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Bound galloylated compounds in persimmon upcycled dietary fiber modulate microbial strains associated to human health after *in vitro* digestion

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ABSTRACT

Persimmon byproduct upcycling was performed by solvent-assisted extraction (SAE) to obtain dietary fiber (DF). The effect of SAE on DF modulation was studied on specific beneficial and pathogenic strains before and after the *in vitro* digestion process. Overall, digested DF samples extracted using acetone as a solvent (dCET) showed higher ($p < 0.05$) prebiotic activity scores (PASs) in beneficial bacteria such as *Bifidobacterium bifidum*, *Lactobacillus casei*, *Lactococcus lactis*, and *Streptococcus salivarius*. Moreover, dCET reduced the tested pathogenic strain populations. Initial cell attachment (ICA) inhibitory activity on biofilm formation by *Pseudomonas putida*, *Staphylococcus aureus* and *Bacillus subitilis* was observed for dCET, as well as inhibition of preformed (PFB) *S. aureus* biofilms. dCET combined with the antibiotics kanamycin (K) or gentamycin (G) exhibited synergistic effects against all tested pathogens, displaying bactericidal effects against *S. aureus*. High-performance liquid chromatography (HPLC-DAD) analysis showed that after hydrolysis, the released gallic acid could have been responsible for the antimicrobial properties registered in DF from the persimmon byproduct. The obtained results provided information about the potential of upcycled persimmon fiber fractions as possible prebiotics, although further research must be performed with complex microbial populations and *in vivo* studies.

1. Introduction

Persimmon fruits (*Diospyros kaki* Thunb.) are increasingly being produced and consumed worldwide due to their taste, nutrients, bioactive compounds, and health-reported properties (Pérez-Burillo, Oliveras, Quesada, Rufián-Henares, & Pastoriza, 2018). China, Japan, Korea, Brazil, and Spain have become the main producers, adding up to more than 4 million tons a year (FAOSTAT, 2021). Persimmon fruits are processed for fruit juice, jam, and vinegar, among other products. This process generates high amounts of byproducts that have been recognized as sources of carotenoids, phenolics, dietary fiber (DF), vitamins, and minerals (Gea-Botella et al., 2021; Matheus, Andrade, Miyahira, & Fai, 2020). In particular, DF has been reported to be high in valuable nonextractable phenolics that tend to be discarded (Benito-González, Martínez-Sanz, Fabra, & López-Rubio, 2019; Ding, Morozova, Scampicchio, & Ferrentino, 2020; Sun et al., 2021). Appropriate processing of food byproducts could allow the reintroduction of valuable food ingredients into the value chain (Akter, Ahmed, & Eun, 2010; Meng, Li, Xiao, Zhang, & Xu, 2017; Praveen, Parvathy, Balasubramanian, & Jayabalan, 2019), in the case of persimmon byproducts

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through the enhancement of DF biological properties.

DF consumption is related to physiological benefits such as regulation of intestinal transit, diabetes prevention, cardiovascular diseases, colon cancer risk reduction, and hepatic steatosis (Cruz-Requena et al., 2019). DF benefits are directly related to DF processing, composition, physicochemical properties, and bound bioactive compounds (Macagnan, Bender, & Speroni, 2018). Beneficial DF effects on the gastrointestinal tract are mediated by modulation of specific microbial communities (Bas-Bellver et al., 2020; Fissore, Santo Domingo, Gerschenson, & Giannuzzi, 2015) that lead to enhanced metabolism of cholesterol and glucose, the regulation of intestinal immunity, restriction of pathogens, and the growth and maintenance of intestinal cells (Farinha et al., 2015; Lambo, Öste, & Nyman, 2005; Meng et al., 2017; Praveen, Parvathy, Jayabalan, & Balasubramanian, 2019; Zarinah, Anis, Napisah, & Shazila, 2018).

Several studies have addressed the impact of DF on modulating the gut pathogenic community (Meng et al., 2017; Palanisamy, Vinosha, Marudhupandi, Rajasekar, & Prabhu, 2017; Yamashita, Sugita-Konishi, & Shimizu, 2001). DF interactions against pathogens may be attributed to biocompounds bound to their matrix that, through enzymatic metabolism, are released and may modulate microbial populations. The mechanisms by which DF regulates pathogen populations have not been fully explored. Pathogens such as *Salmonella enterica*, *Escherichia coli*, *Staphylococcus aureus*, and *Bacillus cereus* have been commonly associated with foodborne outbreaks. These strains also form biofilms, and within them, the cells behave metabolically less actively and less sensitively to the actions of antimicrobial agents (Famuyide, Aro, Fasina, Eloff, & McGaw, 2019; Frassinetti, Gabriele, Moccia, Longo, & Di Gioia, 2020; Jun et al., 2018), which leads to more serious health implications.

A detailed understanding of the interactions between bacteria and DF is required to fully elucidate their effect on health and to allow the institution of better dietary guidelines (Carlson, Erickson, Hess, Gould, & Slavin, 2017; Havlik & Edwards, 2018). The aim of this research was to assess the effect of upcycled persimmon byproducts in beneficial and pathogenic bacterial strains after simulated *in vitro* digestion.

2. Materials and methods

2.1. Chemicals and reagents

Glucose, electrolyte salts, digestive enzymes, antibiotics (kanamycin, gentamycin, and erythromycin), triphenyl tetrazolium chloride (TTC), crystal violet (CV), phosphate buffer solution (PBS), HCl, and gallic acid were all purchased from Sigma–Aldrich (Madrid, Spain). Ethanol, acetone, dimethyl sulfoxide (DMSO) and NaOH were acquired from Panreac (Barcelona, Spain). Ampicillin and amoxicillin discs were purchased from Oxoid (Madrid, Spain). The culture media used consisted of buffered peptone water (BPW) from Scharlab (Barcelona, Spain); tryptic soy broth (TSB) and tryptic soy agar (TSA) from Labkem (Barcelona, Spain); Man, Rogosa & Sharpe broth (MRSB) from Panreac; and Man, Rogosa & Sharpe agar (MRSA) from Biokar (Barcelona, Spain).

2.2. Plant material and DF extraction

'Rojo Brillante' fresh persimmon fruits were purchased from a local market (Orihuela, Spain). Fruits were processed at a pilot-scale facility to obtain persimmon juice according to the process described by Gea--Botella et al. (2021). After filtering, the obtained byproduct consisting of peel, peduncles, and pulp was stored at −20 °C.

Solvent-assisted extraction (SAE) conditions were previously tested and developed considering the optimal purification of upcycled DF, enzymatic inactivation, food safety for human consumption and solvent recovery capabilities. Persimmon byproducts were processed through SAE using three different treatments: distilled water (AQ), 70% ethanol (EtOH) and 70% acetone (CET) at a 1:5 (w/v) ratio. The mixture was heated to 60 °C for 15 min under constant agitation. Then, the mixture was filtered (0.025 mm), and the solid fraction was frozen at −20 °C. Ethanol and acetone were removed and recycled by evaporation prior to freezing the samples. SAE fractions were frozen, dried, ground, and sieved (0.5-mm metal mesh).

2.3. Microbial strains and inoculum preparation

Four microorganisms from Spanish Type Culture Collection (CECT), lactic acid bacteria (LAB) (*Lactobacillus casei* CECT 475, *Lactococcus lactis* subsp. *lactis* CECT 185), *Streptococcus salivarius* subsp. *thermophilus* CECT 7207) and the bifidobacterial *Bifidobacterium bifidum* CECT 870 were used to determine the effect of persimmon DF on potential health-beneficial bacteria. Gram-negative bacteria *S. enterica* CECT 443, *E. coli* CECT 515, and *Pseudomonas putida* CECT 324, as well as the gram-positive bacteria *S. aureus* CECT 59 and *B. cereus* INRA TZ415 (provided by Dr. Frédéric Carlin from Institut National de la Recherche Agronomique, Centre de Recherches d'Avignon, Station de Technologie des produits Végétaux, Domaine Saint-Paul, Site Agroparc, Avignon, France) and *Bacillus subtilis* CECT 39 were used to explore the effects of persimmon DF against human pathogens.

Individual colonies from stock Petri dishes of every microorganism were activated in TSB or MRSB and incubated according to each microorganism's requirement. After incubation, the overnight cell suspensions were adjusted to a 0.5 McFarland [10^8 colony-forming units (CFU)/mL] concentration in 1% BPW using a plate reader at 600 nm. Then, five concentrations from 10^8 to 10^4 CFU were also prepared in serially diluted tubes with 1% BPW.

2.4. In vitro digestion model

An *in vitro* digestion model was used as previously described by Minekus et al. (2014) with some modifications. First, 0.5 g of every sample (AQ, EtOH and CET DF fractions) was subjected to a three-phase simulated digestion. All solutions used in this assay were previously sterilized and tempered at 37 °C. The assay was performed in triplicate. Half of the samples were not digested for future comparative tests.

Oral phase: Samples were mixed in a flask with 3.5 mL of artificial salivary fluid [KCl, KH_2PO_4, $NaHCO_3$, $MgCl_2$ and $(NH_4)_2CO_3$], 0.5 mL of 75 U/mL α-amylase, 25 μL of 0.3 mol/L $CaCl_2$ and 975 μL of double-distilled water (ddH_2O) to form the oral bolus. The pH of bolus was adjusted to 7. The mixture was then incubated at 37 °C for 2 min at 150 rpm in a benchtop incubator shaker (ZHWY-100B, Zhicheng).

Gastric phase: The oral bolus was mixed with 7.5 mL of artificial gastric solution [KCl, KH_2PO_4, $NaHCO_3$, NaCl, $MgCl_2$ and $(NH_4)_2CO_3$], 1.6 mL of 2000 U/mL pepsin, 5 μL of 0.3 mol/L $CaCl_2$ and 695 μL of ddH_2O to form the gastric chyme. The pH was adjusted to 3 with 1 mol/L HCl. The sample was then incubated at 37 °C for 120 min under constant shaking (150 rpm).

Intestinal phase: Gastric chyme was mixed with 11 mL of artificial intestinal solution [KCl, KH_2PO_4, $NaHCO_3$, $MgCl_2$ and NaCl], 5 mL of 100 U/mL pancreatin, 2.5 mL of 10 mmol/L bile extract solution, 40 μL of 0.3 mol/L $CaCl_2$ and 1.31 mL of ddH_2O to form the intestinal chyle. pH was adjusted to 7 with 1 mol/L NaOH. The sample was incubated at 37 °C for 120 min under constant shaking (150 rpm).

Each fraction and control were centrifuged at 948×*g* for 10 min at 4 °C, and the supernatants and pellets were separated. All fractions were immediately frozen (liquid nitrogen) and stored at −80 °C until use. Digested pellets were used for the following assays as they reached the colon and influenced the gut microbiome.

2.5. Prebiotic activity score (PAS) assay

The prebiotic activity assay was performed according to Praveen, Parvathy, Jayabalan, and Balasubramanian (2019) and Zarinah et al. (2018), with slight modifications. The assay was carried out by mixing a

3% (v/v) overnight culture of *B. bifidum*, *L. casei, L. lactis* or *S. salivarius* (10^7 CFU/mL) with 3% (w/v) undigested or digested samples into 5 mL of 1% BPW. Different tubes with 3% (w/v) glucose were included as substrate controls. Simultaneously, the same procedure was performed with the enteric strain *E. coli* (10^7 CFU/mL) in 1% BPW.

All tubes were incubated at 37 °C for 48 h under aerobic (*S. salivarius, L. lactis* and *E. coli*) and anaerobic (*B. bifidum* and *L. casei*) conditions. After incubation, the tubes were serially diluted, and seeding was carried out on the corresponding culture media. Colonies were counted at 0 and 48 h of fermentation. Bacterial growth between 0 and 48 h is expressed as a percentage.

With the results of controls (*E. coli* and glucose), PAS was calculated to determine the potential of persimmon DF to serve as a prebiotic for selected strains that may be useful in identifying combinations that could be added to dairy and other foods (Huebner, Wehling, & Hutkins, 2007). PAS was obtained according to Praveen, Parvathy, Jayabalan, and Balasubramanian (2019) and Zarinah et al. (2018) by the following Eq. (1):

$$PAS = \frac{LogP_X^{48} - LogP_X^0}{LogP_G^{48} - LogP_G^0} - \frac{LogE_X^{48} - LogE_X^0}{LogE_G^{48} - LogE_G^0} \quad (1)$$

where $P_G{}^0$, $P_X{}^0$, $P_G{}^{48}$, and $P_X{}^{48}$ correspond to beneficial strain CFU counts for glucose (*G*) and DF (*X*) samples. $E_G{}^0$, $E_X{}^0$, $E_G{}^{48}$, *and* $E_X{}^{48}$ correspond to the enteric CFU counts for glucose (*G*) and DF (*X*) samples. The assay was performed in triplicate.

2.6. Pathogen-DF-fraction interactions

To explore the effect of persimmon DF fractions against human pathogens, antibacterial activity was assayed before and after *in vitro* digestion. Previously, all samples were dissolved in 1% DMSO and 1% PBS solution to reach a stock concentration of 150 mg/mL. The interaction of DF with pathogens was performed in triplicate.

2.6.1. Antibacterial assays

2.6.1.1. Microdilution assay. A modified colorimetric broth microdilution method was performed as described by Jiménez-Zamora, Pastoriza, and Rufián-Henares (2015). Overnight bacterial suspensions were adjusted with 1% BPW at different concentrations (10^8, 10^6 and 10^4 CFU/mL) before performing the assay. Briefly, 100 μL per well of TSB was added to a 96-well microtiter plate. Then, 100 μL per well of undigested or digested DF solution was added to the first column of the plate. Serial twofold dilutions were made in each well row, with the final aliquot from the tenth well being discarded. Subsequently, 100 μL of one bacterial suspension was added to each well of the corresponding row to reach a final volume of 200 μL per well. DF concentrations per well ranged from 0.07 to 37.5 mg/mL. Kanamycin (200 μg/mL) was used as a positive control. As negative controls, 1% DMSO with 1% PBS solution was used for undigested samples, and a digested blank was used for digested samples. Bacterial inoculum in TSB was used as growth controls. Microplates were incubated at 37 °C for 24 h.

After incubation, 20 μL of each well was serially diluted into 180 μL of TSB. Then, 100 μL of each dilution was spread out on TSA plates, and CFUs were quantified. The growth percentage reduction (GPR) of the pathogenic bacterial population was determined by comparing the final growth of cells treated with DF solutions with that of negative control cells. Bacterial growth was confirmed by adding 10 μL of aqueous 0.5% TTC solution to each well and incubating at 37 °C for 1 h. The color change from clear to red indicated the reduction of TTC to formazan and therefore bacterial growth.

2.6.1.2. Agar-well diffusion method. A pathogenic interaction test was also performed according to Bauer, Kirby, Sherris, and Turck (1996) and Palanisamy et al. (2017). One hundred microliters of bacterial suspension (10^8 CFU/mL) was inoculated on TSA plates. Five-millimeter-diameter wells were made on preincubated agar plates using gel puncture. After 10–15 min, DF fractions (37.5 mg/mL) were loaded in each well. The plates were incubated at 37 °C for 24 h, and then the zone of inhibition was measured. Ampicillin and amoxicillin discs were used as positive controls, while 1% DMSO with 1% PBS solution or digested blank were used as negative controls.

2.6.2. Determination of antibiofilm activity

The assays were performed against three different stages of biofilm formation: initial cell attachment (ICA), preformed biofilm (PFB), and matured biofilm (MB) (Famuyide et al., 2019). First, the potential to prevent ICA was investigated. Briefly, 100 μL of bacterial suspension whose growth was reduced in the previous assay was added to individual 96-well plates and incubated at 37 °C for 4 h. Then, 100 μL of DF fractions at active concentrations were added. The plates were further incubated at 37 °C for 24 h.

The ability of DF fractions to inhibit the development of immature biofilms, as well as to knock down a biofilm mass, was also assessed. For that, two 96-well plates containing 100 μL of bacterial suspension from each microorganism were incubated for 24 and 48 h; after incubation, DF fractions at previously identified active concentrations were added, and then plates were incubated for 24 h more. Gentamicin (20 μg/mL) served as a positive control, while dH_2O or digested blank served as a negative control.

After incubation, the biomass of each well was quantified by CV staining. Samples and cells that had not been attached to the well were discarded, washed four times with dH_2O, and air-dried for 1 h. Wells were stained at room temperature for 15 min with 100 μL of 0.5% CV. Then, the wells were washed four times with dH_2O, air-dried, and finally washed with 125 μL of ethanol for 1 min. Absorbance was measured at 590 nm using a microplate reader (BioTek Instruments, Inc., Vermont, USA). The biofilm inhibition percentage (*BI%*) was calculated by Eq. (2):

$$Biofilm\ \ inhibition\ (BI\%) = \frac{OD_{negative\ \ control} - OD_{experimental}}{OD_{negative\ \ control}} \times 100 \quad (2)$$

where *OD* is the optical density in absorbance units read at 590 nm.

2.6.3. Synergy activity

2.6.3.1. Checkerboard assay. The checkerboard broth microdilution method described previously by Brennan-Krohn and Kirby (2019) and Haroun and Al-Kayali (2016) was used with slight modifications to determine synergism between DF fractions and antibiotics. Twofold serial dilutions of the antibiotic and DF samples were prepared for every combination tested. Fifty microliters of each solution was added to 96-well plates. Then, 100 μL of one bacterial suspension (10^8, 10^6 and 10^4 CFU/mL) was added to each well. After incubation (37 °C for 24 h), 10 μL of an aqueous 0.5% TTC solution was added to each well and further incubated for 1 h.

The synergistic activity was calculated as the fractional inhibitory concentration index (FICI) by Eq. (3):

$$FICI = \frac{S}{C_S} + \frac{A}{MIC_A} \quad (3)$$

where *S* and *A* are the concentration of the DF fraction and the antibiotic present in a single well, while C_S and MIC_A are the lowest concentration of the DF fraction that cause a GPR of a pathogenic strain and the minimum inhibitory concentration (MIC) of antibiotic, respectively. The results were interpreted as follows: FICI <0.5, synergic combination; FICI between 0.5 and 1, additive combination; FICI within 1–4, indifferent combination; and FICI >4, antagonistic combination.

The antibiotics used in this assay were the macrolide erythromycin (0.03–2 μg/mL) and the aminoglycosides gentamicin (0.08–5 μg/mL) and kanamycin (0.78–50 μg/mL).

2.6.3.2. Standard time-kill testing. A standard time-kill test (ASTM, 2016) was performed to obtain information on both inhibition and killing over a 24-h time course and to confirm whether the observed effects were bactericidal. This assay was performed only for the DF fraction-antibiotic combinations and concentrations found to be synergistic or additive in the checkerboard assay.

Five tubes with 5 mL of TSB were prepared. Tube 1 also contained an aliquot of digested DF sample treated with acetone as solvent (dCET) to reach the concentration that had shown synergistic or additive effects; Tube 2 contained an aliquot of antibiotic to reach the concentration that had shown the same effects; Tube 3 contained aliquots of dCET and antibiotic altogether; Tube 4 contained 10^6 CFU/mL bacterial suspension (growth control); and Tube 5 contained only culture medium (blank control). All tubes were vortexed and incubated according to each microorganism's requirements. Concentrations of both the dCET sample and antibiotic were at the subinhibitory level.

At 0, 1, 2, 4, 6, and 24 h, 20-μL aliquots were taken from each tube and serially diluted in a 96-well plate with 180 μL of 1% BPW. CFUs were determined by the drop plate method by taking 10 μL of diluted aliquots from microtiter wells with a multichannel pipette and plating them onto the TSA surface on 8 columns per dish. Plates were incubated at 37 °C for 24 h. After incubation, the bacterial population was quantified.

If the reduction in the bacterial population exposed to the dCET-antibiotic combination after 24 h of incubation was ≥3 log CFU/mL, the combination was considered bactericidal. Moreover, the difference between the bacterial population exposed to the dCET-antibiotic combination versus antibiotic alone after 24 h of incubation was calculated; if this difference was ≥2 log CFU/mL reduction, then the combination was considered synergistic (Brennan-Krohn & Kirby, 2019).

2.7. Preparation of persimmon fiber-bound phenolic extract

Bound phenolics were extracted according to the procedure described by Singh, Negi, and Radha (2013). The antibacterial activity of the bound phenolic extract against pathogenic strains was also evaluated by the broth microdilution (0.03–15.00 μg/μL concentration range) and agar-well diffusion (30 μg) methods. Likewise, GPR was calculated using the MTT solution.

2.8. Identification and quantification of fiber-bound phenolics

Bound phenolic extracts were determined by high-performance liquid chromatography (HPLC-DAD) according to Abu-Reidah et al. (2012) and Salazar-Bermeo et al. (2021). An HPLC Agilent 1200 series instrument (Agilent Technologies, Inc., California, USA) supported with an autosampler, vacuum degasser, quaternary pump, and G1315B diode array detector was used. Chromatographic separation was performed in a Poroshell C18 HPLC column (2.7 μm, 4.6 × 150 mm).

The mobile phases were acetic acid 0.5% v/v as eluent A and acetonitrile as eluent B. The chromatographic method consisted of the following gradient: 0 min, 0% B, 10 min, 20% B, 15 min, 30% B, 20 min, 50% B, 25 min, 75% B, 30 min, 95% B, 33 min 100% B; a 9-min postrun was used after each analysis. The column temperature was kept at 25 °C, and the injection volume was 20 μL. The flow rate was set at 0.70 mL/min throughout the gradient run.

The chromatograms were recorded over the full-range UV/vis spectrum. Quantification was executed by comparing UV absorption spectra and retention times (RT) with authentic standards injected under the same conditions. Control of the system and data evaluation were achieved with Agilent ChemStation for LC version A.00.03.

2.9. Statistical analysis

Experimental results are expressed as the means (n = 3) ± standard error (SE). Where applicable, differences between samples and controls were analyzed using one-way analysis of variance (ANOVA), and significant differences between the means were tested using Tukey's or Dunnett's multiple comparison tests. Differences were considered significant if $p < 0.05$. The statistical software GraphPad Prism 8.0.2 was used to analyze the data.

3. Results

3.1. Assessment of the prebiotic activity of DF from persimmon byproducts

Undigested (u) and digested (d) DF fractions promoted cell proliferation in all beneficial bacteria tested (Fig. 1A). Additionally, higher bacterial growth ($p < 0.05$) after the *in vitro* digestion process was observed. The dCET fraction showed the highest growth stimulation in *L. casei* (54.82 ± 0.80%), followed by *B. bifidum* (38.22 ± 5.54%) and *S. salivarius* (28.90 ± 1.58%). *L. lactis* growth was also highly stimulated (46.21 ± 6.60%) by the dEtOH fraction, whereas the dAQ fraction promoted *B. bifidum* proliferation (23.27 ± 0.77%). The uCET fraction promoted bacterial growth according to the decreasing order *S. salivarius* > *L. casei* > *L. lactis* and *B. bifidum*. uEtOH stimulated proliferation in *L. lactis*, while uAQ did so in *L. casei*. Growth stimulation ranges were higher than those reported for soy (okara) and acerola byproducts, barley β-glucan, grape seed flour, and blueberry juice (Kwon, Kim, Seo, Lee, & Kim, 2018; Lee, Jang, Lee, & Kong, 2020; Vieira, Bedani, Albuquerque, Biscola, & Saad, 2017; Wu et al., 2021).

PAS results revealed the potential of persimmon DFs to act as a carbon source for beneficial host microorganisms in comparison with glucose. For instance, PAS was positive (Fig. 1B) for the dAQ fraction, with significant activity for *B. bifidum* (1.22 ± 0.18). The dCET fraction also showed high effects on *B. bifidum* (2.15 ± 0.26), *L. casei* (1.89 ± 0.01) and *S. salivarius* (0.88 ± 0.04). Regarding L. *lactis*, all DF fractions exhibited a positive PAS, with dEtOH showing the highest value (2.46 ± 0.47). PAS was higher for digested DF fractions ($p < 0.05$). However, interactions of dEtOH with *L. casei* and *S. salivarius* showed a negative PAS. These results suggest that glucose acted as the main carbon source for these bacterial strains; alternatively, *E. coli* could have been more competitive than the others to metabolize persimmon DF fractions.

3.2. Antibacterial activity by the microdilution method

The antibacterial activities of persimmon DF fractions against six pathogenic strains are given in Table 1. A GPR in the tested bacteria was observed after 24 h of incubation in the presence of persimmon DF fractions, especially digested fractions (dEtOH and dCET) ($p < 0.0001$). GPR in gram-negative bacteria was registered at different cell densities (10^8 and 10^6 CFU/mL) and DF concentrations (2.34–37.50 mg/mL), while in gram-positive bacteria, a decrease was observed at low densities (10^6 and 10^4 CFU/mL) and at similar DF concentrations (1.17–37.50 mg/mL). Within gram-positive strains, *S. aureus* at 10^6 UFC/mL was the most sensitive strain in the presence of 37.50 mg/mL dAQ, 1.17 mg/mL dEtOH or 9.38 mg/mL dCET in terms of GPR. Among gram-negative bacteria, *P. putida* showed a high GPR in the presence of dCET ($p < 0.05$).

3.3. Inhibition zones in the agar well diffusion assay

Table 2 summarizes the inhibition zones for all pathogenic bacterial strains tested. Overall, uDF fractions did not show a strong inhibitory effect when compared to dDF fractions; however, digested samples, especially dCET, showed larger inhibition zones in all bacteria ($p < 0.0001$). In *S. aureus*, dCET produced a zone of inhibition greater than both antibiotic controls (11.30 ± 0.14 mm). Additionally, dCET showed similar inhibitions in *P. putida* and *S. enterica* when compared to ampicillin. *E. coli* was the least affected bacterium by DF fractions.

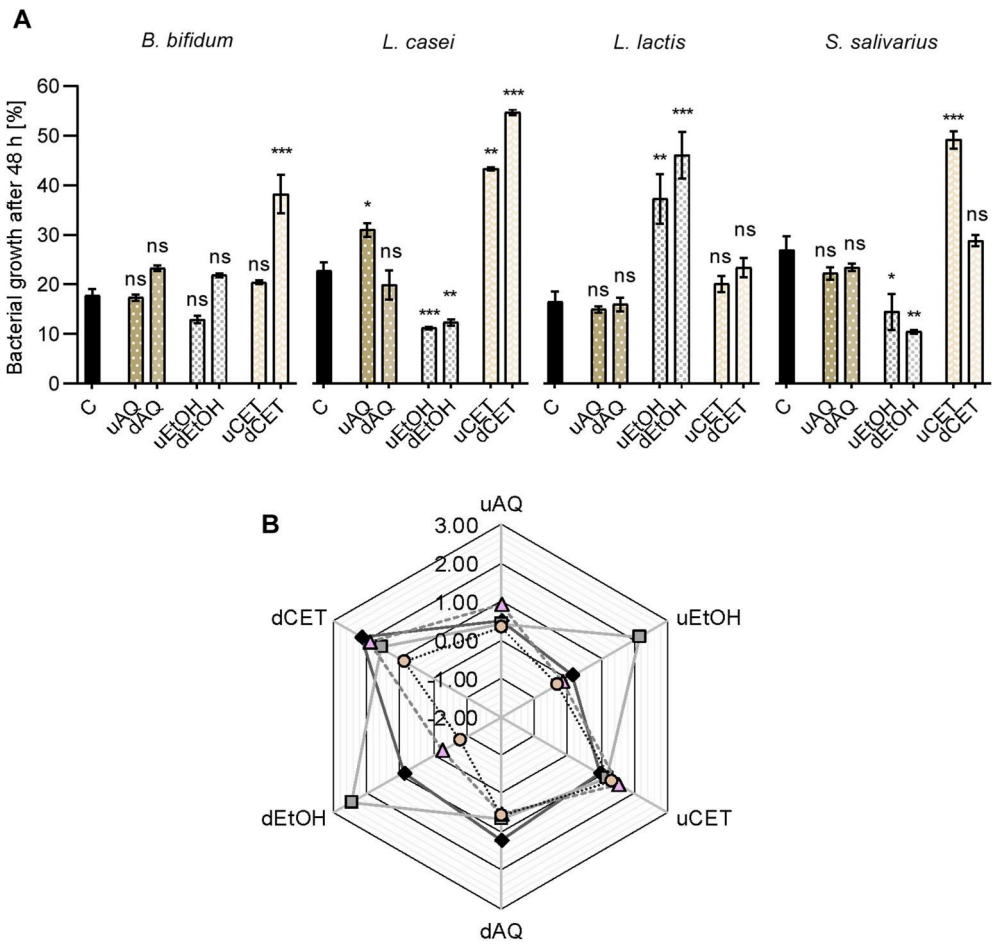

Fig. 1. Effect of dietary fiber (DF) from persimmon byproduct treated with aqueous (AQ), ethanol (EtOH) or acetone (CET) solvents on beneficial host bacteria after 48 h of fermentation. **1A.** Bacterial growth percentage (%) of bacteria exposed to undigested (u) and digested (d) DF fractions. Digested fractions stimulated a higher proliferation of bacteria as compared to glucose control (C) (***$p < 0.001$, **$p < 0.01$, *$p < 0.05$, ns $p > 0.05$ ANOVA with Dunnett's post hoc test). **1B.** Calculated prebiotic activity scores (PAS) for *B. bifidum* (—◆—), *L. casei* (—□—), *L. lactis* (---△---) and *S. salivarius* (····○····). Values > 0 indicate prebiotic activity while values ≤ 0 indicate no prebiotic activity. Values are means (n = 3) ± standard error of the mean.

Table 1
Summary of antibacterial activity data obtained by microdilution method of undigested and digested dietary fiber (DF) from persimmon byproduct treated with aqueous (AQ), ethanol (EtOH) or acetone (CET) solvents.

M.O.	Undigested DF fractions						Digested DF samples					
	uAQ		uEtOH		uCET		dAQ		dEtOH		dCET	
	C	GPR (%)	C	GPR (%)	C	GPR (%)	C	GPR (%)	C	GPR (%)	C	GPR (%)
E. coli	n.r.		37.50	1.92 ± 0.01a, b *	18.75	0.55 ± 0.02a *	18.75	0.79 ± 0.00a *	18.75	5.26 ± 0.01b, c, d, e *	18.75	1.51 ± 0.03a, b *
P. putida	37.50	2.09 ± 0.04a, b *	37.50	7.89 ± 0.04d, e, f, g **	37.50	17.80 ± 0.14k ***	37.50	4.30 ± 0.01a, b, c, d **	18.75	11.58 ± 0.05g, h, i **	37.50	19.25 ± 0.03k, l, m **
S. enterica	37.50	0.60 ± 0.04a *	n.r.		9.38	3.04 ± 0.00a, b, c *	37.50	2.65 ± 0.16a, b *	9.38	0.68 ± 0.02a *	2.34	4.60 ± 0.02a, b, c, d, e *
B. cereus	37.50	11.75 ± 0.28g, h, i ***	18.75	12.88 ± 0.12i, j ***	37.50	10.36 ± 0.13f, g, h, i ***	37.50	16.67 ± 0.02j, k **	37.50	22.45 ± 0.03m, n ***	9.38	16.94 ± 0.10 k **
B. subtilis	37.50	8.61 ± 0.01e, f, g, h ***	18.75	25.09 ± 0.14n ***	37.50	6.99 ± 0.01c, d, e, f **	9.38	12.16 ± 0.10h, i ***	37.50	19.41 ± 0.00k, l, m **	9.38	12.47 ± 0.01h, i **
S. aureus	37.50	30.22 ± 0.10° ***	4.69	21.82 ± 0.03l, m, n ***	0.59	34.88 ± 0.25p ***	37.50	35.70 ± 0.01p **	1.17	17.99 ± 0.01k, l **	9.38	37.85 ± 0.01p **

Digested (d) fractions showed significant differences in growth percentage reduction (GPR) in comparison with undigested (u) samples, especially against gram-positive bacteria. Different superscript letters near GPR values indicate significant differences between DF fractions and tested bacteria ($p < 0.05$ ANOVA with Tukey's post hoc test). 10^8 (*), 10^6 (**) or 10^4 (***) colony forming units (CFU)/mL affected by DF samples. M.O.: microorganism; C: DF active concentration (mg/mL); n.r.: no growth reduction observed. Values are means (n = 3) ± standard error of the mean.

3.4. Antibiofilm activity

Biofilm inhibition results are shown in Fig. 2. According to the criteria established by (Famuyide et al., 2019), *BI%* values < 0% represent enhancement of growth, while values ≥ 50% reflect significant biofilm inhibition. Values between 0 and 50% represent poor inhibition effect. Thus, strong antibiofilm activity during ICA was mainly showcased by digested DF fractions. All digested DF fractions inhibited

Table 2
Inhibition zones (mm) obtained by agar-well diffusion method using dietary fiber (DF) from persimmon byproduct treated with aqueous (AQ), ethanol (EtOH) or acetone (CET) solvents.

M.O.	Inhibition zone (mm)							
	Undigested DF samples			Digested DF samples			Control	
	uAQ	uEtOH	uCET	dAQ	dEtOH	dCET	AMP	AMC
E. coli	n.i.	5.05 ± 0.07a	5.70 ± 0.14a, b	7.40 ± 0.28e, f, g	7.00 ± 0.28d, e, f, g	7.50 ± 0.14f, g, h	8.24 ± 0.84	11.00 ± 0.14
P. putida	6.30 ± 0.14b, c, d	6.60 ± 0.00c, d, e	7.30 ± 0.14e, f, g	7.70 ± 0.14g, h, i	6.70 ± 0.14d, e, f	9.00 ± 0.28j, k	9.33 ± 0.89	11.20 ± 0.14
S. enterica	6.40 ± 0.14b, c, d	n.i.	7.30 ± 0.14e, f, g	8.30 ± 0.42h, i, j	5.80 ± 0.00a, b, c	8.80 ± 0.85j, k	8.67 ± 0.89	12.00 ± 0.55
B. cereus	7.60 ± 0.28g, h	6.70 ± 0.14d, e, f	8.30 ± 0.14h, i, j	7.40 ± 0.28e, f, g	8.50 ± 0.14i, j	10.40 ± 0.00l	8.67 ± 0.55	12.30 ± 0.14
B. subtilis	6.10 ± 0.14b, c	6.30 ± 0.14b, c, d	6.90 ± 0.14d, e, f, g	8.30 ± 0.28h, I, j	8.70 ± 0.14j, k	9.40 ± 0.00k	25.03 ± 0.55	14.67 ± 0.89
S. aureus	5.60 ± 0.00a, b	6.40 ± 0.00b, c, d	7.50 ± 0.14f, g, h	10.60 ± 0.00l, m	9.38 ± 0.16k	11.30 ± 0.14m	9.40 ± 0.55	10.70 ± 0.84

Digested (d) fractions showed greater inhibition zones than undigested (u) samples, especially against Gram-positive bacteria. The inhibition of *B. cereus* and *S. aureus* registered by dEtOH was like the inhibition by ampicillin (AMP) while dCET generated a similar inhibition in *S. aureus* compared to amoxicillin (AMC). Different superscript letters express significant differences between DF fractions and tested bacteria ($p < 0.05$ ANOVA with Tukey's post hoc test). Values are means (n = 3) ± standard error of the mean. M.O: microorganism; n.i.: no inhibition observed.

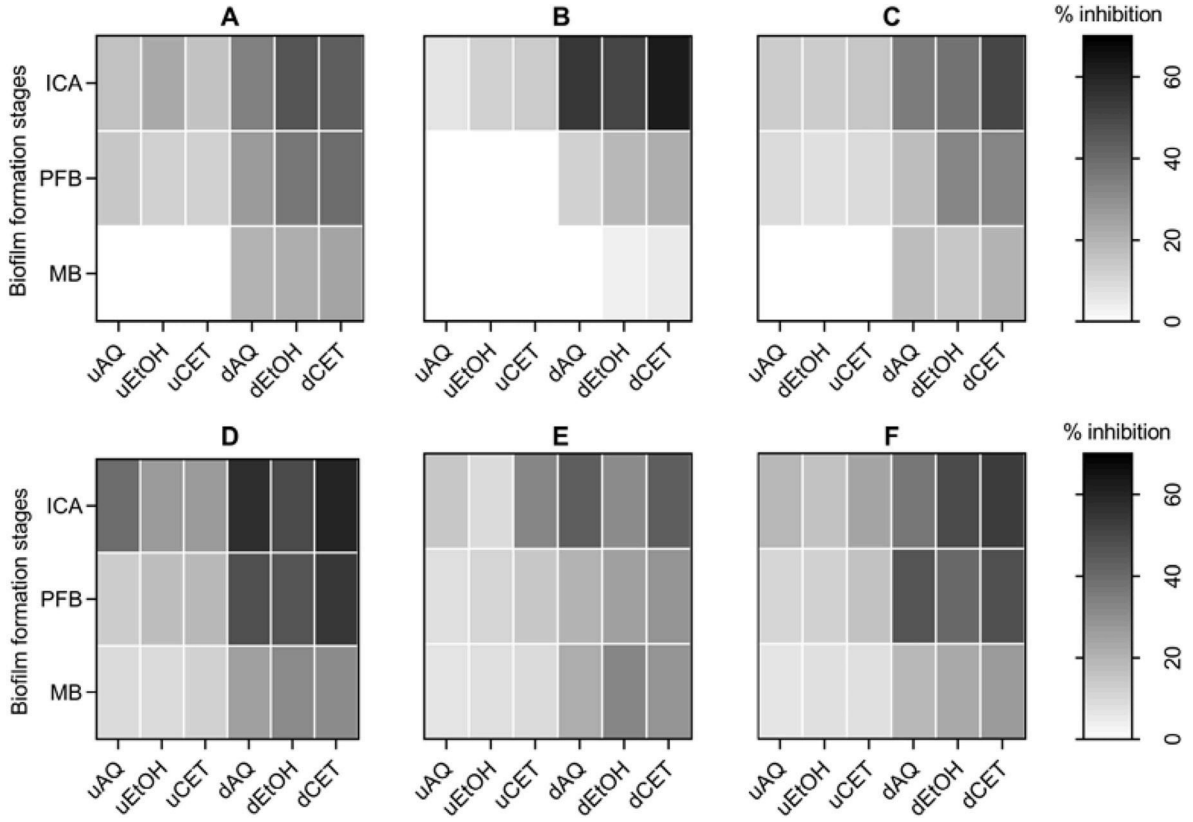

Fig. 2. Biofilm inhibition percentage (*BI%*) of **2A.** *E. coli*, **2B.** *P. putida*, **2C.** *S. enterica*, **2D.** *S. aureus*, **2E.** *B. cereus* and **2F.** *B. subtilis*, caused by undigested (u) and digested (d) dietary fiber (DF) from persimmon byproduct treated with aqueous (AQ), ethanol (EtOH) or acetone (CET) against initial cell attachment (ICA, 4 h), preformed biofilm (PFB, 24 h), and mature biofilm (MB, 48 h). dCET inhibited *P. putida*, *S. aureus* and *B. subtilis* ICAs, as well as *S. aureus* PFB (*BI%* > 50%) (n = 3).

the ICA of *P. putida* (*BI%* > 50%), mainly dCET (62.23 ± 0.86%). *S. aureus* ICA was also inhibited by dAQ and dCET above 50%, with dCET generating the major inhibition on this bacterium (59.66 ± 0.78%). *B. subtilis* ICA was equally inhibited by dCET, with a *BI%* value slightly above 50% (50.29 ± 1.72%).

Inhibition of development in the bacterial PFB after 24 h of incubation and inhibition of MB mass after 48 h of incubation were also evaluated in this assay (Fig. 2). dCET samples expressed significant antibiofilm activity (*BI%* > 50%) against *S. aureus* PFB, whereas low inhibition against PFBs was shown by the other DF fractions. No inhibition of MBs from the tested bacterial strains was observed.

3.5. Synergistic activity and time-kill testing

Since dCET was the DF fraction that generated the highest decrease in pathogenic bacterial growth according to previous assays, its potential synergistic activity was determined in combination with kanamycin (K), gentamycin (G), and erythromycin (E) by the checkerboard assay (Table 3).

The results indicated a synergistic effect expressed by the combination dCET+K (FICI = 0.38) against *B. subtilis* and by the combination dCET+G (FICI = 0.38) against *S. aureus* ($p < 0.0001$). Additive effects were observed for both combinations ($0.5 \leq$ FICI ≤ 1) against other microorganisms. The combination dCET+E was indifferent ($1 <$ FICI ≤ 4) against all the assessed bacterial strains ($p > 0.05$).

To validate the results obtained by the checkerboard assay, a time-

Table 3
Checkerboard results of digested dietary fiber from persimmon byproduct treated with acetone solvent (dCET) in combination with different antibiotics.

Microorganism	FICI					
	dCET+K	Result	dCET+G	Result	dCET+E	Result
E. coli	0.63 ± 0.00b,c	A	0.57 ± 0.00b	A	1.01 ± 0.04d	I
P. putida	0.52 ± 0.10a,b	A	0.75 ± 0.00c	A	1.01 ± 0.04d	I
S. enterica	0.56 ± 0.05b	A	0.53 ± 0.10b	A	1.00 ± 0.04d	I
B. cereus	0.63 ± 0.00b,c	A	0.63 ± 0.00b,c	A	1.02 ± 0.04d	I
B. subtilis	0.38 ± 0.00a	S	0.75 ± 0.04c	A	1.02 ± 0.10d	I
S. aureus	0.63 ± 0.04b,c	A	0.38 ± 0.04a	S	1.02 ± 0.04d	I

According to Fractional Inhibitory Concentration Index (FICI): FICI <0.5, synergy (S); 0.5 ≤ FICI ≤1, additive (A); 1 < FICI ≤4, indifferent (I); FICI >4, antagonist (ANTAG). dCET showed synergistic effect with kanamycin (K) and gentamycin (G) against *B. subtilis* and *S. aureus*, respectively, while an additive effect against the remaining bacteria was observed. An indifferent effect was determined by the combination of dCET with erythromycin (E). Different superscript letters express significant differences between combinations and tested bacteria ($p < 0.05$ ANOVA with Tukey's post hoc test). Values are means ± standard error of the mean (n = 3).

kill test was performed for dCET-antibiotic combinations. The time-kill test (Fig. 3) showed that bacterial cultures that contained dCET or antibiotic alone at subinhibitory concentrations did not display any effect on bacterial proliferation, reaching similar populations to the control. However, when dCET was combined with K or G, a decrease in growth in all bacteria was observed.

According to the time-kill kinetics assay (Table 4), the effects of dCET+K or dCET+G combinations were bacteriostatic (Bt) against almost all assessed bacterial strains. Notwithstanding the above, a bactericidal (Bc) effect was observed on *S. aureus* exposed to dCET+G (3.10 log CFU/mL reduction) ($p < 0.0001$). Moreover, growth differences between bacterial populations exposed to these dCET-antibiotic combinations versus antibiotic alone showed synergistic (S) effects against almost all assessed bacteria. Only the dCET+G combination was additive (A) against *S. enterica* (1.94 log CFU/mL reduction) ($p < 0.05$). The highest synergistic activities (4.71 and 5.74 log CFU/mL reduction) of both dCET-antibiotic combinations were exhibited against *S. aureus* ($p < 0.0001$), while the lowest synergistic activity (2.10 log CFU/mL reduction) was determined for the dCET+K combination against *S. enterica* ($p < 0.05$).

3.6. Antibacterial activity of fiber-bound phenolic extracts

The antibacterial activity of persimmon fiber-bound phenolic extracts against six pathogenic strains is given in Table 5. The minimum inhibitory concentration (MIC) values determined against gram-positive and gram-negative bacteria were 0.23 and 0.47 mg/mL, respectively, while GPRs ranged from 77 to 86% and from 80 to 99% depending on the bacterial species tested. Therefore, gram-positive bacteria showed a clearly higher sensitivity (MIC/2) to the bound phenolic extracts than gram-negative bacteria ($p < 0.05$). Their respective values of minimum bactericidal concentration (MBCs: 0.47 vs. 0.94 mg/mL) confirm this fact.

In addition, inhibition zones of bound phenolic extracts against all pathogenic bacterial strains tested are summarized in Table 5. The size of inhibition zones caused by these extracts in the agar-well diffusion

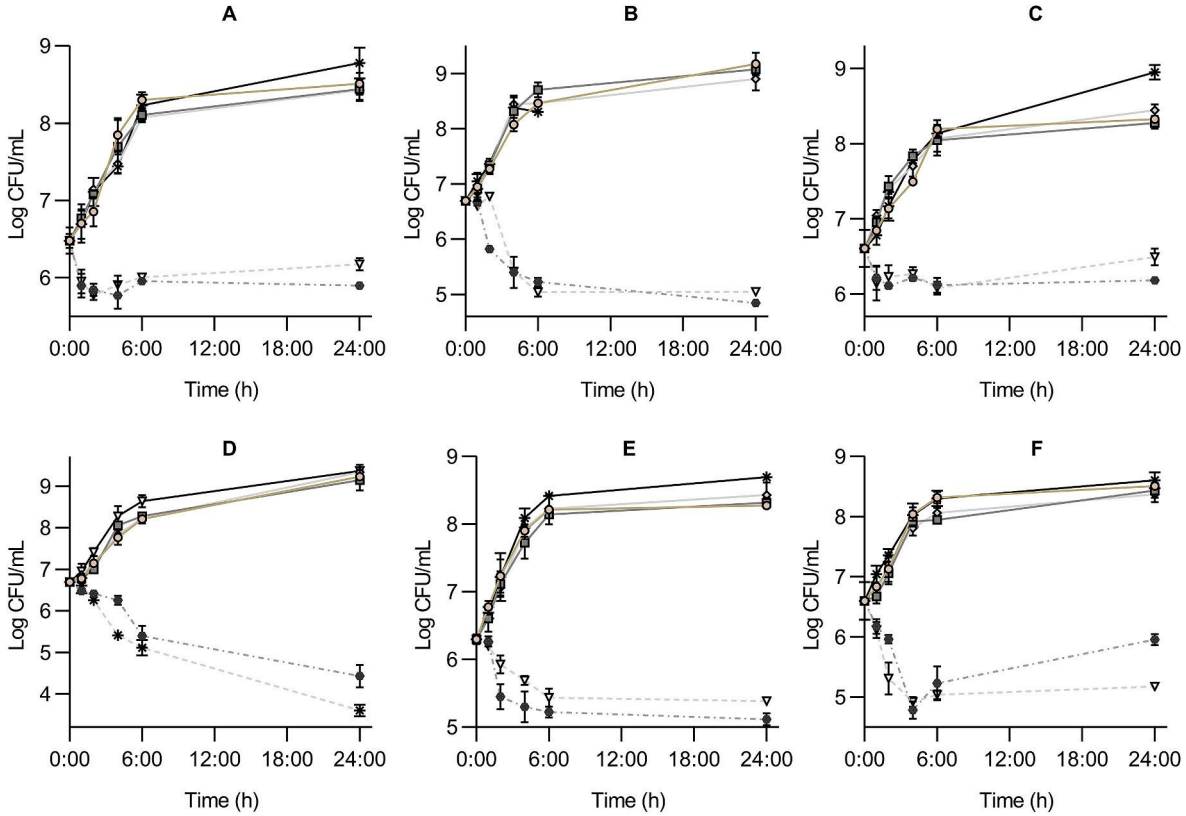

Fig. 3. Time-Kill kinetics of **3A.** *E. coli*, **3B.** *P. putida*, **3C.** *S. enterica*, **3D.** *S. aureus*, **3E.** *B. cereus* and **3F.** *B. subtilis*, in presence of digested dietary fiber treated with acetone (dCET) combined with kanamycin (dCET+K, ●) or gentamicin (dCET+G, ▽). During 24 h of incubation, the growth control (✱) showed the highest proliferation in bacteria, followed by cells exposed only to sub-inhibitory concentrations of dCET (○), K (■) and G (◇), while bacteria population decrease in presence of combinations (dCET with antibiotics). Values are means (n = 3) ± standard error of the mean. SE values < 0.01 were not plotted.

Table 4
Time-kill results of digested dietary fiber from persimmon byproduct treated with acetone solvent (dCET) in combination with different antibiotics.

		Combination[a]		Combination vs. antibiotic[b]	
Microorganism	Antibiotic	Δ (Log CFU)	Category	Δ (Log CFU)	Category
E. coli	K	0.57 ± 0.08a,b,c	Bt	2.54 ± 0.06c	S
	G	0.30 ± 0,04a,b	Bt	2.26 ± 0.03b,c	S
P. putida	K	1.85 ± 0.01f,g	Bt	4.23 ± 0.03f	S
	G	1.62 ± 0.04e,f	Bt	3.82 ± 0.07e	S
S. enterica	K	0.43 ± 0.21a,b,c	Bt	2.10 ± 0.02a,b	S
	G	0.11 ± 0.06a	Bt	1.94 ± 0.10a	A
S. aureus	K	2.27 ± 0.32g	Bt	4.71 ± 0.26g	S
	G	3.10 ± 0.09h	Bc	5.74 ± 0.16h	S
B. cereus	K	1.19 ± 0.02d,e	Bt	3.21 ± 0.01d	S
	G	0.92 ± 0.02c,d	Bt	3.05 ± 0.11d	S
B. subtilis	K	0.65 ± 0.22b,c	Bt	2.48 ± 0.02c	S
	G	1.43 ± 0.38e,f	Bt	3.19 ± 0.10d	S

Different superscript letters in the same column express significative differences between combinations and tested bacteria ($p < 0.05$ ANOVA with Tukey's post hoc test). Values are means ± standard error of the mean (n = 3). Concentrations of combinations and antibiotics were at sub-inhibitory levels.

[a] Difference in bacterial population exposed to combination after 24 h of incubation. The combination is considered bactericidal (Bc) at ≥ 3 log CFU/mL reduction, if not, bacteriostatic (Bt).

[b] Difference between bacterial population exposed to combination vs. antibiotic alone after 24 h of incubation. The effect was considered synergistic (S) at ≥ 2 log CFU/mL reduction, if not, additive (A).

Table 5
Antimicrobial activity of fiber-bound phenolic extract of persimmon byproduct.

Microorganism	Agar-well diffusion assay	Microdilution assay		
	Inhibition zone	MBC	MIC	Inhibition
	(mm)	(mg/mL)	(mg/mL)	(%)
B. cereus	9.00 ± 1.26a, b	0.47	0.23	80.18 ± 4.75c
B. subtilis	7.83 ± 0.75b, c	0.47	0.23	85.89 ± 2.64b, c
S. aureus	10.33 ± 0.52a	0.47	0.23	76.97 ± 8.23c
E. coli	7.67 ± 0.52c	0.94	0.47	98.54 ± 3.20a, b
P. putida	9.53 ± 0.59a, b	0.94	0.47	98.74 ± 5.20a
S. enterica	9.02 ± 0.63a, b, c	0.94	0.47	80.20 ± 1.46c

Fiber-bound phenolic extract obtained from persimmon byproduct exerted antibacterial activity against pathogenic bacteria, especially against *S. aureus*. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) (99.9% < MBC) were registered by microdilution method. Lower MICs were determined against gram-positive bacteria while higher inhibitions were determined against gram-negative pathogens. Different superscript letters between inhibition zone or inhibition percentage values indicate significant differences in antibacterial activity against all microorganisms evaluated ($p < 0.05$ ANOVA with Tukey's post hoc test). Values are mean (n = 3) ± standard error of the mean.

assay (Fig. 4B) was similar to that registered for digested DF fractions, especially the dCET fraction. In both cases, *S. aureus* was the most sensitive strain (10.33 ± 0.52 mm) ($p < 0.0001$), followed by *B. cereus* (9.00 ± 1.26 mm). The inhibitory effect of bound phenolic extracts on most of the tested pathogenic populations was also comparable to that of the ampicillin control (Table 2).

3.7. Identification of bound phenolics

The HPLC-DAD chromatograms of compounds identified in persimmon fiber-bound phenolic extracts are shown in Fig. 4A. Four phenolic compounds, gallic acid, sinapic acid, salicylic acid and ellagic acid, were identified by RTs corresponding to the respective commercial standards. Gallic acid was the main fiber-bound phenolic compound extracted (88.20 ± 2.60 mg/g), while the others were detected at lower concentrations (<0.01 mg/g).

4. Discussion

This work evaluated the impact of the SAE of DF from persimmon byproducts together with its subsequent simulated *in vitro* gastrointestinal digestion on some microorganisms commonly associated with human health. We first studied the effects of the different DF fractions on the growth and proliferation of four selected strains of human beneficial host bacteria. Undigested and especially digested DF fractions promoted cell proliferation in all strains tested. Overall, DF fractions were shown to be a potential carbon source for the proliferation of *B. bifidum*, *L. casei*, *L. lactis* and *S. salivarius*.

Nondigestible or slowly digestible carbohydrates are associated with a positive modulating activity in the gut microbiota composition and activity, among other beneficial physiological effects (Hernandez-Hernandez, Olano, Rastall, & Moreno, 2019). Nevertheless, the results showed a direct effect of SAE on the growth of beneficial host bacteria. Effectiveness of retention of probiotic bacteria by DF is essential to their survival and gut colonization mainly due to their faster passage through the acidic gastric environment and providing substrate in the colon. Microbial adherence to hydrocarbons could depend on not only the origin of the fiber (Holko & Hrabě, 2012) but also its processing, as suggested in this study. In addition, the low content of soluble fiber (with high amounts of carbon easily metabolized by gut microbiota in the uAQ fraction) could have limited the proliferation of most tested bacterial strains (Harris et al., 2020).

Since all persimmon DF fractions exhibited prebiotic activity on *L. lactis*, this bacterium was able to grow using the oligosaccharides provided in DF. Similar to L. *lactis*, the *Lactobacillus* and bifidobacterial species degrade fructooligosaccharides and polydextrose (Farinha et al., 2015; Ziyuan et al., 2021), whose contents increased after the *in vitro* digestion process. According to the above, the dCET fraction exhibited higher prebiotic activity on *L. casei* and *B. bifidum*, while the dEtOH fraction showed its highest growth capacity on *L. lactis*. Substrate specificity and growth rate are both species- and strain-specific. However, the modulation of gut microbiota should consider the different and complex interrelations between its numerous members; for this reason, it would be essential to carry out *in vitro* and *in vivo* studies considering the entire microbiota (Gibson et al., 2017).

The antimicrobial activity of DF has not been sufficiently evaluated, although, as we have just seen above, it could show a direct effect as a modulating agent of the populations of the gut microbial community, including potential pathogens that can cause serious infections. The study of the antimicrobial activity of different DF fractions from persimmon byproducts by broth microdilution and agar-well diffusion methods highlighted an inhibitory effect, particularly after *in vitro* digestion and on *S. aureus*. Moreover, according to the time-kill test (ASTM, 2016), a bactericidal (Bc) effect of the dCET+G combination against *S. aureus* was observed. Simultaneously, the effects of dCET+K or dCET+G combinations were bacteriostatic (Bt) against the remaining

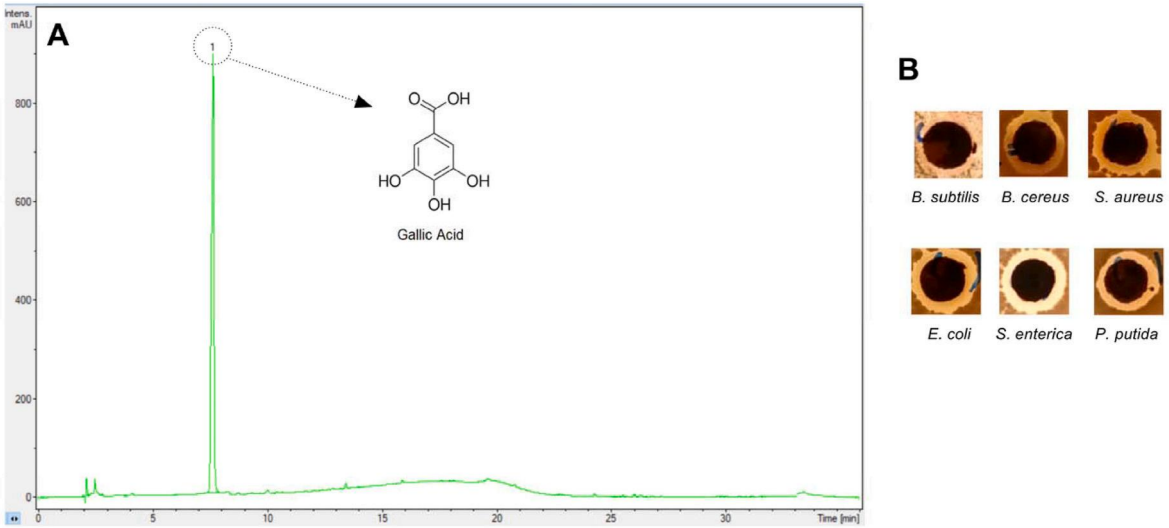

Fig. 4. Effect of fiber-bound phenolic extract from persimmon dietary fiber (DF) against pathogens. **4A.** HPLC-DAD chromatogram of bound phenolic extract. **4B.** Antibacterial activity of bound phenolic extract in agar-well diffusion method. Gallic acid was the main compound identified in the bound phenolic extract from upcycled persimmon DF. Its effect was similar to registered results for the digested DF treated with acetone (dCET) and the ampicillin (AMP) control.

bacterial strains evaluated. However, the growth differences between bacterial populations exposed to these dCET-antibiotic combinations versus antibiotic alone showed synergistic (S) effects against almost all assessed bacteria. Finally, ICA inhibitory activities on biofilm formation by *P. putida*, *S. aureus* and *B. subitilis* were observed for dCET, as well as inhibition of *S. aureus* PFB. Therefore, the multistep process leading to biofilm formation is closely associated with the adhesion of the different selected pathogenic strains to the surface of persimmon DF (Holko & Hrabě, 2012).

The effects of various polysaccharides, such as locust bean, xanthan, agar, carrageenan-maltodextrin, and pectin gums, have been reported by Karlton-Senaye, Ayad, Davis, Khatiwada, and Williams (2016) on the growth of foodborne pathogens, such as *S. enterica*, *S. aureus*, *Listeria monocytogenes*, and *E. coli* O157:H7. Additionally, the combined effects of these gums and eight antimicrobial agents, including kanamycin, were also analyzed. Regarding dCET+K and dCET+G combinations, their results could potentially lead to a synergistic application of polysaccharides and antimicrobial agents to enhance the effect and reduce the recovery period of the pathogenic infections produced by the microorganisms.

The *in vitro* digestion process increased the contents of fructooligosaccharides, polydextrose, and free monosaccharides. The hydrolysis of the digested DF released matrix-bound phenolic compounds such as *p*-coumaric acid, epicatechin, catechin, kaempferol, quercetin, resveratrol, ellagic acid, and gallic acid (Salazar-Bermeo et al., 2021). Fiber-bound phenolic extracts showed antibacterial activity in the agar-well diffusion method similar to the dCET fraction with almost identical inhibition zones against the six pathogenic strains tested. The results suggest that persimmon fiber-bound phenolic extracts were mainly involved in the antimicrobial effect of the dCET fraction. Duda-Chodak (2012) and del Valle et al. (2016) reported inhibitory effects of natural polyphenols on gut microbiota. HPLC-DAD analysis showed that the bound phenolic extract mainly consisted of gallic acid. Indeed, gallic acid has been reported to provide a strong antibacterial effect and can induce irreversible changes in the membrane properties of *E. coli*, *Pseudomonas aeruginosa*, *S. aureus* and *L. monocytogenes* (Borges, Ferreira, Saavedra, & Simões, 2013). As a result, the galloyl groups bonded to the DF matrix could influence bacterial adherence to polysaccharides by affecting their structural and physicochemical properties, and after hydrolysis, the antibacterial activity of persimmon DF was obtained from upcycled byproducts.

5. Conclusions

The obtained results showed upcycled DF from persimmon byproducts as a potential modulator agent of microbial populations. Among the DF fractions tested, dCET exhibited the highest prebiotic, antibacterial, and antibiofilm activities, as well as a synergistic effect in combination with kanamycin or gentamycin against most of the tested bacterial strains. The antibacterial activity displayed by the persimmon dCET fraction may have been due to the presence of fiber-bound galloylated compounds, which after release showed the antibacterial effects assessed, especially gallic acid. These findings suggest that upcycling persimmon byproducts through suitable SAE processing could be applied in developing new ingredients for the food, antimicrobial, prebiotic, and nutraceutical industries; however, variables such as optimal scaling up conditions, solvent recovery technologies, and food safety for human consumption should be addressed in future feasibility studies.

CRediT authorship contribution statement

Bryan Moreno-Chamba: Investigation, Methodology, Formal analysis, Writing – original draft. **Julio Salazar-Bermeo:** Investigation, Methodology, Formal analysis, Writing – original draft. **María Concepción Martínez-Madrid:** Conceptualization, Visualization, Supervision. **Victoria Lizama:** Methodology, Formal analysis. **Francisco Martín-Bermudo:** Conceptualization, Supervision. **Genoveva Berná:** Visualization, Supervision. **Madalina Neacsu:** Conceptualization, Supervision, English proofreader. **Domingo Saura:** Conceptualization, Validation, Project administration. **Nuria Martí:** Conceptualization, Supervision, Validation. **Manuel Valero:** Conceptualization, Formal analysis, Data curation, Writing – original draft, Writing – review & editing, Project administration.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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