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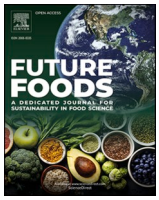

Yield *vs.* functionality: Enzyme-assisted extraction of mango peel reduces antioxidant dietary fiber quality

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ABSTRACT

This study explores enzymatic-assisted extraction (EAE) of fibers from mango peels and their prebiotic potential. Mango peels were treated with Pectinex®, Pectinex Smash XXL®, and Viscozyme® at various temperatures (30–50 °C) and times (2–6 h). Ethanolic precipitable matter (EPM) was used to estimate soluble fiber yield. Viscozyme® at 40 °C for 2 h produced the highest EPM (~15 g/100 g dry matter). However, control samples (30 °C, 2 h) preserved the most phenolic compounds (250 mg GAE/g EPM) and antioxidant activity in the EPM. *In vitro* fermentation of EPM showed that all fibers induced the production of SCFA, with the phenolic-richer fibers promoting a greater propionic acid production. These findings indicate that EAE is a valuable strategy to extract fibers from mango peels, but that enzymatic conditions must be fine-tuned to preserve higher concentration of phenolic compounds. The interaction between phenolics and fiber, or antioxidant dietary fiber, appears pivotal in modulating gut microbiota functionality.

1. Introduction

Mango (*Mangifera indica* L.) is a massively produced tropical fruit exported worldwide. The extensive cultivation of mangoes (1.73 million Tn produced only in 2021) generates a substantial quantity of peels. About 7–24 % of mango consists of peels, and peels represent a serious problem of disposal (Aggarwal et al., 2017). The high amount of dietary fiber and phenolic compounds in mango peels presents a valuable opportunity for their upcycling.

Recent research has explored upcycling mango peels by extracting functional compounds using enzymatic-assisted extraction (EAE), but also chemical extractions and more direct extractions such as drying and powdering (Marçal and Pintado, 2021). EAE is an environmentally friendly approach to extract valuable bioactive compounds such as phenolic compounds and fibers from different materials (Gligor et al., 2019; Vilas-Franquesa et al., 2024). Recently, we showed that EAE could be a useful technique to produce soluble fibers from mango peels by using Pectinex and Viscozyme (Vilas-Franquesa et al., 2024b).

The solubilization of fibers by EAE results in variations in fiber size and profile, with differing levels of phenolic compounds. Fibers containing bound phenolics have been associated with greater health benefits (Gutiérrez-Díaz et al., 2021) and labeled as antioxidant dietary fiber (ADF) by Saura-Calixto (2011). The ADF can have variable concentrations of phenolic compounds, which can be heterogeneously bound to the fiber matrix (Vilas-Franquesa et al., 2024a). In any case, the fiber acts as protective agent of these phenolic compounds, only releasing them upon degradation by the gut microbiota (Pérez-Jiménez et al., 2025). The ADF can be derived from various plant matrices, with greater presence in those sources that exhibit higher concentrations of phenolic compounds, such as peels or seeds. Although it was shown before that mango fibers can promote the growth of lactic acid bacteria (LAB), the specific effects of different enzymatic preparations (like Pectinex or Viscozyme) on the extraction and recovery of ADF from mango peels with potential prebiotic properties remain unclear.

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The present research aims at identifying which commercially available enzymatic combinations are most effective in producing ADF. Ethanolic precipitable matter (EPM) was used to quantify the soluble fibers extracted by each treatment. The phenolic content and antioxidant activity of the resulting EPM were analyzed to assess differences in the ADF potential among samples. Our hypothesis was that EAE would result in higher solubilization of (formerly) insoluble materials but not necessarily in higher EPM production. Additionally, we hypothesized that EPM with higher phenolic content could stimulate the metabolic activity of gut bacteria, thus showing higher prebiotic effects.

2. Materials and methods

2.1. Materials

Frozen mango peels (MP) from our previous research were used in the present experiment (Vilas-Franquesa et al., 2024b). Briefly, the fruits, of Kent variety (Peru), had been purchased at a local supermarket (Jumbo, Wageningen, The Netherlands), peeled with a conventional peeler and the peels were immediately frozen. MP had been stored at −20 °C for 4 months at the time of the experiment.

Viscozyme® L (VZ), Pectinex® XXL (PX) and Pectinex® Smash XXL (PXS), provided by Novozyme (Bagsvaerd, Denmark) were selected based on earlier findings (Vilas-Franquesa et al., 2024b). VZ is a blend of beta-glucanase (endo-1,3(4)-) with side activities of xylanase, cellulase and hemi-cellulase. PX's reported main activity is pectin lyase activity with side activity of polygalacturonase, whereas PXS mainly presents pectin lyase activity.

Sodium carbonate 99 % (Na_2CO_3), sodium chloride (NaCl), sodium hydroxyde (NaOH), Folin-Ciocalteu's phenol reagent, DPPH (2,2-Diphenyl-1-picrylhydrazyl), gallic acid 97.5–102.5 %, Trolox 97 % ((±)-6-Hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid), TPTZ 99 % (2,4,6-Tris(2-pyridyl)-s-triazine), sodium acetate 99 %, acetic acid glacial and glucose monohydrate, were obtained from Sigma Aldrich-Merck KGaA (Darmstadt, Germany). Iron(III)chloride 97 % (FeCl3) was obtained from Honeywell (Seelze, Germany), HPLC-grade methanol was obtained from Actua-All Chemicals (Oss, Netherlands), hydrochloric acid 37 % (HCl) and ethanol 96 % were obtained from VWR International (Rosny-sous-Bois, France). Tryptone, MRS broth and MRS agar were obtained from Oxoid LTD (Hampshire, England). Peptone physiological salt solution tubes from Tritium Microbiologie B.V. (Eindhoven, The Netherlands).

2.2. Enzymatic hydrolysis of mango peels

Frozen MP were washed as explained elsewhere (Vilas-Franquesa et al., 2024b). Then, frozen MP were directly blended with demineralized water at ratio 1:3 MP:water (w/w). This solution was used for enzymatic hydrolysis. A total of 30 g of MP solution was poured into a Greiner tube of 50 mL total volume. Thereafter, each enzyme was added at the highest dosage as recommended by the manufacturer (Supplementary Material), or without any addition (control, CO). The pH was adjusted in each tube according to an averaged pH value as recommended by the manufacturer (Supplementary Material), using 2 M HCl solution. The optimal pH for PXS was set at 3.5, as no optimal pH was provided by the manufacturer and its current application is on citrus fruits, which usually has a pH below 4 (El-Otmani et al., 2011). The pH was measured at 20 °C using a pHenomenal® pH1000L (VWR International B.V., Netherlands) pH-meter.

The samples were then immediately incubated at different temperatures (30, 40 and 50 °C) over different times (2 and 6 h). At the end of the incubation, the samples were heated at 100 °C for 10 min to stop the hydrolysis (Wongkaew et al., 2021; Wu et al., 2012), prior to being transferred to an ice-water bath for 30 min. Next, the samples were centrifuged at 4700 g for 15 min at 20 °C. Then, the supernatant, containing the solubilized material, was transferred into 50 mL standing Greiner tubes. The pellet was discarded, while the supernatant was stored at −20 °C until further analyses. All conditions were tested in triplicate.

2.3. Dry matter (DM) and ethanolic precipitable matter (EPM)

DM was quantified according to the AOAC methods of analysis (AOAC 950.46; AOAC, 2005) and EPM was quantified as reported by Vilas-Franquesa et al., 2024b. In both cases, supernatants were thawed overnight at 4 °C and placed in a water bath at RT (~25 °C) for 20 min before the analysis. For the EPM quantification, a minor change was included after obtaining the wet EPM pellet. The pellet was dried at 60 °C for 16 h in a conventional oven drier (Binder, Germany). Once dried, the pellet was weighted and EPM was quantified by weight difference. DM and EPM were performed in triplicate, one measurement in each EAE sample.

2.4. Antioxidant activity (DPPH and FRAP) and total phenolic content (TPC) of the EPM

The EPM produced (0.5 g) was solubilized in mili-Q water (5 mL) following the process described before (Vilas-Franquesa et al., 2024b). No additional dilutions were applied to the extract before running either of the antioxidant analyses. Solubilized EPM was frozen at −20 °C for two weeks prior to analysis. Samples were thawed overnight at 4 °C and used immediately after for analysis. The antioxidant activity was assessed by both techniques on the same day after sample thawing. The antioxidant activity and the TPC assays were performed exactly as previously detailed in the same matrix (Vilas-Franquesa et al., 2024b). Antioxidant activity was tested twice in each EAE sample.

2.5. Upscaling EPM production

The EPM production process was scaled up for its *in vitro* colonic fermentation. The *in vitro* colonic fermentation was performed with a final concentration of 0.4 g EPM / 100 mL in the flasks. Therefore, a 4 % stock solution was prepared. To that end, a total of 1.5 and 3 g of EPM was produced from the CO (30 °C, 2 h) and VI (40 °C, 6 h) selected samples, respectively. The EPM of each sample was prepared according to the protocol detailed in Section 2.3 (but with higher volumes according to the needed g of EPM) and was solubilized with 32.5 and 75 mL of mili-Q water, respectively. The upscaled EPM solution was used for the *in vitro* batch colonic fermentation and for the LC-MS/MS quantification of gallic acid and mangiferin.

2.6. Quantification of gallic acid and mangiferin in EPM

The EPM solution from the upscaling was firstly diluted 1:10 with mili-Q water then filtered using a RC 0.2 μm pore size filter Whatmann™ (Buckinghamshire, U.K.). Next, the sample was processed by LC-MS/MS using the analytical conditions detailed in previous works (Vilas-Franquesa et al., 2024b). Briefly, the analysis was performed using a Nexera UPLC system coupled to an LCMS-8050 triple quadrupole mass spectrometer (Shimadzu). UPLC components included an autosampler, solvent delivery module, degasser, column oven, and valve unit. Separation was achieved on an Acquity Premier BEH C18 column (1.7 μm, 2.1 × 100 mm) with a VanGuard pre-column (Waters), using a gradient of 0.1 % formic acid (A) and acetonitrile with 0.1 % formic acid (B). The flow rate was 0.3 mL/min and the column was maintained at 40 °C. Data was processed in the same manner with the same software. Specific phenolics were quantified twice in each EAE sample.

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

A High-Performance Anion Exchange Chromatography (HPAEC)

system equipped with a CarboPac PA-1 column (2 × 250 mm) in combination with a CarboPac PA guard column (2 × 25 mm) and a pulsed electrochemical detector in pulsed amperometric detection mode (Dionex, Sunnyvale, USA) was used for this purpose. EPM was diluted 1:10 with mili-Q water (*v/v*). The elution, flow rate, standards and everything else were used as described before (Vilas-Franquesa et al., 2024b). Sugars, GalA and GluA were quantified twice in each EAE condition.

2.8. In vitro batch colonic fermentation

The samples showing the highest (CO, 30 °C, 2 h, labeled MFCO) and lowest (VZ, 40 °C, 6 h, labeled MFVZ) antioxidant activity values were used for the *in vitro* batch colonic fermentation. The *in vitro* colonic fermentation assay was performed following the method used by (Pérez-Burillo et al. (2021) with some modifications. Fresh fecal samples were collected from three healthy, non-smoker, active adults, 20–28 y/o, with a body mass index between 18 and 25. None of the donors had any history of gastrointestinal disorders or antibiotic treatment for at least 3 months before this study. Fecal slurries were prepared within 2 h after defecation following the method described by Huang et al. (2021). Batch-culture fermentation glass vessels (10 mL working volume) were sterilized and filled with 4.3 mL of sterile basal colonic nutrient medium, consisting of 5.22 g/L K_2HPO_4, 16.32 g/L KH_2PO_4, 2.0 g/L $NaHCO_3$, 2.0 g/L yeast extract, 1.0 g/L mucin, 0.5 g/L-cysteine HCl, and 2.0 mL/L Tween 80. No peptone was added to the mix and sterile conditions were maintained during all sample management. Next, 2 mL of EPM (1.4 % stock solution concentration, MFVZ or MFCO) was added to the vessel. Commercial pectin from apple (Merck, Darmstadt, Germany) was used as positive control (1.4 % stock solution, labeled MFPE) and mili-Q water was used as negative control (without external addition of carbon sources, labeled MF). The final concentration of fibers in the fermentation vessel was 0.4 %. The vessels were then inoculated with 0.7 mL of fecal slurry and immediately flushed with N_2 gas to create anaerobic conditions. The vessels were sealed tight and placed in an incubator at 37 °C under mild shaking conditions (100 rpm) for 24 h. Fermented samples were taken at the specified time points and the reaction was stopped by using liquid nitrogen. The fermented samples were stored at −20 °C until SCFA analysis. This study did not require ethical approval, as confirmed by the Medical Ethical Committee of East Netherlands and the Medical Ethical Advisory Committee of Wageningen University (METC-WU, METC 2023–16,718).

2.9. Short-chain fatty acids analysis (SCFA)

SCFA was analyzed following the method described by Huang et al. (2021) with some modifications. The fecal fermented samples were thawed on the same day of SCFA analysis. For thawing, the fermented samples were placed in a water bath at 30 °C for 40 min. Thawed samples were centrifuged at 10,000 g and 4 °C for 5 min and subsequently filtered using a 0.2 μm regenerated cellulose (RC) syringe filter. A precise volume of 150 μL of the sample (MF, MFPE, MFVZ or MFCO) was added to a chromatographic insert-containing vial. Next, 75 μL of the internal standard solution (0.225 mg/mL 2-ethylbutyric acid in 0.15 M HCl and 0.45 M oxalic acid) was added to each sample. A 5-point calibration curve was prepared for each of the SCFA analyzed (acetate, propionate, butyrate, valerate, isobutyrate, and isovalerate), ranging from 0.015 to 0.7 mg/mL.

SCFA measurement was performed using a Shimadzu GC-2014 (Kyoto, Japan) equipped with a flame-ionization detector, a capillary fatty acid-free Stabil wax-DA column (1 μm × 0.32 mm × 30 m) (Restek, Bellefonte, PA, USA) and a split injector. The injection volume was 0.5 μL and the carrier gas was N_2. Temperatures of the injector and detector were set at 100 and 250 °C, respectively. The temperature profile started at 100 °C, increased to 172 °C by 10.8 °C/min, then to 200 °C by 50 °C/min, and held for 1 min at this temperature. The results were processed using the software Chromeleon 7.2.10 (Thermo Fisher Scientific, San Jose, *CA*). All samples were analytically quantified in triplicate.

2.10. Statistical analysis

Statistical Analysis was performed with R Statistical Software 4.3.1. A two-way ANOVA was used for all analysis. The distribution and homogeneity of the data was tested visually through boxplots and computed through Levene's Test. Shapiro-Wilks test was performed on the residuals. In case of significance of the ANOVAs, the data were further investigated with post-hoc Tukey's test. The p-value adopted for statistical significance was <0.05. A Kruskal-Wallis test was run in the data from oligosaccharide quantification due to the non-normal distribution of the data. The statistical significance was still set at <0.05

For the principal component analysis (PCA), the Kaiser-Meyer-Olkin (KMO) measure verified the sampling adequacy for the analysis. Bartlett's test of sphericity indicated that correlations between items were sufficiently large for PCA. An initial analysis was run to obtain eigenvalues for each of the components in the data. Two of the components showed eigenvalues over Kaiser's criterion of 1 and in combination explained 88.32 % of the variance (Supplementary Material). The scree plot confirmed the first two components to be the most relevant for explaining the variance of the statistical model. Therefore, the first two components were retained for the final analysis. No rotation was applied since the first two principal components showed a great fit with the raw data. The items that were clustered on the first principal component were DM, DPPH and TPC. The items that were clustered on the second component were EPM and FRAP (Supplementary Material). The raw dataset used for this article can be found in the following repository: DOI:10.17632/z2vs6gwbmk.1.

3. Results

3.1. Solubilized material and produced EPM after enzymatic hydrolysis

Data in Fig. 1 shows the amount of solubilized material roughly doubling in all enzymatically treated samples compared to the control. All enzymes had a slightly lower performance after 2 h of incubation at 30 °C compared to 6 h. PX was the enzyme exhibiting the highest material solubilization at 6 h and 40 °C, which was 4 % higher than VZ under the same conditions (Fig. 1). These results were in line with those obtained in our previous study (Vilas-Franquesa et al., 2024b), in which the use of PX or VZ resulted in a similar solubilization percentage.

Sharif and colleagues (2023) found higher solubilization using alcalase when compared to pectinases, when using ultrasounds as pretreatment. This may happen because ultrasounds can facilitate the access of alcalase to the protein found in the middle lamella that is bound to hemicellulose and pectin polysaccharides. However, Sharif et al. (2023) did not investigate whether without ultrasounds the results would be similar. Finally, PXS was the enzyme with the lowest solubilization (Fig. 1). PX is a blend of pectinases, hemicellulases and arabinases, whereas PXS is mainly pectin lyase. The better performance by PX is expected as the cell wall not only consists of pectin but also other carbohydrates (*e.g.* hemicellulose).

The amount of soluble fiber, estimated by measuring ethanol precipitable matter (EPM), obtained after each enzymatic treatment, is reported in Fig. 2. Overall, operating at 40 °C shows potential in producing soluble fiber regardless of the duration of the treatment. The EPM obtained at 2 h at 40 °C when using VZ was significantly higher than any other combination. The use of enzymatic treatment resulted in higher EPM recovery than the control, showcasing the efficacy of EAE in solubilizing fibers from mango peels. The use of different classes of enzymes to produce oligosaccharides from formerly insoluble material in pectin-rich matrices has been well documented (Manthei et al., 2024; Vilas-Franquesa et al., 2024; Martínez Sabajanes et al., 2012).

While enzymatic treatments significantly enhanced EPM yield compared to controls, especially at 30 and 40 °C, higher temperature (at

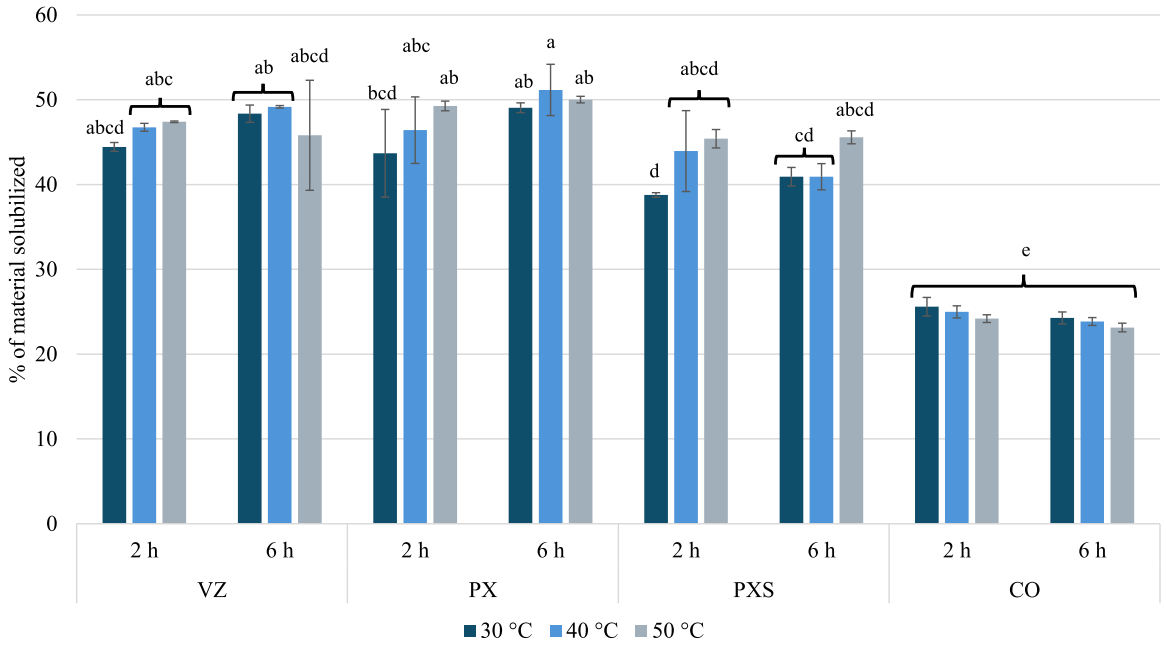

Fig. 1. Percentage of solubilized material from mango peels (dry matter) in the recovered supernatant using different enzymatic treatments related to the starting dry matter. VZ: Viscozyme, PX: Pectinex, PXS: Pectinex XXL, CO: control or no-enzyme. Bars show average values ± SD. Different letters mean statistical differences at $p<.05$.

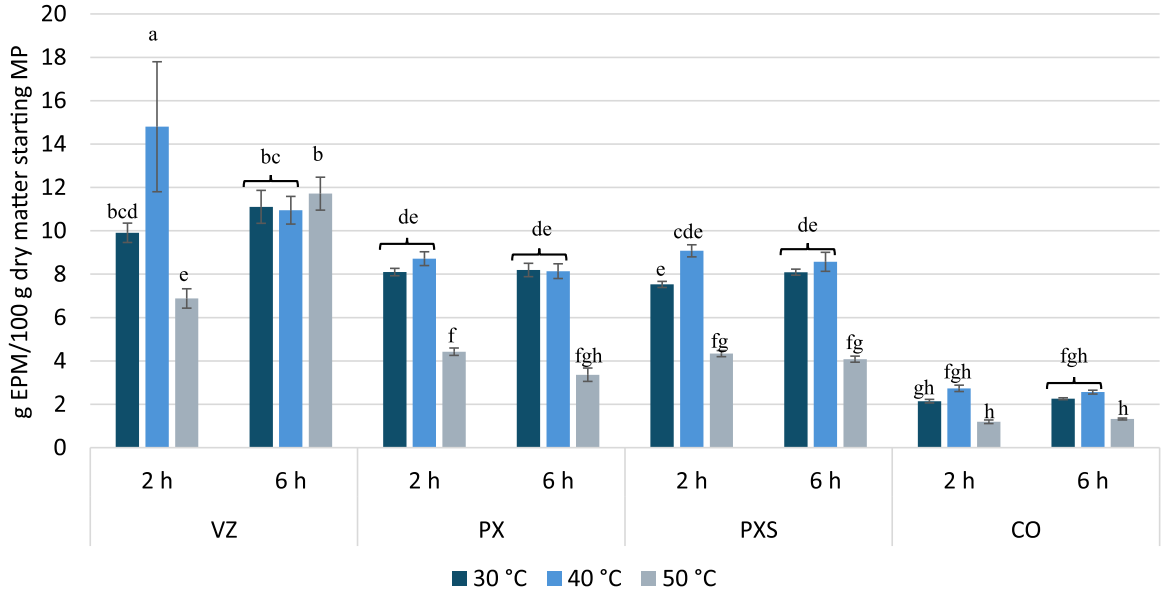

Fig. 2. Soluble fiber production (in g of EPM obtained from 100 g of dry matter of mango peels) after different enzymatic treatments at 30, 40 and 50 °C for 2 and 6 h. Bars show mean and SD. Different letters mean statistical significance at p<.05. VZ: Viscozyme, PX: Pectinex, PXS: Pectinex Smash XXL, CO: Control (no enzyme), EPM: ethanolic precipitable matter, MP: mango peels.

50 °C) negatively affected EPM precipitation, likely by altering the solubility or aggregation behavior of pectic compounds. This indicates that temperature exerts a dominant influence at this level (Fig. 2). Enzymes may also contribute by reducing the molecular weight of pectic oligosaccharides, which can further impair ethanol precipitation, but the effect of temperature appears to be the overriding factor at 50 °C.

3.2. Total and specific phenolics, antioxidant activity and sugar profile in the EPM fraction

The results presented in Fig. 3 indicate that the EPM fraction from the control sample incubated at 30 °C for 2 h had the highest concentration of phenolic compounds and greatest antioxidant activity. This is because the EAE has a direct effect on the breakdown of polysaccharide chains and can be useful in releasing phenolic aglycones from mango peels (Vilas-Franquesa et al., 2024b), depleting the mango peel fibers of its main antioxidant components, therefore resulting in lower antioxidant activity (Fig. 3). Whereas phenolic solubilization may be beneficial to produce phenolic extracts, it can be counterproductive when aiming at the extraction of antioxidant dietary fiber (ADF), which is the joint product resulting from the interaction between phenolic compounds and fibers (Saura-Calixto, 2011). Phenolic compounds can be bound to fiber covalently or non-covalently. In the former scenario, the phenolic compounds are physically entrapped and are released when

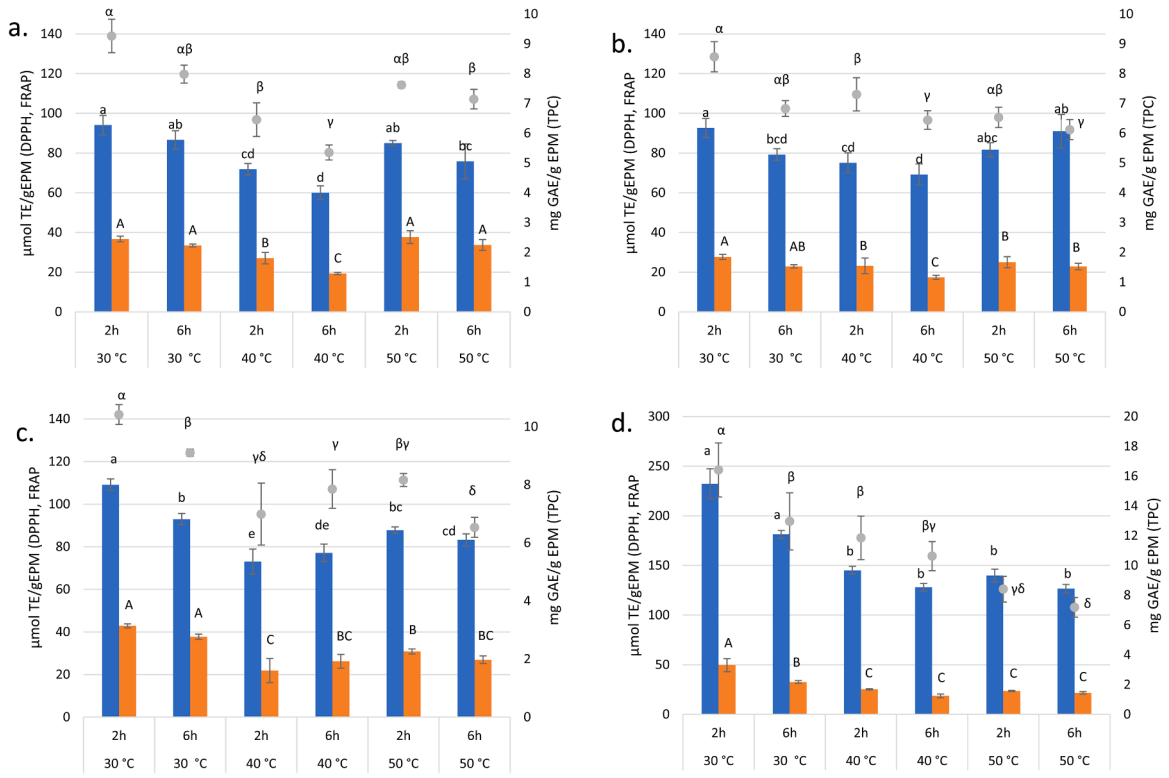

Fig. 3. Antioxidant activity by DPPH (orange bars) and FRAP (blue bars), and total phenolic compounds (TPC, grey dots) of the EPM extracts produced by Viscozyme (a), Pectinex (b), Pectinex Smash XXL (c), and control (d) at different temperatures and times. Antioxidant activity is expressed as µmol Trolox Equivalent/g EPM using DPPH (blue bars) and FRAP (orange bars), and total phenolic content (grey dots) is expressed as mg Gallic Acid Equivalent/g EPM. Bars and dots show mean and SD. Different letters mean statistical significance at p<.05 across the same enzyme and parameter (*i.e.* CO and TPC).

the fiber is hydrolyzed in smaller chains, whereas in the latter, side esterase activities of enzymatic preparations are key to hydrolyze the covalent bonds between phenolic compounds and fiber (Rocchetti et al., 2022).

Fig. 3d illustrates a significant decline in phenolic compounds and antioxidant activity over time in the control samples. Like what was observed in EAE samples, higher temperatures enhance solubilization, which reduces the concentration of antioxidant compounds—such as phenolics—in the remaining insoluble fiber (EPM). Given that the temperatures applied in this experiment are not high enough to cause degradation of phenolic compounds (Natali et al., 2022), it is likely that their reduction in the fiber is primarily due to solubilization rather than degradation.

Fig. 3 also shows a significant drop in antioxidant activity and phenolic compounds in EPM derived from enzyme activity from 30 to 40 °C. Nevertheless, similar results are observed at 40 and 50 °C regardless of the time of incubation. This means that there is an increase in solubilization at this temperature due to enzyme efficiency (as seen in Fig. 1), translating into lower phenolic retention in the recovered EPM (Fig. 3). The results are also consistent with our previous work on mango peels (Vilas-Franquesa et al., 2024b). Islam and colleagues (2023) also showed that there is a significant increase in the recovery of phenolic compounds when digesting banana peels with VZ at 45–55 °C (Islam et al., 2023).

The use of VZ resulted in the lowest phenolic concentration in the EPM at 40 °C for 6 h (<80 mg GAE/g EPM), and the lowest antioxidant activity by FRAP (<20 µmol TE/ g EPM) and DPPH (<60 µmol TE/ g EPM (Fig. 3). Interestingly, VZ simultaneously led to the highest overall EPM yield (Fig. 2). This suggests that VZ promotes more aggressive cleavage compared to other enzymes, thereby facilitating a greater release of phenolic compounds. Similar behavior has been reported in other matrices (Zhang et al., 2021).

The quantification of gallic acid and mangiferin was performed in the EPM fractions with the highest (CO, 30 °C, 2 h) and lowest (VZ, 40 °C, 6 h) antioxidant activity. These fractions were also used for SCFA analysis. Regarding the most important phenolic compounds in the extracted fibers, gallic acid and mangiferin were present in higher amounts in CO samples than VZ (Table 1).

Differences in the sugar profile from the EPM product were also detected (Table 2). The use of enzymes was not only linked to higher EPM quantity, but also to higher solubilization of material (Fig. 1, 2). The results clearly indicate that the EPM produced by EAE also contained a higher percentage of sugars (Table 2).

3.4. Interactions between solubilization, phenolics and antioxidant activity

A PCA summarizing the main characteristics of the various mango peel extracts is reported in Fig. 4. Dimension 1 explained a great part of the variance from dry matter, DPPH, and TPC variables, whereas dimension 2 explained part of the EPM and FRAP variances (Supplementary Material). The data indicate that EPM is more strongly associated with FRAP antioxidant activity than with DPPH. The enzymatically treated samples are separated from the control along both axes, with a more pronounced distinction in Dimension 1. This suggests that enzyme applications resulted in EPM extracts with lower antioxidant contribution (FRAP) compared to the control (Fig. 1). This pattern suggested that

Table 1
Concentration of gallic acid and mangiferin aglycones in the EPM extracts used in the *in vitro* colonic fermentation.

Sample	Gallic acid (ppm)	Mangiferin (ppm)
CO	$1.304^{a} \pm 0.020$	$0.031^{a} \pm 0.000$
VZ	$0.489^{b} \pm 0.013$	$0.017^{b} \pm 0.000$

Table 2
Concentration of glucuronic and galacturonic acids, and monomeric sugars in the EPM extracts used in the *in vitro* colonic fermentation.

Sample/ Sugar	Fucose (μg/g)	Rhamnose (μg/g)	Arabinose (μg/g)	Galactose (μg/g)	Glucose (mg/g)	Mannose (μg/g)	Xylose (μg/g)	Galacturonic acid (mg/g)	Glucuronic acid (μg/g)
CPE	13.82 ± 0.63	ND	491.50 ± 2.73	17.59 ± 1.89	0.64 ± 0.00	ND	ND	ND	ND
CO	ND	ND	ND	14.34 ± 0.21	10.55 ± 0.12	ND	ND	0.12 ± 0.17	7.87 ± 11.12
VZ	10.02 ± 0.92	23.15 ± 0.16	91.93 ± 3.06	182.00 ± 1.58	7.74 ± 0.05	ND	ND	14.07 ± 0.20	ND

Values represent the mean ± SD. CPE: commercial pectin, CO: control samples incubated at 30 °C for 2 h, VZ: samples extracted by Viscozyme at 40 °C for 6 h, ND: Non-Detected.

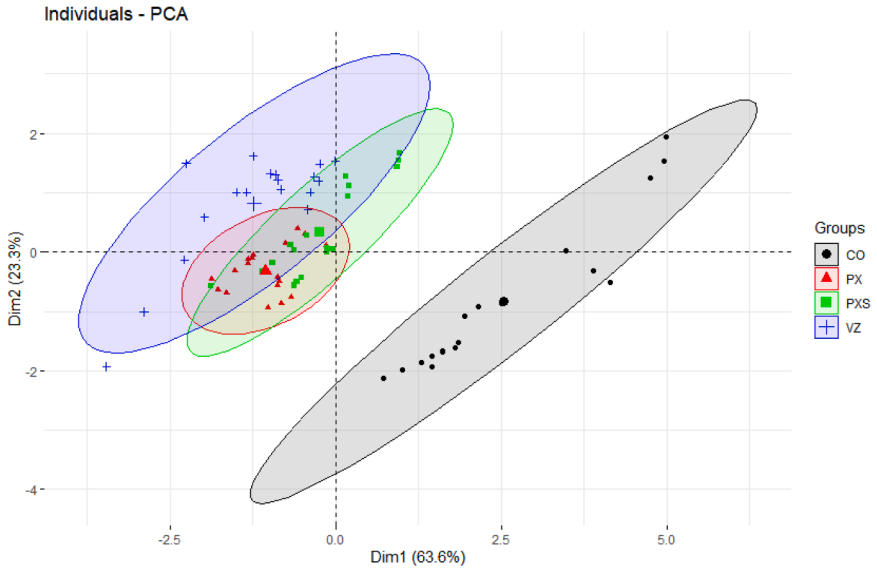

Fig. 4. Two-dimension PCA mapping of the samples produced by different enzymatic treatments, and the control. Dimension 1 (dry matter, DPPH, TPC), Dimension 2 (EPM, FRAP).

the enzymatic treatments applied were less effective in producing antioxidant dietary fiber (ADF), as defined by both fiber yield and its associated antioxidant potential.

It is known that the use of enzymes could help in extracting soluble dietary fiber (*e.g.* from apple pomace (Li et al., 2014)) when compared to other technologies. This is because enzymes break down the cell wall, and keep on further breaking down large molecules, such as hemicellulose or cellulose, into smaller molecules (*e.g.* XOS, FOS…), making them soluble. When it comes to ADF production, the usefulness of enzymes is not that clear, as excessive incubation – and degradation of fibers – may lead to the release of bound phenolics, which is the principal source of antioxidant activity in the ADF (Angulo-López et al., 2022). Figs. 3 and 4 suggest that the most effective approach for producing ADF – defined herein as EPM with associated antioxidant activity – may lie in specific conditions in control samples, rather than in enzymatic treatments, even at mild temperatures. However, the quantity of EPM triples when using enzymatic treatments, as shown in Fig. 2. Therefore, a trade-off must be considered between the extent of solubilization achieved and the amount of phenolic compounds lost, making the application of enzymes under mild conditions potentially beneficial for the obtention of ADF.

3.5. *In vitro batch colonic fermentation of EPM from mango peels*

The *in vitro* batch colonic fermentation was explored in the EPM fractions with the highest (CO, 30 °C, 2 h) and lowest (VZ, 40 °C, 6 h) antioxidant activity as well. Mango peel fiber, whether processed with or without enzymatic treatment, stimulated SCFA production after 24 h of incubation.

There was a higher production of acetic acid in the MFPE samples (Fig. 5a). This was expected as the metabolization of pectin by the gut microbiota leads to high amounts of acetic acid after 24 h of fermentation (Gulfi et al., 2005), and the MFCO and MFVZ samples produced are not pure pectin chains. Interestingly, the amount of propionic acid was significantly higher in MFCO compared to MFPE or MFVZ (Fig. 5). The concentration of phenolic compounds in MFCO was far superior to those in MFVZ. Propionic acid production has been linked to lower local inflammation, to improved insulin sensitivity, and could contribute to satiety (Al-Lahham et al., 2010). However, recent research has shown that propionic acid levels in plasma could be linked to increased odds of cognitive decline (Neuffer et al., 2022). Therefore, the fact that a product with higher ADF potential leads to higher production of propionic acid needs to be carefully considered.

Interestingly, the higher presence of free monomeric sugars in the EPM (Table 2) did not translate into higher production of SCFA. This could be important in elucidating the role of phenolic compounds, and ADF, to the production of SCFA and overall gut health. Other authors have recently shown that soluble dietary fiber extracts from citrus peels can promote the production of propionic acid in fecal fermentations (Gu et al., 2023). However, they linked this increase to the higher arabinose concentration in the extracts, an association that is not seen here.

Our results show that EAE can be a useful technique to extract fibers from mango peels that influence SCFA production. In contrast, recent publications have found the opposite, with oligosaccharide-rich EAE extracts from apple bagasse and orange peel showing no effect on SCFA production (Manthei et al., 2024). The use of enzymes is important in

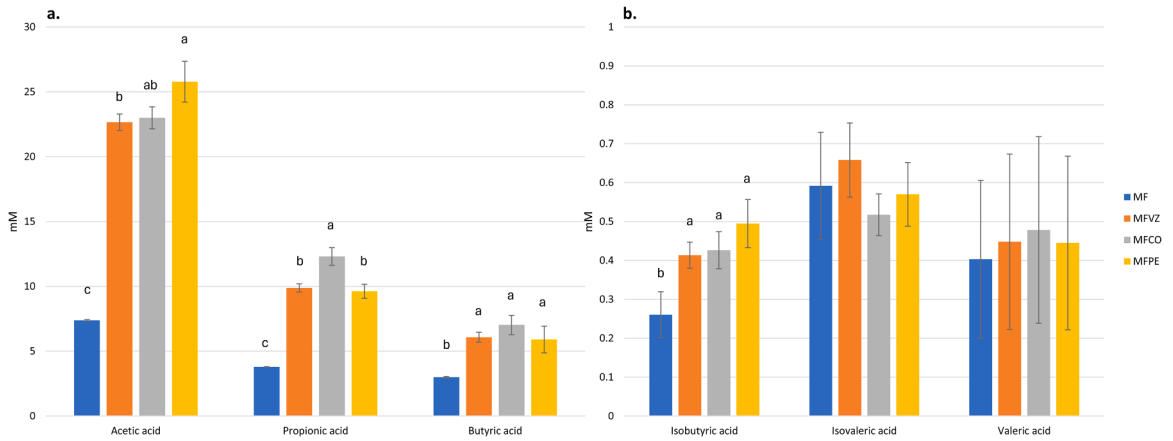

Fig. 5. Production of (a) acetic, propionic and butyric acid, and (b) isobutyric, isovaleric and valeric acid by gut microbiota in a solution with 0.4 % of soluble fibers (EPM) from mango peel with high (MFCO, 30 °C, 2 h) and low (MFVZ, 40 °C, 6 h) antioxidant capacity. MF is the production of SCFA without any fiber source added, and MFPE is the production of SCFA with 0.4 % commercial pectin. Bars indicate the SE of the mean. Statistical significance is shown with different letters at $p<.05$.

the higher production of EPM, but the method of extraction should be optimized for better functionality. Additionally, different concentrations of phenolic compounds in the extracted fibers can have a direct effect on the proportion of SCFA, and very likely to the production of metabolites by the human gut microbiota. This effect possibly comes from the concentration and type of interaction between the phenolic compounds and the fiber (*i.e.* ADF).

4. Conclusion

The EAE of mango peels results in higher solubilization of formerly insoluble materials. All tested enzymes contributed to this effect, proving the former hypothesis. The resulting EAE extract is rich in soluble fibers (*e.g.*, EPM), with Viscozyme being the most effective for producing such fibers. The recovery of fibers from mango peels can help reduce the negative environmental impact of by-products from mango processing by upcycling it into dietary fiber ingredients for food applications, such as bakery or beverage fortification. However, the soluble fibers obtained via EAE exhibit a lower concentration of phenolic compounds and reduced antioxidant capacity compared to control, which diminishes the potential of the process to address the ADF production.

Our findings indicate that EAE can be a good alternative to produce soluble fibers from mango peels that promote SCFA production, suggesting a prebiotic effect. The findings also indicate that differences in phenolic concentration in the fibers can lead to different proportions of SCFA. A higher concentration of phenolic compounds led to increased propionic acid production by gut bacteria, suggesting that the phenolic content of the fibers can influence the resulting SCFA profile. This study opens new avenues for using EAE to extract soluble fibers from other food by-products. Production of fiber through EAE of mango peels could be advantageous due to its technological applications and efficiency, and it could be optimized to produce high amounts of soluble fibers with great concentrations of phenolic compounds.

Ethical statement

The ethical approval for studies in humans and animals was not required for the present research.

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CRediT authorship contribution statement

Arnau Vilas-Franquesa: Writing – review & editing, Writing – original draft, Supervision, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Sibilla Bensi:** Writing – original draft, Methodology, Investigation, Formal analysis, Data curation. **Shiqi Yang:** Writing – review & editing, Methodology, Investigation, Formal analysis. **Vincenzo Fogliano:** Writing – review & editing, Supervision, Resources, Project administration, Conceptualization.

Declaration of competing interest

The authors declare no conflicts of interest.

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Supplementary materials

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Data availability

The data has been published in a repository and the DOI link made available within the manuscript

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