



Upcycling mango peels into a functional ingredient by combining fermentation and enzymatic-assisted extraction

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ABSTRACT

This study aims at upcycling mango peels by a sequential application of enzymatic hydrolysis, using Viscozyme and Pectinex at 50 °C for 2 h; and fermentation, using *L. plantarum* and *B. animalis* at 48 h for 37 °C. The use of Viscozyme led to a considerable increase in the concentration of galacturonic and glucuronic acids in the unfermented samples (308.96 and 12.97 mg/100 ml higher than control, respectively), whereas the use of Pectinex resulted in higher oligosaccharide solubilization (5.3 % more than control). None of the enzymes influenced microbiological growth. The recovery of gallic acid aglycone increased 17-fold over the control when Pectinex and *B. animalis* were used. Similarly, the recovery of mangiferin aglycone increased by 60 % after fermentation by either bacteria. The results indicate that this sequential processing strategy might be utilized to extract phenolic aglycones and produce functional ingredients from mango peels.

1. Introduction

Mango (*Mangifera indica* L.) is one of the major tropical fruits in terms of production and export (FAO, 2022). It is estimated that 1.73 million tons were produced by 2021. The processing of mangoes to end-products such as mango juice, mango flesh or other applications generates 30 to 50% of waste. This translates into high amounts of mango peels (7–24%) and seeds (9–40%), which represent a serious problem of disposal (Aggarwal et al., 2017). The number of research articles focusing on the upcycling of mango peels has increased from 2015, with higher interest in its applications within the food industry (Marçal & Pintado, 2021). The predominant approach employed for the upcycling of these peels involves their dehydration and subsequent processing into a powdered form, which serves as a valuable fiber-rich ingredient. This method is more appealing due to the extended shelf life it provides compared to using fresh peels. The incorporation of mango peel powder into various food formulations has exhibited improvements in terms of

nutritional composition, as well as improvements in functional and physical attributes of the resulting food product. Additionally, contemporary techniques encompass the extraction of bioactive compounds, such as phenolic compounds, carotenoids, or pectin, from mango peel powder (Marçal & Pintado, 2021).

Compositionally, up to 75% of mango peels dry matter (DM) is composed of carbohydrates, of which the major part is insoluble dietary fiber (up to 50% of DM in some cultivars), and reduced amounts of protein (1.5–6.6%) and fat (1.6–3.7%) (Marçal & Pintado, 2021). Pectic polysaccharides make up the majority of the dietary fibers found in mango peels. These fibers contain significant levels of bioactive substances, particularly phenolic compounds (PCs; 14.85–127.6 mg gallic acid equivalents (GAE)/g DM) (Marçal & Pintado, 2021). The most significant PCs in mango peels, other than gallic acid, is mangiferin, which is present in various amounts and forms (Quintana et al., 2021) and has recently been investigated for its potential health benefits (Mei et al., 2021). However, these PCs could be tightly bound or trapped

Abbreviations: ADF, antioxidant dietary fiber; VI, Viscozyme®; PE, Pectinex® XXL; PES, Pectinex® Smash XXL; CE, Celluclast®; LP01, *Lactiplantibacillus plantarum* LMG P-21021; B501, *Bifidobacterium animalis* spp lactis LMG P-21384; NET, non-enzymatically treated; MPS, mango peel solution; TPC, total phenolic compounds; GAE, gallic acid equivalents; TE, trolox equivalents; ESM, ethanolic soluble matter; EPM, ethanolic precipitable matter; GaA, galacturonic acid; GluA, glucuronic acid; MRS(A), deMan Rogosa and Sharpe (Agar).

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inside the fiber polysaccharides, reducing their bioavailability after ingestion. The bioavailability of PCs from mango peels must then be improved using a solubilization procedure of these hitherto insoluble fibers.

There are two possible main solubilization strategies to improve the bioaccessibility of PCs from agro-industrial by-products; (1) breaking down the fiber to which the PC is bound releasing PC-rich short-chain fiber components (i.e. oligosaccharides), and (2) cleaving the PC-fiber bond – and releasing the PC molecule (Vilas-Franquesa et al., 2023). Biotechnological methods, such as bacterial fermentation and enzymatic hydrolysis procedures, have recently been used for this purpose since they are more environmentally friendly than traditional solvent extractions. Enzymatic hydrolysis can be effective for producing PC-rich oligosaccharides, commonly known as antioxidant dietary fiber (ADF) (Saura-Calixto, 1998), while fermentation can release PCs thanks to the microorganisms' esterase activity also in synergy with plant enzyme activities (Filannino, Di Cagno, & Gobbetti, 2018). The ADF idea is currently gaining more interest because it has been recently demonstrated that the PC-oligosaccharide bond is advantageous for the PC bioaccessibility. This is because fiber can protect PCs from degradation or transformation until they reach the lower intestine, where they are released by the metabolic activity of the gut microbiota and can have positive effects. (Jakobek & Matic, 2019).

The most preferred bacterial strains for fermenting agricultural and industrial byproducts are lactic acid bacteria (LAB). Due primarily to the release of PCs, the application of LAB fermentation can successfully improve the antioxidant activity of the initial substrate (Saadoun et al., 2021). In addition, *Bifidobacterium* spp. can also be used for the same purpose, extending their application to the upcycling of mango peels. In fact, predigested mango peels have been found to enhance the number of *Bifidobacterium* and *Lactobacillus* genera in a dynamic *in vitro* model of human colon (Sáyago-Ayerdi et al., 2019). Nevertheless, the use of LAB and *Bifidobacterium* spp. for the fermentation of a fresh mango peel solution can be thwarted due to the scarce availability of important carbon sources (i.e. free sugars) which are used by these microorganisms, especially in the initial stages of their growth.

Besides being a good tool for the production of ADF, enzymatic hydrolysis can be employed as a pretreatment to increase the free sugars. This would favor a successive fermentation by LAB, as they would use the sugars as energy source, reducing their concentration in the final product, increasing its value. In fact, the combination of enzymatic hydrolysis with LAB fermentation has been successfully applied in cassava tuber for starch release (Adetunji et al., 2016), and extended to canola oil-pressed cake, observing an increased solubilization of proteins due to a dissociation of the PC-protein complex (Tian et al., 2023). The sequential application of enzymatic hydrolysis and fermentation could be a good strategy for the solubilization of bioactive compounds from mango peels.

This study aims to evaluate the potential sequential application of different enzymatic cocktails and probiotic strains (*Lactiplantibacillus plantarum* and *Bifidobacterium animalis*) to increase the solubilization of PCs in aqueous extracts from mango peels, and to investigate the potential production of ADF by ethanolic precipitation of the aqueous extracts. Based on the data provided, we hypothesize that the enzymatic pretreatment can be used as a tool to enhance the growth and fermentation of mango peels by *B. animalis* and *L. plantarum*, subsequently improving the recovery of PCs. Elucidating this is the first step towards understanding the applicability of a combinative treatment to upcycle mango peels into functional ingredients. To that aim, the total phenolic content (TPC) in aqueous extracts, ethanolic precipitable matter (EPM) and ethanolic soluble matter (ESM) fractions was determined by the Folin-Ciocalteu assay, and the anti-oxidant activity was determined by the DPPH and FRAP assays. The contents of gallic acid and mangiferin, the major PC in mango peels, were measured by LC-MS/MS. Important parameters of the fermentation process such as the microbiological growth and pH were also monitored.

2. Materials and methods

2.1. Bacterial preparation

The commercial probiotic strains *Lactiplantibacillus plantarum* LMG P-21021 (LP01) and *Bifidobacterium animalis* spp. lactis LMG P-21384 (B501) were provided by Probiotical S.p.A. (Novara, Italy). The strains were stored in a 20% glycerol MRS solution at -20°C until use. The glycerol solution containing viable strain was pipetted (300 μl) to 7 ml of MRS broth (Fisher Scientific NL, Landsmeer, Netherlands) in a 15 ml Greiner tube, vortexed, and left undisturbed for 16 h at 37°C . A pellet was formed after centrifuging the Greiner tube for 10 min at 3,000 rpm. The supernatant (MRS broth) was then discarded and the pellet was washed using 7 ml of saline solution 0.9%. Subsequently the pellet was resuspended with 7 ml of 0.9% saline solution to produce the strain stock solution. The cell density was checked at 600 nm with a Cary 60 UV-Vis spectrophotometer (Agilent Technologies, California, U.S.). The resulting value was checked against a calibration curve plotting the optical density (OD) at 600 nm against the CFUs/ml of the corresponding strain. The strain stock solution was used to achieve the desired concentration of cells in the mango peel solution (log 6 CFUs/ml). To assertively quantify the CFUs/ml, an aliquot of 1 ml of the stock solution was serially diluted and plated in MRS agar (MRSA, Fisher Scientific NL, Landsmeer, Netherlands) by inclusion. The subsequent CFUs/ml count was performed after incubating the MRSA plates for 48 h days at 37°C . All plates were produced in duplicate.

2.2. Experimental design

2.2.1. Mango peel source and composition

A total amount of 80 ready-to-eat, green peel, small-sized mangoes (≈ 550 g in weight) from the variety Kent harvested in Peru were purchased from a local supermarket (Jumbo, Wageningen, Netherlands) at two different days, two months apart (July and September of 2022). The mangoes were peeled with conventional peeler, and the peels were mixed and immediately frozen (-20°C) until the preparation of mango peel solution (MPS).

For the proximate composition analysis, mango peels were thawed, cut to 1–2 cm^2 pieces using scissors, and ground to fine powder using a miller 6875D Freezer/Mill® (Spex Sample Prep, United States). The settings for grinding were; precooling time 10 min, run time 4 min, cool time 2 min, 2 cycles, and rate 15 cycles per second. The processed powder was then freeze dried and used for compositional analysis, with the exception of moisture content, which was performed on fresh (not frozen) mango peel pieces. All analyses were performed in triplicate. Most of the analyses were performed according to AOAC methods of analysis (AOAC, 2005).

Moisture content was quantified by weight difference after oven-drying 2 g of fresh mango peel pieces into aluminum trays for 24 h at 105°C (AOAC 950.46). Total crude protein content was determined by the Dumas method (AOAC 968.06) by weighing 15 mg of freeze dried mango peel powder and applying a conversion factor of 6.25*N after combustion. Ash content was determined by weight difference after incinerating 1 g of freeze dried mango peel powder in a muffle furnace at 550°C for 3 h (AOAC 942.05). Soluble, insoluble and total dietary fiber were determined following the AOAC protocol 991.43 using the Total dietary Fiber Assay Kit purchased from Megazyme (Neogen Europe Ltd., Ayr, United Kingdom). However, 0.4 g of freeze dried mango peels were used instead of 1 g, to facilitate the filtering step and avoid clogging. Total fat was determined by exhaustion using an automated Soxtherm SOX 406 apparatus (Gerhardt GmbH & Co., Königswinter, Germany). Precisely 1 g of freeze dried mango peel powder was weighted and placed in a cellulose-based thimble (Whatman™ 603), and the top was covered with grease-free cotton wool. The thimble was then placed in a thimble holder and immediately after into the extraction beaker (200 ml total volume). A total of 150 ml of *n*-hexane were added to the extraction

beaker, which was then placed in an automated. The extraction process was run for 1 h and the extraction temperature was set at 170 °C. After extraction, samples were left under a fume hood overnight for the evaporation of the remaining solvent. Fat content was calculated by weight difference. At last, carbohydrate content was calculated by weight difference of the dry matter fraction (FAO, 2003).

2.2.2. Mango peel solution preparation and preliminary experiment

The preparation of the fermented samples is presented in Fig. 1. First, mango peels were chemically treated to reduce the microbial load

(Jarvis et al., 2001). In brief, mango peels (100 g) were immersed in 2% sodium carbonate (1 L) for 1 min. Next, they were rinsed with and immersed into 70% ethanol (1 L) for 1 min more. Lastly, rinsed mango peels were washed with demineralized water (1 L) for 2 min. The washed mango peels were then mixed with demi water (1:1, w/w) and blended using a conventional Thermomix® (Vorwerk, Wuppertal, Germany) until an homogeneous puree was produced (MPS). The MPS was stored at −20 °C until its use for enzymatic treatment or fermentation.

The choice of the enzymatic pretreatment was based on the solubilization of material by different enzymatic cocktails. All enzymatic

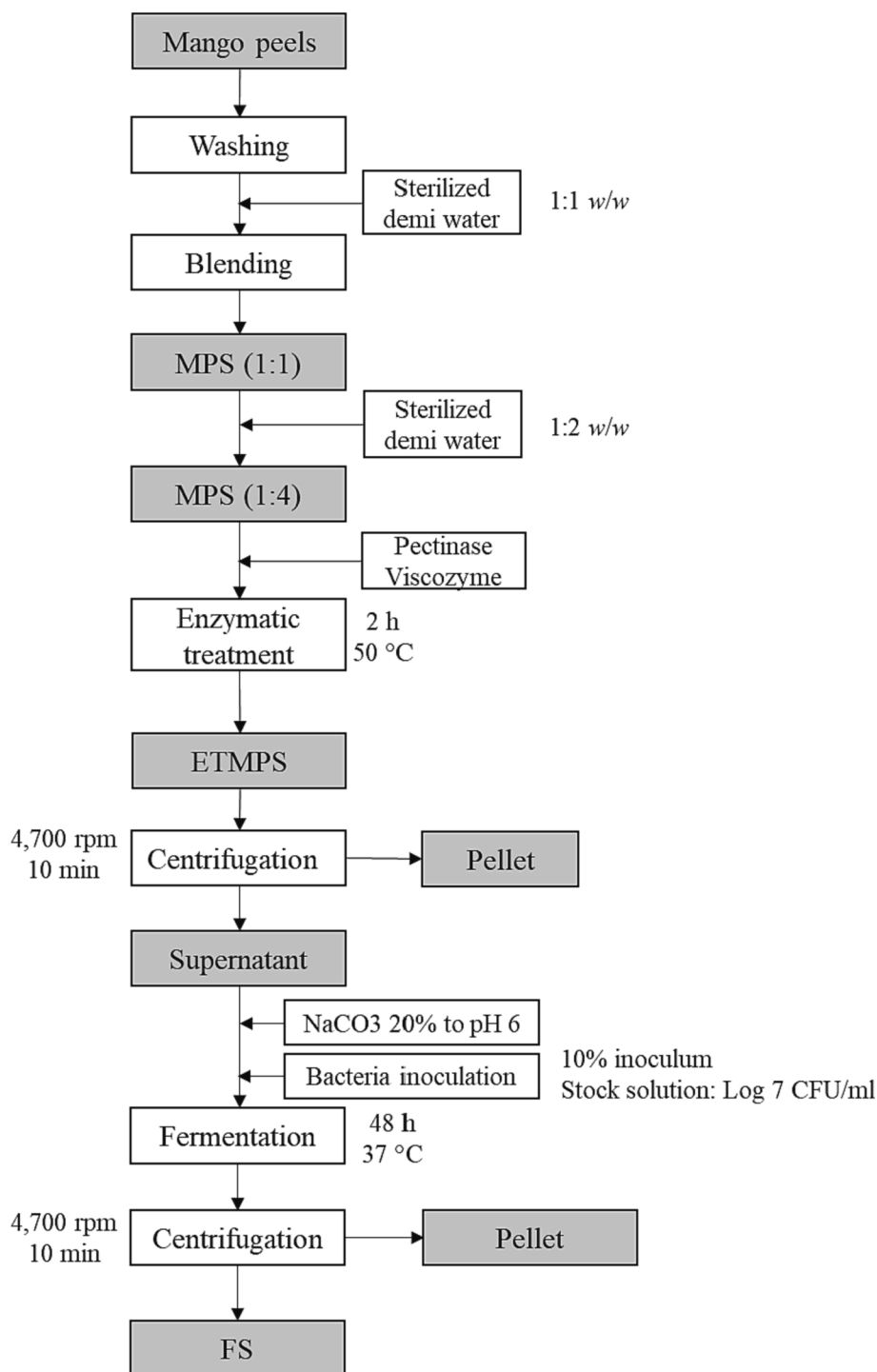


Fig. 1. Flow chart of the upcycling process of mango peels. MPS: mango peel solution; ETMPS: enzymatically treated mango peel solution; FS: fermented supernatant. Black boxes – products, white boxes – processes.

mixtures used herein were kindly provided by Novozymes (Bagsværd, Denmark). The MPS (1:1, w/w) was used for the solubilization test. Viscozyme®L (VI; 100 fungal β -glucanase units/g, batch number: KTN02327), Pectinex®XXL (PE; 10,000 pectinase units/g, batch number: KON10082), Celluclast® (CE; 700 endoglucanase units/g, batch number: CCN03212), Pectinex® Smash XXL (PES; 19,400 pectinase units/g, batch number: KON00286) and a mixture of VI:PE:CE were used for this preliminary experiment. The concentration of each enzymatic cocktail in the final solution was the highest according to the manufacturer: 250, 60, 200 and 120 μ l/kg of MPS for VI, PE, CE and PES, respectively. The mixture of VI:PE:CE was also used at the highest concentration of those enzymatic cocktails forming the mixture, therefore leading to a ratio of 49:12:39 of VI, PE and CE respectively. The preliminary enzymatic treatment was run for 2 and 6 h at constant temperature of 50 °C in a shaking water bath (100 rpm). The outcome of this test on MPS determined which enzymes to use as a pretreatment for the fermentation.

2.2.3. Main experiment

The MPS was mixed with water to achieve a final ratio of 1:4 (w/w, diluted MPS), as this dilution was sufficient to allow the growth of microorganisms used (results not shown). The enzymatic treatment was performed by adding VI and PE at the highest concentrations recommended by the manufacturer. The enzymatic digestion was run for 2 h at 50 °C in a shaking water bath (100 rpm). A condition without enzymatic treatment (NET) was included as a comparison alongside the VI and PE samples. Next, the supernatant was recovered by centrifugation (4,700 rpm for 10 min at 25 °C), obtaining the raw material for the fermentation (Fig. 1). The pH of the supernatant was changed to 6 using 20% aqueous NaCO₃ solution. VI, PE, and NET supernatants were incubated with or without inoculum (LP01 and B501) to a final cell density of log 6 CFUs/ml, resulting in a total of 9 conditions. The samples were incubated at 37 °C for 48 h. The fermented and non-fermented supernatants (FS in both cases for the sake of clarity) were immediately used for testing the microbiological growth and pH. Finally, the FS was centrifuged (4,700 rpm, 10 min, 25 °C) and stored at –20 °C for DM, EPM and ESM analyses and at –80 °C for all other analyses. All experiments were performed in triplicate, and all analyses were run in triplicate except when indicated otherwise.

2.3. Microbiological growth and pH

Microbial growth was quantified visually and expressed as CFUs/ml after plating 1 ml of a serially diluted FS (using 9 ml PBS tubes (Tritium Microbiologie, Eindhoven, Netherlands)) in MRSA by inclusion and incubating the plates at 37 °C for 48 h. The pH of the FS was checked with a table pH meter pHenomenal® pH 1100L (VWR International, Boxmeer, Netherlands).

2.4. Dry matter, EPM and ESM

Dry matter was determined on 2 g of FS following the protocol AOAC 950.46 (AOAC, 2005). EPM was quantified by weight difference after enzymatic digestion of the sample. In this case, 10 g of FS were precisely weighted in 50 ml centrifuge tubes. Subsequently, 12.5 μ l of α -amylase was added to the tube and incubated at 100 °C for 30 min and stirred at low speed. The sample was then cooled to 60 °C and vigorously agitated. 25 μ l of protease was then added and the sample was incubated for 30 min with continuous agitation. After protease incubation, the pH of the sample was checked and corrected to pH 4.1 to 4.8 when needed by using 5% NaOH solution. A total of 50 μ l of amyloglucosidase was added to the sample and incubated for 30 min at 60 °C under continuous agitation. The resulting product was then precipitated by adding pre-heated ethanol (95 %, 60 °C) to the sample at ratio 4:1 (v/v). The sample was then left undisturbed for 60 min at room temperature, and subsequently centrifuged at 3,000 rpm for 10 min to separate the EPM from

the ESM. The resulting pellet was freeze-dried and weighted again (for EPM quantification) and subsequently stored at –20 °C until resuspension with water, whereas the ESM fraction was directly stored at –20 °C until antioxidant activity and total phenolic analyses. The freeze-dried pellet (EPM) was resuspended the day of the analysis by adding small volumes of milli-Q water ($\approx$ 100 μ l) and carefully pressing the pellet pieces against the tube, to the final volume of 10 ml. All enzymes used in the present test were purchased from Neogen Europe Ltd. (Michigan, U.S.) under the product name Total Dietary Fiber Assay Kit (AACC Method 32–05.01). In order to standardize the results of antioxidant activity and phenolic content assays for the ESM fraction, it was necessary to determine the weight of this fraction. To achieve this, the weight of the ESM fraction was calculated by subtracting the value of the EPM fraction from the DM of the FS.

2.5. Antioxidant activity (DPPH, FRAP)

The DPPH and FRAP methodologies were adapted from Suárez-Jacobo et al. (2011). In both cases, the FS was first centrifuged at 4,700 rpm for 10 min at room temperature (25 °C) and diluted in milli-Q water at ratio 1:10 (v/v). Conversely, the ESM and the resuspended EPM were used without prior dilutions. For the DPPH analysis, the sample was added at 2.5% in individual wells of a total capacity of 300 μ l, subsequently filling the well to maximum volume with a 0.1 mM methanolic solution of 2,2-Diphenyl-1-picrylhydrazyl (DPPH·) (Merck Life Science NV, Amsterdam, Netherlands). The plate was gently shaken and kept at complete darkness for one hour. Absorbance was then read at 515 nm using a Cary 60 UV–Vis spectrophotometer (Agilent Technologies, California, U.S.). The absorbance results were checked against a five-point calibration curve (0.2 to 1.5 mM) produced from a 2 mM methanolic stock solution of ($\pm$)-6-Hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox, 97%, Merck KGaA, Darmstadt, Germany). The final results were expressed as mM of Trolox Equivalents (TE) on FS samples and as mg TE/100 mg of DM for ESM and EPM samples.

For the FRAP analysis, different solutions were prepared. A solution of 10 mM of 2,4,6-tripyridyl-s-triazine (TPTZ, $\geq$ 98% purity, Merck KGaA, Darmstadt, Germany) was prepared using an aqueous mixture comprising 80% HCl 0.05 M and 20% milli-Q water. Next, a solution of 20 mM FeCl₃ (97 % purity, Merck KGaA, Darmstadt, Germany) was prepared using milli-Q water. Acetate buffer (pH = 3.6) was prepared by mixing the appropriate amounts of acetic acid glacial and sodium acetate (Merck Life Science NV, Amsterdam, Netherlands). The buffer solution can be stored for up to 6 months. Every solution used for FRAP analysis was freshly prepared each day of analysis, except for the buffer. The FRAP solution was prepared by mixing 2.5 ml of the TPTZ solution, 2.5 ml of the FeCl₃ solution and 25 ml of acetate buffer, and the resulting mix was warmed up to 37 °C prior to its use. The sample was pipetted to a 300 μ l well (2.94% of the total volume), where was mixed with 8.82% of milli-Q water and 88.24% of warmed FRAP solution, as indicated by previous authors (Stracke et al., 2009). The 96 well-plate was stored at room temperature under dark conditions for 30 min. The absorbance was then read at 593 nm and the resulting value was compared against a five-point calibration curve (35 to 280 μ M) obtained from a 350 μ M methanolic stock solution of Trolox. The antioxidant activity was measured by both assays on the FS, EPM and ESM fractions. The final results were expressed as mM of Trolox Equivalents (TE) on FS samples and as mg TE/100 mg of DM for ESM and EPM samples.

2.6. Total phenolics

The total phenolic method was adapted from the validated technique proposed by Singleton et al. (1999). The FS was centrifuged (4,700 rpm, 10 min, 25 °C) and then diluted in milli-Q water at ratio 1:10 (v/v), whereas the ESM and the resuspended EPM were used without prior dilutions. Using 96-well plates with an individual well capacity of 300 μ l, 188 μ l of milli-Q water were added in the well. Subsequently, 20 μ l of

sample were added in the well and mixed gently. Finally, 11 μ l of F-C reagent (2 N, Merck Life Science NV, Amsterdam, Netherlands) was added followed by 31 μ l of 20% NaCO₃ ($\geq 99.5\%$ purity, Merck Life Science NV, Amsterdam, Netherlands). The plate was then gently mixed and kept in complete darkness for 60 min at room temperature. The absorbance was read at 760 nm using a Cary 60 UV-Vis spectrophotometer (Agilent Technologies, Amstelveen, Netherlands), and the resulting value was compared against a five-point calibration curve (25 to 250 ppm) obtained from a 500 ppm aqueous stock solution of gallic acid (97.5–102.5% purity, Merck Life Science NV, Amsterdam, Netherlands). The final results were expressed as mg GAE/ 100 mg DM.

2.7. LC-MS/MS quantification of gallic acid and mangiferin

FS diluted with milli-Q water (1/10, v/v) was used for LC-MS quantification of gallic acid and mangiferin. A Nexera UPLC system coupled with an LCMS-8050 triple quadrupole mass spectrometer (Shimadzu Corporation, Kyoto, Japan) was used for this purpose. The UPLC unit consisted of a SIL-30AC autosampler, an LC-20ADXR solvent delivery module, a DGU-20ASR degassing unit, a CTO-20AC column oven and an FCV-20AH2 valve unit.

The UPLC separation of both gallic acid and mangiferin was performed injecting 5 μ l of sample to an Acquity Premier BEH C18 Column, 1.7 μ m, 2.1 $\times$ 100 mm BEH, connected to an Acquity UPLC BEH C18 VanGuard Pre-column, 130 $\text{\AA}$, 1.7 μ m, 2.1 mm $\times$ 5 mm (Waters Chromatography B.V., 4879 AH Etten-Leur, the Netherlands). The flow rate was set at 0.3 ml/min and the column temperature at 40 °C. The mobile phases consisted of 0.1 % formic acid (solvent A), acetonitrile with 0.1 % formic acid (solvent B) with the following elution profile (t in [min]/[% B]): (0.0/5), (2.0/35), (7.5/95), (9.5/95) and (9.6/5). Optimization of the multiple reaction monitoring parameters was performed by flow injection analysis of gallic acid and mangiferin ($\geq 98\%$ purity, Merck Life Science NV, Amsterdam, Netherlands) standard compounds (20 ppm in H₂O/ACN, 1:1 v/v) of the multiple reaction monitoring transitions (Supplementary Table 2) using the support software (LabSolutions, Shimadzu Corporation, Kyoto, Japan). The system ran for 13.5 min per each sample and MS data was collected from 1 to 1.9 min for gallic acid and 4 to 6.2 min for mangiferin. Negative ionization mode was used for both MS analyses. The voltage of the turbo ion-spray ionization was 4.0 kV. The electrospray ionization probe was set at 300 °C, the desolvation line at 250 °C and the heat block at 400 °C. The pressure of the collision-induced dissociation gas was 4 kPa and the flow rates of the drying gas, nebulizer gas and heating gas were set at 10 ml/min, 3 ml/min and 10 ml/min, respectively.

Data was processed with LabSolutions Insight software (Shimadzu Corporation, Kyoto, Japan). A 5-point calibration curve was constructed from 0.05 to 5 ppm for gallic acid ($R^2 = 0.998$) and from 0.0033 to 0.1 ppm for mangiferin ($R^2 = 0.997$). The matrix effect was $98.60 \pm 2.35\%$ and was calculated using the Equation (1). LOD and LOQ were calculated using the standard deviation of the intercept from the calibration curve. LOD and LOQ values were 0.027 and 0.081 ppm for gallic acid and 0.018 and 0.054 ppm for mangiferin.

Matrix effect(%)

$$= \left(\frac{\text{Response of the analyte spiked into the matrix}}{\text{Response of the analyte in the aqueous solution}} \right) * 100 \quad (1)$$

2.8. Quantification of sugars, galacturonic acid (GalA) and glucuronic acid (GluA)

The FS was diluted with milli-Q water (1:100, v/v) prior to the analysis. The amount of monomeric sugars, glucuronic and galacturonic acid was measured according to Gilbert-López et al. (2015) with minor modifications. A High-Performance Anion Exchange Chromatography (HPAEC) system equipped with a CarboPac PA-1 column (2 $\times$ 250 mm) in combination with a CarboPac PA guard column (2 $\times$ 25 mm) and a

pulsed electrochemical detector in pulsed amperometric detection mode (Dionex, Sunnyvale, USA) was used. The elution pattern was adapted for oligosaccharide detection. The flow rate was set at 0.25 ml/min and NaOH 21 mM was used for column equilibration. Elution was performed as follows: 0–20 min, 21 mM NaOH; 20–30 min, 21–100 mM NaOH; 30–90 min, 0–300 mM sodium acetate in 100 mM NaOH; 90–91 min, 300–500 mM sodium acetate in 100 mM NaOH; 91–96 min, 500 mM sodium acetate in 100 mM NaOH; 96–97 min, 500 mM sodium acetate in 100–21 mM NaOH. Glucuronic and galacturonic acids ($\geq 97\%$ and $\geq 98\%$ purity respectively) and all monomeric sugars (purity of $\geq 99\%$) were purchased from Merck Life Science NV (Amsterdam, Netherlands) and were used as standards for quantification. This analysis was performed in duplicate.

2.8.1. Percentage of oligosaccharides

The percentage of oligosaccharides was calculated based on the relative peak area that eluted after all mono and disaccharide compounds. The peaks of GalA and GluA were also subtracted from the overall oligosaccharide percentage (Equation (2)).

$$\% \text{area oligosaccharides} = \frac{\Delta A_{\text{oligo}} - (A_{\text{GalA}} + A_{\text{GluA}})}{\Delta A A} * 100 \quad (2)$$

Where ΔA_{oligo} is the sum of the areas of the peaks eluted from the 30th to the 100th minute of the chromatogram (see Supplementary Fig. 2), A_{GalA} is the area of GalA, A_{GluA} is the area of GluA, and $\Delta A A$ is the sum of all areas of the chromatogram (including sugars).

2.9. Statistical analysis

Data were statistically analyzed with the software R-4.0. Assumptions were checked by first visually interpreting the Q-Q and boxplots from all analyses. Normality was double-checked by the Shapiro-Wilk test. One-way ANOVAs were performed per each parameter analyzed, and the Tukey's post hoc test was used to find significant differences between samples. In all analyses, the significance threshold was set at $p < .05$.

3. Results and discussion

3.1. Microbiological growth and pH

The fermentation of enzymatically treated MPS resulted in bacterial growth of about one log when using both LP01 and B501 strains (Table 1). The strain LP01 grew better in the VI-treated matrix rather than the PE-treated matrix. The uninoculated samples showed high presence of LAB as well, which could be derived from the high presence of *Lactobacillus* sp. in fresh mango peels (Taïbi et al., 2022). The autochthonous flora did also have an effect on pH, which dropped from 6 to approximately 4.9 in all uninoculated samples. The initial LAB cell concentration in diluted MPS was approximately 2 log CFU/ml (results not shown), providing support to these findings. Despite the fact that the differences were not statistically significant, LP01 had a higher incidence in dropping the pH when compared to B501, probably deriving from their different growing rate and metabolic activity (Supplementary Fig. 1). These findings indicate that the use of an enzymatic pretreatment was not able to improve bacterial growth.

The bacterial metabolic activity can be very different depending on the available substrate, subsequently having different effects on the fermented matrix ('fermentation effect'). The composition of the raw material used for the present study can be found in Supplementary Table 1. The 'fermentation effect' has to be proven by the growth of the inoculated microorganism, otherwise the results may be derived from other biochemical or biological processes. Recent data suggests that LAB can grow in a diluted aqueous solution of freeze-dried mango peels (Lee et al. 2021), yet these results cannot be inferred to fresh ingredients. To the best of our knowledge, this is the first work that shows the fermentability of

Table 1

Microbiological growth, pH values, dry matter (DM), ethanolic precipitable matter (EPM) and the respective percentage of different fermented mango peel solutions – with and without enzymatic pretreatment.

Enzyme	Strain	Log ₁₀ CFU/ml	pH	g DM/100 ml	mg EPM/100 ml	Ratio EPM/DM (%)
VI	LP01	7.72 ± 0.05	4.61 ^{ab} ± 0.10	2.03 ± 0.09	355.00 ^{ab} ± 7.07	17.50
	B501	7.68 ± 0.06	4.57 ^{ab} ± 0.04	2.02 ± 0.13	250.00 ^{cd} ± 0.01	12.38
	Uninoculated	7.70 ± 0.92	4.91 ^a ± 0.20	1.99 ± 0.17	170.00 ^d ± 56.57	8.52
PE	LP01	7.70 ± 0.03	4.14 ^b ± 0.02	1.96 ± 0.11	283.33 ^{bc} ± 55.08	14.45
	B501	7.76 ± 0.01	4.34 ^b ± 0.06	2.02 ± 0.17	455.00 ^{ab} ± 21.21	22.53
	Uninoculated	7.67 ± 0.96	4.95 ^a ± 0.08	2.02 ± 0.20	443.33 ^{ab} ± 35.12	21.97
NET	LP01	8.03 ± 0.19	4.19 ^b ± 0.07	1.69 ± 0.16	485.00 ^a ± 21.21	28.75
	B501	7.54 ± 0.15	4.49 ^{ab} ± 0.44	1.92 ± 0.18	490.00 ^a ± 70.71	25.57
	Uninoculated	7.12 ± 0.04	4.95 ^a ± 0.06	1.61 ± 0.02	446.67 ^{ab} ± 92.92	27.74

Initial cell density was measured to be 6.22 and 6.58 log₁₀ CFU/ml for LP01 and B501 respectively. Values represent the mean ± SD. Different superscripts show significant differences at $p < .05$. VI: Viscozyme, PE: Pectinex, NET: Non-enzymatically treated, LP01: *Lactiplantibacillus plantarum* 01, B501: *Bifidobacterium animalis* 01: CFU: Colony Forming Units.

fresh mango peel and the success of the process, as other authors have attempted the fermentation of the same product (i.e. fresh mango peels by-products) without reporting the microorganism growth after the fermentation (Munishamanna et al., 2017; Ojokoh, 2007).

3.2. Dry matter and ethanol precipitable matter (EPM)

Compared to the other enzyme mixtures that were evaluated, both VI and PE demonstrated a noteworthy ability to dissolve cell wall material from a 1:1 (w/w) mixture of MPS, as shown in Fig. 2. Compared to the control, the solubilization of material doubled after both enzymatic treatments. The combination of enzymes (VI:CE:PE) did not result in enhancements in the breakdown of mango peel materials and it was therefore excluded as a pretreatment for fermentation. There were no discernible changes between the 2-hour and 4-hour incubation periods, implying that the 2-hour treatment was sufficient to achieve optimal solubilization outcomes (Fig. 2). Accordingly, VI and PE were utilized as pretreatments for the fermentation of MPS 1:4 (w/w).

Data in Table 1 indicate the enzymes used were not able to significantly increase the solubilization of material from a MPS 1:4 (w/w) when compared to the control. In the present research, the solubilization

of material is exclusively due to the enzymatic process, as the fermentation was applied to the enzymatically treated supernatant after centrifugation (Fig. 1). These findings differ than those from other studies that report a two-fold increase in the extraction yield of mango peels when using pectinase (Sharif et al., 2021). The variations in solubility might arise from differences in the operational conditions during the process, such as the concentration or activity of the enzyme used, the use of buffered solutions or the combinative effect with ultrasounds, and the water ratio – as demonstrated in the preliminary enzymatic solubilization test.

The use of enzymes slightly increased the dry matter content and decreased the EPM concentration in the samples, especially when using VI (Table 1). It should be noted that, although EPM fraction cannot be fully considered as soluble fiber (Mafias & Saura-Calixto, 1993), it could be used as an indicator. These results illustrate that the higher solubilization of matter that happens when using enzymatic treatments is primarily due to the solubilization of sugars or other non-ethanolic precipitable compounds rather than soluble fiber – or higher molecular weight compounds. This effect has also been recently reported in kiwifruit extracts when comparing enzymatic against acid or alkali extraction of fiber (Wang et al., 2021).

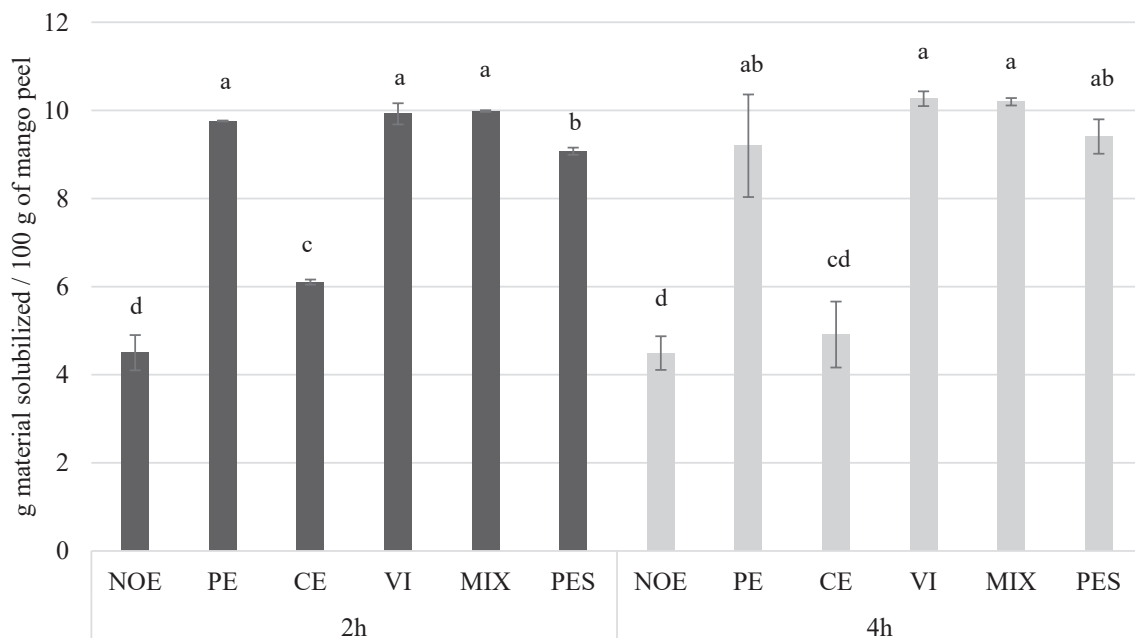


Fig. 2. Solubilization of dry matter after enzymatic treatment using different enzymes at different incubation times. The samples were MPS at 1:1 dilution factor and incubation temperature was 50 °C. Error bars represent SD from the mean value. Different letters show statistical significance at $p < .05$. NOE: non-enzymatically treated, PE: Pectinex, CE: Celluclast, VI: Viscozyme, MIX: mixture of PE-CE-VI, PES: Pectinex Smash.

The fermentation did not influence the EPM concentration in NET samples. The use of *L. plantarum* has a positive effect on the EPM concentration in samples pretreated with VI, and *B. animalis* has a positive effect in samples pretreated with PE. These were also established as the best growing conditions for each bacteria in the preliminary experiments (Supplementary Fig. 1). This demonstrates that the better the growth, the lower the concentration of EPM. The matrix-dependent growth of potentially probiotic LAB was previously reported in beetroot and carrot juice (Malik et al., 2019), and possibly derived from (1) different metabolic activity, (2) the variation in the sugar profile after applying different enzymatic treatments, and (3) the bacteria's affinity to these carbon sources. The bacterial growth and metabolic activity are dependent on the concentration of EPM, which is directly linked to the enzyme used.

Lastly, the EPM of the uninoculated VI sample was significantly lower compared to their uninoculated counterparts (PE, NET), lowering the percentage of EPM more than 12% (Table 1). This could be explained by (1) the higher enzymatic activity of the VI cocktail, which shortened the chain of the potential oligosaccharides and therefore reducing the EPM fraction, or (2) by the presence of autochthonous flora. Mango peel has an autochthonous microbiological profile, with *Leuconostoc mesenteroides* being the dominant bacteria (~80%), and *Pediococcus pentosaceus* and *Lactobacillus brevis* being also identified (Liao et al., 2016). The washing process used on mango peels was insufficient to completely remove the naturally occurring LAB, resulting in a cell density of 2 log CFU/ml in the solution. Furthermore, the commercial enzymatic solution introduced the possibility of additional bacterial sources, partly adding to the bacterial count, as indicated in the manufacturer's datasheet. The subsequent processing created conducive conditions for the growth of LAB, which explains the high concentration of CFUs and the decrease in EPM in the samples treated with VI. In these VI-treated samples, the autochthonous LAB may have thrived due to their preference for the provided environment.

3.3. Antioxidant activity

Antioxidant activity measured by DPPH showed increased values of 15% and 12% after LP01 and B501 fermentations in NET conditions, respectively, results that were in line with the FRAP readings. In the samples pretreated with PE, the fermentation by LP01 increased the antioxidant activity up to 57% and 36% when using DPPH and FRAP, respectively, and the fermentation by B501 increased the antioxidant activity up to 31 and 48%. This is in line with what other authors showed, as lactic acid fermentation has been extensively used as a tool to increase the antioxidant activity of aqueous vegetable matrices (Fessard et al., 2017).

The enzymatic treatment with VI resulted to a significant increase on the antioxidant activity compared to the NET samples (Table 2). Mango peels contain pectin, which makes up to 20% of their dry weight and is the most valuable soluble fiber (Marçal & Pintado, 2021). Additionally, mango peels are a good source of phenolic compounds, compounds that

can boost antioxidant activity, making mango peel a potentially beneficial source of antioxidants and ADF. According to Ajila et al. (2007), the main enzymes found in mango peels are phenolic oxidases, peroxidase and protease, but amylase and xylanase are also present in low quantities. Although these enzymes could contribute to browning, fruit maturation and production of volatile compounds, they have limited effect on the degradation of the dietary fiber present in mango peels – and subsequently on the release of antioxidant compounds. VI is an enzymatic cocktail composed of β -glucanases, pectinases, hemicellulases and xylanases, that could efficiently cleave the long pectin and hemicellulose chains, solubilizing parts of them together with the phenolic compounds that might be attached. This could increase the antioxidant activity of the FS, as observed in the present experiment (Table 2).

3.4. Phenolic profile

The highest TPC values were found in LP01 and B501 VI samples, and the uninoculated NET sample (Table 2). The use of fermentation increased the antioxidant activity, possibly due to the metabolic activity of the strains, which could result in depolymerization of phenolic polymers or glycosylated phenolic compounds into their basic building blocks or aglycones (Saadoun et al., 2021). As a consequence, more phenolics can react with the coloring reagent, translating into higher quantification (Table 2).

When analyzing individual phenolics, the concentration of both gallic acid and mangiferin increased in the fermented FS when compared to the non-fermented counterpart (Table 2). The increase in gallic acid through fermentation could possibly come from the ability of *L. plantarum* and *B. animalis* to produce extracellular esterases (Landete et al., 2021). These esterases would specifically cleave the ester bond of galloylated phenolics containing gallic acid (e.g. penta-*O*-galloyl-glucoside) and mangiferin (e.g. mangiferin-6-*O*-gallate) that are present in mango peels (Quintana et al., 2021), or would cleave the ester bond of the gallic acid or mangiferin with hemicellulose or pectic oligosaccharides. The increase rate is much lower for mangiferin (up to 93% on VI and 140% on PE samples) compared to gallic acid (up to 151% on VI and 1,614% on PE samples), likely because the PCs containing gallic acid are far more abundant in mango peels (Quintana et al., 2021). Nonetheless, it should be noted that as a xanthone, mangiferin could also be linked to or 'entrapped' within polysaccharide chains by means of electrostatic or van der Waals interactions (Palafox-Carlos et al., 2011), making it less likely to be cleaved by phenolic esterase – because there would be no covalent ester bond to break – and more likely to be released by hemicellulose-degrading enzymes or other external factors. The fairly great amounts of TPC observed in the uninoculated NET sample (Table 2) could come from the phenolic compounds that were still covalently bond to other molecules, making it possible to react with the FC reagent but not to be quantified as individual phenolics. In addition, there are other – phenolic – compounds that might be present in the mango peel solution that are not targeted in the present experiment and

Table 2

Antioxidant activity, total phenolic concentration and specific phenolic profile of mango peel fermented supernatant - with and without enzymatic treatment.

Enzyme	Strain	FRAP (mM TE)	DPPH (mM TE)	TPC (mg GAE/100 mg DM)	Gallic acid (ppm)	Mangiferin (ppm)
VI	LP01	1.088 ^{ab} ± 0.112	2.706 ^{ab} ± 0.726	3.276 ^a ± 0.316	35.816 ^a ± 1.987	0.526 ^a ± 0.034
	B501	1.220 ^a ± 0.156	3.270 ^a ± 0.209	3.180 ^a ± 0.407	37.172 ^a ± 1.369	0.444 ^{ab} ± 0.021
	Uninoculated	1.074 ^{ab} ± 0.180	3.222 ^a ± 0.791	2.629 ^{ab} ± 0.134	14.792 ^b ± 4.608	0.272 ^c ± 0.002
PE	LP01	0.798 ^{bc} ± 0.140	2.166 ^{ab} ± 0.455	2.649 ^{ab} ± 0.421	15.456 ^b ± 4.241	0.369 ^{bc} ± 0.027
	B501	0.764 ^{bc} ± 0.103	2.046 ^{ab} ± 0.238	2.556 ^{ab} ± 0.229	15.544 ^b ± 3.593	0.377 ^{bc} ± 0.055
	Uninoculated	0.585 ^c ± 0.095	1.383 ^b ± 0.295	2.106 ^b ± 0.190	0.907 ^c ± 0.588	0.157 ^d ± 0.004
NET	LP01	0.635 ^{bc} ± 0.054	1.666 ^{ab} ± 0.336	2.895 ^{ab} ± 0.064	3.758 ^c ± 1.641	0.305 ^c ± 0.058
	B501	0.689 ^{bc} ± 0.151	1.616 ^b ± 0.377	2.660 ^{ab} ± 0.538	0.504 ^c ± 0.370	0.310 ^c ± 0.039
	Uninoculated	0.454 ^c ± 0.075	1.444 ^b ± 0.440	3.209 ^a ± 0.330	ND	ND

Different superscripts mean statistical differences between conditions at $p < 0.05$. VI: Viscozyme pretreatment, PE: Pectinex pretreatment, NET: Non-enzymatically treated, LP01: *Lactiplantibacillus plantarum* 01, B501: *Bifidobacterium animalis* 01, TE: Trolox Equivalents, GAE: Gallic Acid Equivalents.

could have an impact on the TPC readings (Quintana et al., 2021). The greater production of phenolic aglycones observed in the present experiment after fermentation was not observed in a similar study focused on canola seed pressed cake (Tian et al., 2023). A matrix effect on the enzymatic and fermentation processes may account for this variation.

3.5. Free sugars, GalA and GluA

Data in Table 3 shows that the major effect on free sugar concentration resides on the fermented samples rather than the combination of treatments or enzymatic treatment. In fact, the amount of free sugars was not significantly different in PE nor VI samples when compared to NET samples. Similarly, the use of VI in citrus peels did not have an effect on simple sugar concentrations, in some cases observing the same decreasing trend when compared to the acid-hydrolyzed samples (Sabater et al., 2019). Interestingly, higher glucose and sucrose concentration were found in NET samples compared to VI or PE samples (Table 3). This could be attributed to (1) the enzymes naturally present in mango peels (Ajila et al., 2007) and (2) the metabolic activity of the microorganisms used. In fact, VI and PE pretreatments could initially lead to higher sugar concentration, providing the microorganisms with a sugar source that they could later use for their growth (Supplementary Fig. 1), therefore triggering this substantial drop in glucose and sucrose concentration. Monitoring the free sugar concentration is a technique that helps at understanding the enzymatic and fermentation efficiency. In that way, the inoculation of LP01 and B501 in MPS proved to be a good strategy for the significant reduction of glucose in all samples compared to the uninoculated samples. This should be regarded as an important processing attribute for the end-product.

The GluA and GalA concentrations were significantly higher in the VI samples compared to PE or NET samples (Table 3). This phenomenon could be ascribed to the varied enzymatic composition of VI, which results in an increased number of potential cleavage sites and enhanced collaboration among the constituent enzymes within this enzyme cocktail. Consequently, this interplay leads to the improved dissolution of a greater quantity of monomeric compounds.

The higher concentration of galactose, rhamnose and arabinose found in the VI samples could also support this hypothesis. The homogalacturonan polymeric fraction of pectin is primarily composed by GalA, whereas the rhamnogalacturonan I (RGI) polymeric fraction contains higher levels of rhamnose. In addition, RGI could also present attached galactose side-chains bound to arabinose monomers (Kim et al., 2022). The complex composition of this polysaccharide explains the direct increase of these monomers when using VI. The same trend was also observed with PE, although the differences in GluA and GalA were less noticeable. This effect might have originated from either the different composition of the cocktail (consisting solely of pectinases, hemicellulases, and arabinases) or the reduced concentration of the enzyme cocktail employed in the MPS matrix, even though the concentration used adhered to the manufacturer's recommended usage instructions.

3.6. Oligosaccharide estimation

The HPLC analysis of free sugars allowed to perform an estimation of the oligosaccharide percentage in the matrix. Data showed a fermentation and enzymatic treatment effect on the percentage of oligosaccharides (Supplementary Fig. 2). The highest percentage of oligosaccharides was observed on PE and fermented samples, followed by the same enzymatic treatment but uninoculated samples. Therefore, the enzymatic treatment demonstrated a more pronounced effect compared to the impact attributed to the utilized bacteria. It should be noted that the VI yielded lower percentage of total oligosaccharides when compared to all NET samples (Supplementary Fig. 2), suggesting that VI was more efficient in producing lower molecular weight

Table 3
Concentration of sugars, galacturonic and glucuronic acid, and the oligosaccharide percentage of different fermented mango peel solutions – with and without enzymatic pretreatment.

Enzyme	Strain	Fucose	Rhamnose	Arabinose	Galactose	Glucose	Mannose	Xylose	Fructose	Sucrose	Δ free sugars	Galacturonic acid	Glucuronic acid
VI	LP01	1.078 ^A ± 0.049	1.135 ^A ± 0.030	47.671 ^A ± 0.239	13.490 ^A ± 0.082	85.384 ^C ± 15.443	1.406 ± 0.219	2.106 ^A ± 0.009	451.613 ± 12.623	148.942 ^{BC} ± 0.566	752.824 ^{AB} ± 27.350	306.368 ^A ± 5.838	13.175 ^A ± 0.012
	B501	0.938 ^A ± 0.200	0.978 ^A ± 0.245	41.019 ^A ± 9.343	11.505 ^A ± 2.659	83.689 ^C ± 9.684	ND	0.963 ^B ± 0.193	400.376 ± 75.074	129.662 ^C ± 22.592	669.130 ^B ± 119.991	275.188 ^A ± 2.813	11.459 ^A ± 2.813
	Uninoculated	1.078 ^A ± 0.013	1.192 ^A ± 0.069	45.967 ^A ± 4.927	13.206 ^A ± 0.895	146.870 ^{AB} ± 5.845	ND	1.032 ^B ± 0.002	468.781 ± 8.504	156.377 ^{BC} ± 0.458	834.503 ^{AB} ± 3.678	311.486 ^A ± 3.683	13.218 ^A ± 2.258
PE	LP01	0.562 ^B ± 0.003	0.245 ^B ± 0.024	44.599 ^A ± 1.527	4.066 ^B ± 0.167	107.940 ^{BC} ± 9.873	ND	1.131 ^B ± 0.004	475.475 ± 14.728	176.707 ^{BC} ± 23.595	810.726 ^{AB} ± 49.531	78.006 ^B ± 0.217	1.592 ^B ± 0.101
	B501	0.593 ^B ± 0.031	0.244 ^B ± 0.014	43.483 ^A ± 1.469	3.655 ^B ± 0.028	133.254 ^{BC} ± 5.652	ND	1.163 ^B ± 0.070	469.554 ± 17.377	182.533 ^{ABC} ± 25.731	834.481 ^{AB} ± 50.373	74.276 ^B ± 1.703	1.314 ^B ± 0.058
	Uninoculated	0.564 ^B ± 0.082	0.248 ^B ± 0.055	37.608 ^A ± 6.512	3.361 ^B ± 0.624	189.456 ^A ± 32.941	ND	1.090 ^B ± 0.144	478.536 ± 60.403	200.719 ^B ± 16.918	911.583 ^{AB} ± 117.680	69.672 ^{BC} ± 14.558	1.883 ^B ± 0.282
NET	LP01	0.562 ^B ± 0.009	0.050 ^B ± 0.071	0.368 ^B ± 0.521	1.228 ^B ± 0.008	103.558 ^{BC} ± 2.621	ND	1.225 ^B ± 0.095	485.345 ± 6.263	255.911 ^A ± 22.271	848.247 ^{AB} ± 30.642	4.318 ^{CD} ± 0.290	0.254 ^B ± 0.072
	B501	0.561 ^B ± 0.012	0.073 ^B ± 0.001	0.104 ^B ± 0.029	1.185 ^B ± 0.012	87.236 ^C ± 0.812	ND	1.211 ^B ± 0.029	466.023 ± 5.081	212.670 ^{AB} ± 15.509	769.062 ^{AB} ± 19.755	0.304 ^D ± 0.013	0.224 ^B ± 0.006
	Uninoculated	0.585 ^B ± 0.012	0.064 ^B ± 0.002	0.132 ^B ± 0.045	1.143 ^B ± 0.001	192.397 ^A ± 13.408	ND	1.090 ^B ± 0.005	510.022 ± 1.280	252.478 ^A ± 1.895	957.911 ^A ± 10.196	2.523 ^{CD} ± 1.507	0.246 ^B ± 0.112

Values are expressed as mg / 100 ml of FS ± SD. Statistical differences are depicted with different superscript on each of the analytes at $p < 0.05$. Measurements were performed in duplicate. VI: Visczyme pretreatment, PE: Pectinex pretreatment, NET: Non-enzymatically treated, LP01: *Lactiplantibacillus plantarum* 01, B501: *Bifidobacterium animalis* 01, TE: Trolox Equivalents, GAE: Gallic Acid Equivalents.

compounds (as discussed before). This can be further verified through the substantial increase in GalA and GluA levels in comparison to the other samples (Table 3).

Interestingly, the results reveal that fermentation by both strains led to an increase in the overall oligosaccharide content when employing PE and NET as pretreatments. This observation could imply that PE and NET created an environment in which the introduced strain could amplify the proportion of oligosaccharides present, rather than reducing them as recently suggested (Wongkaew et al., 2021). This, together with a decrease in the percentage of oligosaccharides on the VI samples after fermentation, suggests that the produced oligosaccharides were not the main source for the growth of the used strains (also observed in Table 3). Although the use of VI did not lead to higher oligosaccharide concentration, it is important to highlight the potential for achieving greater oligosaccharide percentages through the application of milder conditions and the optimization of the enzymatic process.

3.7. Production of ESM and EPM fractions: ADF potential

The interaction of bound phenolics with dietary fiber is crucial to understand their bioavailability and impact on human health. Recently it has been reported that phenolic compounds bound to fiber (bound phenolics, ADF concept) could be more bioavailable, as they could be somewhat 'protected' by the fiber (to which they are bound) through the digestion process and thus reach the lower parts of the digestive system where they have the major effect (Jakobek & Matić, 2019). In addition, the bound phenolic fraction contributes the most to the antioxidant activity of dietary fiber (Liu et al., 2019). Hence, exploring the feasibility of ADF isolation using varied matrices and methods would be a worthwhile pursuit. In the present research we studied if the combination of enzymatic treatment and bacterial fermentation could be used as a technique to produce ADF. To investigate this, the antioxidant activity and the TPC were tested on the EPM and ESM fractions of the outcoming product (FS).

The lower antioxidant values in the ESM fraction were found to be in the uninoculated samples, followed by the B501 and LP01 in that order (Table 4). Conversely, the LP01 samples always showed the lower antioxidant activity, followed by B501 and uninoculated samples in the EPM fraction. Both fermentation and enzymatic treatment enhanced the antioxidant activity in the ESM fraction with the subsequent decrease in

the EPM fraction. PCs – especially mangiferin and gallic acid – are being released from the insoluble fraction during enzymatic treatment of mango peels, and from the solubilized fiber through the fermentation process (in this case), which increases their concentration in the FS (Table 2, Table 4). These solubilization processes had been previously reported on durum wheat fiber and barley spent grain using *Trichoderma* sp. derived enzymes (showing increases of 2.5 and 1.2 mg GAE/g (Napolitano et al., 2006)), in wheat bran by LAB fermentation (showing increases of 0.379 mg GAE/g after 48 h of fermentation (Spaggiari et al., 2020)), and in the press cake of canola after oil extraction using both enzymatic and LAB fermentation strategies (showing increases of aprox. 0.2 mg GAE/g after VI enzymatic treatment (Tian et al., 2023)). The same trends in both ESM and EPM fractions were observed for TPC (Table 4). These results show that neither enzymatic or fermentation processes could be used alone nor in combination for the production of ADF from mango peels. However, other authors successfully used endoxylanases to produce feruloylated oligosaccharides with higher antioxidant activity compared to the untreated samples (Katapodis et al., 2003), therefore demonstrating the possibility of producing ADF through enzymatic processes. This could derive from the use of a more selective and less active enzyme when compared to the enzymatic cocktails used herein. Therefore, the use of gentler or alternative conditions during enzymatic treatment of mango peels should be explored in order to obtain ADF.

4. Conclusions

This study investigated the impact of sequentially treating mango peels using enzymatic and fermentation techniques. The hypothesis of enhancing the fermentation by enzymatic pretreatment was not verified in mango peels, as the growth of the bacteria was not influenced by the enzymatic pretreatment. In addition, the ADF production by this process is not efficient, as both enzymatic and fermentation processes depleted the antioxidant fiber from its bound phenolic compounds. Nevertheless, both bacterial strains used in the present experiment (i.e. B501 and LP01) were able to grow by one log in all conditions, leading to substantial changes in the recovery of PCs. The recovery of gallic acid and mangiferin aglycones was significantly higher when VI was combined with any of the strains, at least 2.4 and 1.6 times when compared to the unfermented control, respectively, while their recovery in the fermented

Table 4

Antioxidant activity (DPPH, FRAP) and total phenolic compounds (TPC) from the ethanolic precipitable matter (EPM) and the ethanolic soluble matter (ESM) fractions of different fermented mango peel solutions – with and without enzymatic pretreatment.

Fraction / Enzyme	Strain	DPPH (mg TE/100 mg DM)	FRAP (mg TE/100 mg DM)	TPC (mg GAE/100 mg DM)
ESM	LP01	1.735 ^a ± 0.147	2.798 ^a ± 0.347	17.472 ^a ± 0.993
	B501	1.371 ^b ± 0.037	2.618 ^a ± 0.278	15.975 ^a ± 1.220
	Uninoculated	0.869 ^c ± 0.047	1.881 ^b ± 0.056	13.577 ^{ab} ± 3.156
PE	LP01	1.021 ^c ± 0.083	1.953 ^b ± 0.108	12.905 ^{ab} ± 0.800
	B501	0.954 ^c ± 0.136	1.927 ^{bc} ± 0.240	13.058 ^{ab} ± 0.969
	Uninoculated	0.623 ^d ± 0.107	1.575 ^{bc} ± 0.217	9.463 ^{bc} ± 0.572
NET	LP01	0.782 ^d ± 0.100	1.959 ^b ± 0.088	13.719 ^{ab} ± 2.102
	B501	0.535 ^{de} ± 0.087	1.307 ^c ± 0.051	8.930 ^{bc} ± 2.984
	Uninoculated	0.294 ^e ± 0.119	1.227 ^c ± 0.342	7.686 ^c ± 0.397
EPM	LP01	0.052 ^{de} ± 0.147	2.007 ^c ± 0.064	10.892 ^b ± 0.452
	B501	0.254 ^d ± 0.037	1.988 ^c ± 0.142	13.557 ^b ± 4.875
	Uninoculated	ND	3.882 ^b ± 1.147	25.178 ^b ± 7.334
PE	LP01	ND	2.113 ^c ± 0.211	17.325 ^b ± 1.363
	B501	0.290 ^d ± 0.021	1.642 ^c ± 0.206	15.528 ^b ± 2.641
	Uninoculated	0.775 ^c ± 0.260	2.598 ^c ± 0.402	24.136 ^b ± 5.443
NET	LP01	1.138 ^b ± 0.191	2.680 ^c ± 0.070	25.510 ^b ± 6.936
	B501	1.305 ^b ± 0.167	2.617 ^c ± 0.288	26.792 ^b ± 0.958
	Uninoculated	2.974 ^a ± 0.102	5.680 ^a ± 0.120	82.684 ^a ± 15.867

Different superscripts mean statistical differences between conditions at $p < .05$. VI: Viscozyme pretreatment, PE: Pectinex pretreatment, NET: Non-enzymatically treated, LP01: *Lactiplantibacillus plantarum* 01, B501: *Bifidobacterium animalis* 01, TE: Trolox Equivalents, GAE: Gallic Acid Equivalents.

PE samples was 17 and 2.4 times higher, respectively. Furthermore, the use of VI, and any of the strains, considerably increased the antioxidant activity of the FS.

Given that the enzymatic treatment was kept brief (2 h) and the fermentation was carried out using common probiotic bacterial strains, this approach could have industrial applications to yield functional food ingredients. The recovery of phenolic compounds by enzymatically assisted fermentation methods employing other agro-industrial by-products merits additional investigation, whilst optimization processes or other approaches should be pursued for the manufacture of ADF from mango peels.

Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this work the author(s) used QuillBot in order to improve readability. After using this tool/service, the author(s) reviewed and edited the content as needed and take(s) full responsibility for the content of the publication.

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Arnau Vilas-Franquesa: Conceptualization, Investigation, Methodology, Validation, Formal analysis, Visualization, Writing – original draft, Writing – review & editing. **Christos Fryganis:** Methodology, Validation, Writing – review & editing. **Melania Casertano:** Conceptualization, Writing – review & editing. **Marco Montemurro:** Conceptualization, Writing – review & editing. **Vincenzo Fogliano:** Conceptualization, Writing – review & editing, Supervision.

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.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

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