifolius Raddi) *in vitro* and in cheese experimentally contaminated with *Listeria monocytogenes*. Innov. Food Sci. Emerg. Technol. 36, 120–127.
Wu, S.C., Yang, Z.Q., Liu, F., Peng, W.J., Qu, S.Q., Li, Q., Song, X.B., Zhu, K., Shen, J.Z., 2019. Antibacterial effect and mode of action of flavonoids from licorice against methicillin-resistant *Staphylococcus aureus*. Front. Microbiol. 10, 2489.
Yang, X., Jiang, Y., Yang, J., He, J., Sun, J., Chen, F., Zhang, M., Yang, B., 2015. Prenylated flavonoids, promising nutraceuticals with impressive biological activities. Trends Food Sci. Technol. 44, 93–104.
Yemmireddy, V.K., Cason, C., Moreira, J., Adhikari, A., 2020. Effect of pecan variety and the method of extraction on the antimicrobial activity of pecan shell extracts against different foodborne pathogens and their efficacy on food matrices. Food Control 112, 107098.
Yuan, W., Lee, H.W., Yuk, H.G., 2017. Antimicrobial efficacy of *Cinnamomum javanicum* plant extract against *Listeria monocytogenes* and its application potential with smoked salmon. Int. J. Food Microbiol. 260, 42–50.
Zhang, H., Yamamoto, E., Murphy, J., Locas, A., 2020. Microbiological safety of ready-to-eat fresh-cut fruits and vegetables sold on the Canadian retail market. Int. J. Food Microbiol. 335, 108855.