

## Effects of soluble and insoluble fractions of fruit pomaces on short-chain fatty acid generation by fecal microbiota *in vitro*

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### ABSTRACT

This study investigated the dietary fiber composition, monosaccharide profile, and prebiotic potential of fruit pomaces (apple, apricot, peach, and grape). Insoluble fiber (IF) was predominant in all samples, with apple (74.37%) and grape pomace (74.06%) having the highest IF contents. The highest soluble fiber (SF) was detected in peach pomace (18.71%), while grape pomace had the lowest (6.55%). Monosaccharide analysis showed that glucose was the major sugar in both SF and IF fractions, followed by arabinose, galactose, mannose, xylose, and rhamnose. Fucose was not detected. Apricot and apple pomaces exhibited the highest levels of neutral sugars and uronic acids, suggesting a richer presence of pectic substances and hemicelluloses. Despite grape pomace having the highest phenolic content and antioxidant capacity, its low SF content limited SCFA (short-chain fatty acid) production during in-vitro fecal fermentation. In contrast, apple pomace—with a more balanced SF/IF profile and richer monosaccharide diversity—induced significantly higher acetate, propionate, and butyrate levels ( $P < 0.05$ ). These results highlight the importance of not just fiber quantity, but also solubility and sugar composition, in determining the prebiotic efficacy of fruit pomace fibers. Among the pomaces evaluated, apple pomace displayed compositional characteristics that may warrant further investigation for potential prebiotic applications.

**Keywords:** fruit pomace, dietary fiber, in vitro fermentation, short-chain fatty acids (SCFA), monosaccharide composition

### INTRODUCTION

The growing interest in novel prebiotic compounds—driven by consumer awareness and environmental concerns—has accelerated research into recovering bioactive ingredients from biological sources, particularly fruit pomaces (Rodriguez-Costa et al., 2018). Fruit and vegetable pomaces, rich in nutrients, are increasingly used as functional food components. These materials contain substances such as dietary fibers, phenolics, essential fatty acids, and minerals, making them valuable candidates for the development of sustainable food and feed applications or for use in biotechnological processes (Rangaraj et al., 2021; Granucci et al., 2023).

Recent studies have explored the enzymatic and microbial valorization of fruit pomace as a source of fermentable fiber and bioactive compounds (Fayaz et al., 2022). As a major output of juice and puree production, fruit pomace offers a cost-effective alternative for recovering non-digestible polysaccharides that can be subjected to microbial fermentation. Rather than disposal through conventional means, such valorization supports circular bioeconomy strategies by utilizing pomace as a resource (Karim et al., 2025).

Fermentation of dietary fibers by gut microbiota produces short-chain fatty acids (SCFAs), primarily acetate, propionate, and butyrate. These metabolites are frequently used as indicators of microbial fermentation activity *in vitro*, and their profiles can differ depending on the structure and origin of the fiber source (Davis et al., 2010; Yang et al., 2013). Fruit pomace fibers, particularly from apple, have been reported to exhibit high fermentability and a balanced ratio of soluble and insoluble components, which may influence their microbial degradability (Chen et al., 2022; Ge et al., 2022).

In Türkiye, fruits such as apple, grape, peach, and apricot are widely cultivated and processed into juice, generating large amounts of pomace. According to TÜİK (2017), these fruits account for the majority of juice production in the country. Apple pomace alone constitutes approximately 25% of the fresh fruit weight and contains sugars, carbohydrates, proteins, pectin, dietary fiber, and minerals (Kosseva, 2009). Similarly, grape pomace is rich in dietary fiber (cellulose, hemicellulose, pectin), while peach and apricot pomaces offer additional pectin-rich fiber and bioactive content (O'Shea et al., 2012; Coşkuner et al., 2025).

While most studies have examined whole pomace, emerging evidence suggests that soluble (SF) and insoluble (IF) fiber fractions may exhibit distinct fermentation characteristics. This study investigates the effects of soluble (SF) and insoluble (IF) dietary fiber fractions from apple, grape, peach, and apricot pomaces on microbial fermentation activity through SCFA production in an *in vitro* colon model, offering compositional and functional insights into their fermentability.

This study evaluates the fermentation potential of dietary fiber fractions from apple, grape, peach, and apricot pomaces through SCFA production in an *in vitro* colon model. While SCFA levels provide an indicator of microbial fermentation activity, this work does not extend to assessing microbiota composition or direct health outcomes. Future studies are warranted to investigate the effects of these fiber fractions on gut microbiota

composition and host health *in vivo*. The results contribute to understanding the fermentability of pomace-derived fibers and support their potential valorization in food, feed, or biotechnological applications.

## MATERIAL AND METHODS

### *Substrate preparation*

Apricot (*Prunus armeniaca*), peach (*Prunus Persica*), grape (*Vitis vinifera*), and apple (*Malus domestica*) pomaces used in this study were obtained from the Dimes Fruit Juice Factory in Tokat, Türkiye. Peach and apricot pomaces were collected in July 2019, while grape and apple pomaces were collected in September 2019. The pomace samples were dried in a vacuum oven at 55 °C and 110 mbar pressure until a constant weight was achieved. After drying, the samples were ground using a coffee grinder to reduce particle size and stored in sealed bags at -18 °C until analysis.

### *Proximate analysis of fruit pomaces*

The weight loss, expressed as a percentage, resulting from holding dried fruit pomace samples (3–5 g) at 105 °C until a constant weight was achieved, was calculated according to Helrich (1990). Nitrogen content was determined by the Dumas method, and protein content was calculated by multiplying it by 6.25, following Helrich (1990). Total fat content was determined using the Soxhlet method and expressed as a percentage of dry matter, in accordance with Helrich (1990). Ash content was determined by burning 3–5 g of sample at 550 °C until no brown spots remained, and the percentage was calculated based on weight loss, as outlined in Helrich (1990). For pH analysis, 3 g of dried pomace was mixed with 50 mL of distilled water and vortexed for 2 min. The filtrate obtained through filter paper was used to measure pH with a pH meter. Titratable acidity was determined by titration with 0.1 N sodium hydroxide until pH 8.2 was reached. The acidity was calculated as a percentage based on the predominant organic acid in each fruit: malic acid for apple, citric acid for apricot and peach, and tartaric acid for grape.

### **Total phenolic content and antiradical scavenging activity of fruit pomaces**

For sample extraction, 1 g of each sample was mixed with 50 mL acidified methanol (0.1% HCl) and homogenized using an Ultra-Turrax for 30 min ( $2 \times 15$  min) in an ice bath ( $\sim 4$  °C) (Rockenbach et al., 2011). The extract was then filtered under vacuum through Whatman No.1 filter paper for use in total phenolic content and antiradical activity analyses. Total phenolic content was determined by measuring the color developed by phenolic compounds in an alkaline environment with Folin-Ciocalteu reagent using spectrophotometry, following the method previously applied in fruit sample analysis. Calculations were based on a standard curve of gallic acid, and results were expressed as mg of gallic acid equivalents (mg GAE/g).

Antiradical scavenging activity was assessed using the DPPH (2,2-diphenyl-1-picrylhydrazyl) method, as described in previous fruit pomace analyses (Demirkol and Tarakci, 2018). Diluted extracts were incubated with DPPH-methanol solution ( $10^{-3}$  mol/L) in the dark for 30 min, and absorbance was measured at 515 nm. Antioxidant capacity was expressed as  $\mu\text{g}$  of Trolox equivalent per mg of dry matter ( $\mu\text{g TE}/\text{mg DM}$ ). Each sample was analyzed in triplicate.

### **The extraction of IDF (Insoluble Fraction) and SDF (Soluble Fraction) from fruit pomaces**

Dietary fibers were extracted from fruit pomaces using an *in vitro* upper gastrointestinal digestion protocol described by Tuncil et al. (2018), which includes the removal of proteins and fats from dried samples. Initially, dried samples were defatted with hexane using a Soxhlet apparatus. Then, 12 g of the defatted sample was mixed with 84 mL phosphate buffer solution and adjusted to 37 °C. The pH was lowered to  $2.5 \pm 0.2$  using 6M HCl, and 2 mL of pepsin solution was added per 12 g sample. Samples were incubated at 37 °C for 30 min with continuous stirring. Following pepsin digestion, the pH was adjusted to  $6.9 \pm 0.2$  using 6M NaOH, and 4 mL of pancreatin solution was added per 12 g sample. Incubation continued at 37 °C for 90 min under

continuous stirring. After digestion, samples were heated to  $85 \pm 5$  °C for 20 min to deactivate enzymes. Insoluble dietary fibers were separated by centrifugation, while soluble fibers were isolated via ethanol precipitation. The centrifuged insoluble fractions were dried at  $60 \pm 5$  °C for 4 hours, then ground using a coffee grinder. The ethanol-precipitated soluble fractions were filtered, and the residue on the filter paper was also dried under the same conditions.

### **Neutral sugar composition**

Monosaccharide compositions of soluble and insoluble fractions were quantitatively determined using gas chromatography (GC; Shimadzu GC-2030) equipped with an SP2330 column, following the alditol acetate derivatization method described by Pettolino et al. (2012). Detailed GC operating conditions, including injector and column temperatures and run times, were adopted from Tuncil et al. (2016).

Samples were injected using an autosampler (Shimadzu AOC-20i) into a gas chromatograph fitted with a Restek Rtx-2330 column (30 m length, 0.25 mm inner diameter, 0.2  $\mu\text{m}$  film thickness) and a flame ionization detector (GC-FID 7890A, Agilent Technologies, Inc., Santa Clara, CA). The GC parameters were as follows: injector temperature 240 °C; injection volume 1  $\mu\text{L}$ ; split ratio 1:10; column flow rate 1.14 mL/min; carrier gas helium. initial column temperature, 160 °C; column temperature program, with a gradient as follows: gradient 1, 160 °C, 7.15 min; gradient 2, slope 4.00 °C/min, 220 °C, 4.10 min; gradient 3, slope 2.90 °C/min, 240 °C, 5.15 min; gradient 4, slope 10.80 °C/min, 260 °C, 5.10 min. Pure sugar standards—rhamnose, xylose, arabinose, fucose, mannose, galactose, and glucose—were subjected to alditol acetate derivatization prior to analysis. Each sample was analyzed in duplicate.

### **Determination of uronic acid (Acidic monosaccharides)**

The determination of acidic monosaccharides (galacturonic acid and glucuronic acid) in the soluble and insoluble fractions was performed using the AACC 32-25.01 uronic acid method (Uppsala method) as described by Theander et al. (1995).

Briefly, 50 mg of each dietary fiber fraction was treated with 0.3 mL of 12 M sulfuric acid at 30 °C for 1 hour. Then, 8.4 mL of distilled water was added, and the mixture was autoclaved at 125 °C for 1 h. After autoclaving, 100 µL of the filtrate was mixed with 100 µL of sodium chloride/boric acid solution, followed by the addition of 1.6 mL of 18 M sulfuric acid. The mixture was incubated at 70 °C for 40 min. Subsequently, 80 µL of 3,5-dimethylphenol solution was added, and absorbance was measured at 400 nm and 450 nm using a spectrophotometer.

A galacturonic acid monohydrate standard solution was used to construct the calibration curve, and acidic monosaccharide content was expressed as a percentage by weight. Each sample was analyzed in duplicate.

### ***In vitro* fermentation**

The prebiotic potential of dietary fibers from dried and powdered fruit pomaces was evaluated *in vitro* using the method described by Sayar et al. (2007). Fecal samples were collected from three healthy individuals (two females aged 30 and 36, and one male aged 35) who had not used antibiotics for at least three months. All participants followed an omnivorous diet, which was considered appropriate to ensure a representative fecal inoculum for SCFA production in the *in vitro* colon model.

50 mg of dietary fiber were placed in fermentation tubes and incubated with 4 mL of anaerobic medium (BHI broth without dextrose, distilled water, Na<sub>2</sub>CO<sub>3</sub>, L-cysteine hydrochloride, and resazurin) at 0, 6, 12, and 24 h. Fecal samples, homogenized in BHI broth (1:3 w/v) and filtered through cheesecloth, were pooled and 1 mL was added to each tube. Tubes were sealed and incubated anaerobically (80% N<sub>2</sub>, 20% CO<sub>2</sub>) at 37 °C and 120 rpm for 24 h.

SCFA analysis was conducted on 1 mL aliquots, with lactulose serving as a positive control. Ethical approval for this study was obtained from the Ordu University Clinical Research Ethics Committee (code: 2019/115).

### ***SCFA measurements***

SCFAs were isolated from fecal samples following Tuncil et al. (2017) and analyzed by gas chromatography (Shimadzu GC-2030) to quantify acetic, propionic, and butyric acid concentrations. Samples were mixed with 100 µL of an internal standard solution—prepared from 157.5 µL 4-methylvaleric acid, 1.47 mL 85% phosphoric acid, 39 mg copper sulfate pentahydrate, and distilled water up to 25 mL—and stored at -20 °C until analysis.

Before analysis, frozen samples were thawed at room temperature and centrifuged at 13,000 rpm for 10 min. GC analysis was performed using a Stabilwax-DA fused silica capillary column with the following conditions: injector temperature 230 °C; injection volume 3 µL; split ratio 1:50; oven temperature held at 100 °C for 1 minute, then increased at 8 °C/min to 200 °C and held for 5.5 min; flame ionization detector temperature 250 °C. SCFA concentrations were quantified using 4-methylvaleric acid as an internal standard and expressed in millimoles per liter (mM).

### ***Statistical analysis***

The data were statistically analyzed using analysis of variance (ANOVA) with the SPSS 20 software package. Tukey's post-hoc test was employed to identify significant differences between groups, with a significance level of  $\alpha = 0.05$ . Determinations were carried out in duplicate.

## **RESULTS AND DISCUSSION**

### ***Physicochemical properties of dried fruit pomaces***

The physicochemical properties of the fruit pomaces dried in a vacuum oven are given in Table 1. Dry matter content values varied between 88.85% and 93.64%. The highest dry matter content was found in apple and grape pomace, and the lowest dry matter content was found in peach pomace. Ash content varied between 1.29% and 3.62%, with the highest ash content in apricot and peach pomace and the lowest ash content in apple pomace.

Seker et al. (2009) reported moisture contents of 5.2% and 5.1%, and ash contents of 3.08% and 3.23% for apple and apricot powders, respectively, which are in agreement with the values observed in the present study. Similarly, González-Centeno et al. (2010) found ash values in grapes comparable to those observed in this study. Pagan and Ibarz (1999) reported an ash content of 3% for fresh peach pomace, while Kasapoğlu et al. (2020) showed ash values for apricot pomace ranging from 0.60% to 5.34%. These literature data are consistent with the results obtained in the current study.

The oil content of fruit pomaces varied between 0.85% and 3.02%, with apricot pomace containing the highest fat and peach pomace the lowest. Protein concentrations varied between 3.63% and 8.77%, where apricot pomace had the least and apple pomace the highest protein content.

Deng et al. (2011) reported fat contents ranging from 1.14% to 6.33% across five grape varieties, while Karnopp et al. (2017) found protein and fat contents of 11.44% and 7.69% in grape pomace flour, respectively. Fatima et al. (2018) reported typical protein and fat ratios in apricot fruit flesh as 1.4–2.0% and 0.4–0.6%, respectively, noting that the flesh contains low amounts of proteins and lipids, whereas apricot kernels are rich in both (20–30% protein and 40–52% fat) (Alpaslan and Hayta, 2006).

Another study indicated fat and protein contents in apricot pomace varying from 0.2% to 3.12% and 1.06% to 4.87%, respectively (Kasapoğlu et al., 2020), these values are in agreement with those determined in this study. Pagan and Ibarz (1999) reported protein and fat values of 7.5% and less than 3%, respectively, for fresh peach pomace. Figuerola et al. (2005) found protein contents of 3.12 and 8.42 g/100 g dry matter in apple and grape pomace, respectively.

The titratable acidity values of the pomaces showed that apple pomace had the lowest acidity (1.11%), while apricot pomace had the highest (4.76%). Apricot pomace pH values have been reported between 2.56 and 5.63 (Kasapoğlu et al., 2020). There was no significant difference in titratable acidity between apricot and peach pomaces ( $P>0.05$ ). As shown in Table 1, apple and grape pomaces had similar pH values, followed by peach pomace, with apricot pomace exhibiting the lowest pH.

Total phenolic contents (TPC) of extracts from powdered pomaces ranged from 284.3 to 4574.84 mg GAE/100 g dry matter, while antiradical activity values ranged between 2.86 and 33.49  $\mu\text{g TE/mg}$ . Grape pomace exhibited the highest TPC and antioxidant capacity ( $P<0.05$ ), with no significant differences among the other pomaces ( $P>0.05$ ).

**Table 1.** Proximate compositions of dried fruit pomaces

Components	Content (% , dry weight basis)*			
	Apple pomace	Apricot pomace	Peach pomace	Grape pomace
DM (%)	93.64 $\pm$ 0.03 <sup>A</sup>	92.63 $\pm$ 0.18 <sup>B</sup>	88.85 $\pm$ 0.2 <sup>C</sup>	93.55 $\pm$ 0.09 <sup>A</sup>
ash (%)	1.29 $\pm$ 0.13 <sup>C</sup>	3.35 $\pm$ 0.00 <sup>A</sup>	3.62 $\pm$ 0.05 <sup>A</sup>	2.63 $\pm$ 0.09 <sup>B</sup>
oil (%)	2.61 $\pm$ 0.10 <sup>B</sup>	3.02 $\pm$ 0.12 <sup>A</sup>	0.85 $\pm$ 0.06 <sup>C</sup>	2.85 $\pm$ 0.08 <sup>A</sup> <sup>B</sup>
protein (%)	8.77 $\pm$ 0.04 <sup>A</sup>	3.63 $\pm$ 0.01 <sup>D</sup>	4.62 $\pm$ 0.05 <sup>C</sup>	7.20 $\pm$ 0.05 <sup>B</sup>
TA (%)	1.11 $\pm$ 0.08 <sup>B</sup>	4.76 $\pm$ 0.15 <sup>A</sup>	4.00 $\pm$ 0.45 <sup>A</sup>	1.84 $\pm$ 0.17 <sup>B</sup>
pH	3.93 $\pm$ 0.00 <sup>A</sup>	3.75 $\pm$ 0.00 <sup>C</sup>	3.81 $\pm$ 0.00 <sup>B</sup>	3.93 $\pm$ 0.02 <sup>A</sup>
DPPH ( $\mu\text{g TE/mg}$ )	3.09 $\pm$ 0.08 <sup>B</sup>	3.55 $\pm$ 0.21 <sup>B</sup>	2.86 $\pm$ 1.07 <sup>B</sup>	33.49 $\pm$ 2.34 <sup>A</sup>
TPC (mg GAE/100g DM)	345.69 $\pm$ 7.73 <sup>B</sup>	464.31 $\pm$ 4.91 <sup>B</sup>	284.3 $\pm$ 12.56 <sup>B</sup>	4577.84 $\pm$ 313.12 <sup>A</sup>

<sup>A-D</sup> Values with different letters in the same row differ from one another at  $P<0.05$  level. Abbreviations: DM: Dry matter; TA: Titratable acidity; TPC: Total phenolic content.

Wang et al. (2019) reported an apple pomace powder TPC of 289.1 mg GAE/100 g, whereas Cerda-Tapia et al. (2015) found values of 275.44 and 150.72 mg GAE/100 g in different apple pomace samples, all lower than the 345.69 mg GAE/100 g measured in this study. Karnopp et al. (2017) reported a grape pomace TPC of 2179.68 mg GAE/100 g. De Moraes Crizel et al. (2013) found TPC values of 118.66 mg/g and 124.97 mg/g in orange pomace and peel dietary fibers, respectively. Variations in TPC across studies may result from differences in extraction methods, plant materials, solvents, and temperatures (Li et al., 2006).

### Dietary fibre content and composition

The amounts of water-soluble and insoluble fractions in fruit pomaces are presented in Table 2. Among the pomaces, the soluble fraction (SF) content ranged from 6.55% in grape to 18.71% in peach pomace, while the insoluble fraction (IF) varied more widely, with the highest value in apple pomace (74.37%) and the lowest in peach pomace (42.74%). These values indicate that although peach pomace had the highest soluble fraction, it had the lowest insoluble fraction, resulting in a lower overall fiber contribution compared to apple and grape pomaces. In all pomaces, IF content was higher than SF. Peach pomace had the highest SF, whereas grape pomace had the lowest. Conversely, the highest IF levels were observed in grape and apple pomaces, while peach pomace had the lowest IF content.

The considerable amounts of both fiber fractions indicate that fruit pomaces are structurally rich in fermentable and non-fermentable polysaccharides. Soluble and insoluble fractions are known to differ in their physicochemical properties, which may influence their behavior during fermentation and gastrointestinal transit (Grigelmo-Miguel et al., 1999).

Cerda-Tapia et al. (2015) reported that apple pomace contains 70.9% total dietary fiber, with 59.8% insoluble and 11.1% soluble fiber. Similarly, Renard and Thibault (1991) found apple pomace fiber content to be 88.5%, consisting of 69.9% insoluble and 18.6% soluble fiber. Llobera and Cañellas (2007) reported that "Manto

Negro" red grape pomace has 77.27% total dietary fiber, predominantly insoluble (73.5%) compared to soluble (3.77%).

Grigelmo-Miguel et al. (1999) showed that 'Sudanell' peach contains 30.7–36.1% total dietary fiber, with 23.8% insoluble and 12.3% soluble fiber. Apricot pomace, a by-product of apricot nectar processing, was reported to have 7.82% total dietary fiber (Kang et al., 1999). Adil et al. (2007) found that peach peel has higher phenolic content than peach flesh, with a total phenolic content of 84.07 mg GAE/100 g fresh fruit.

**Table 2.** Soluble and insoluble fractions of dried fruit pomaces

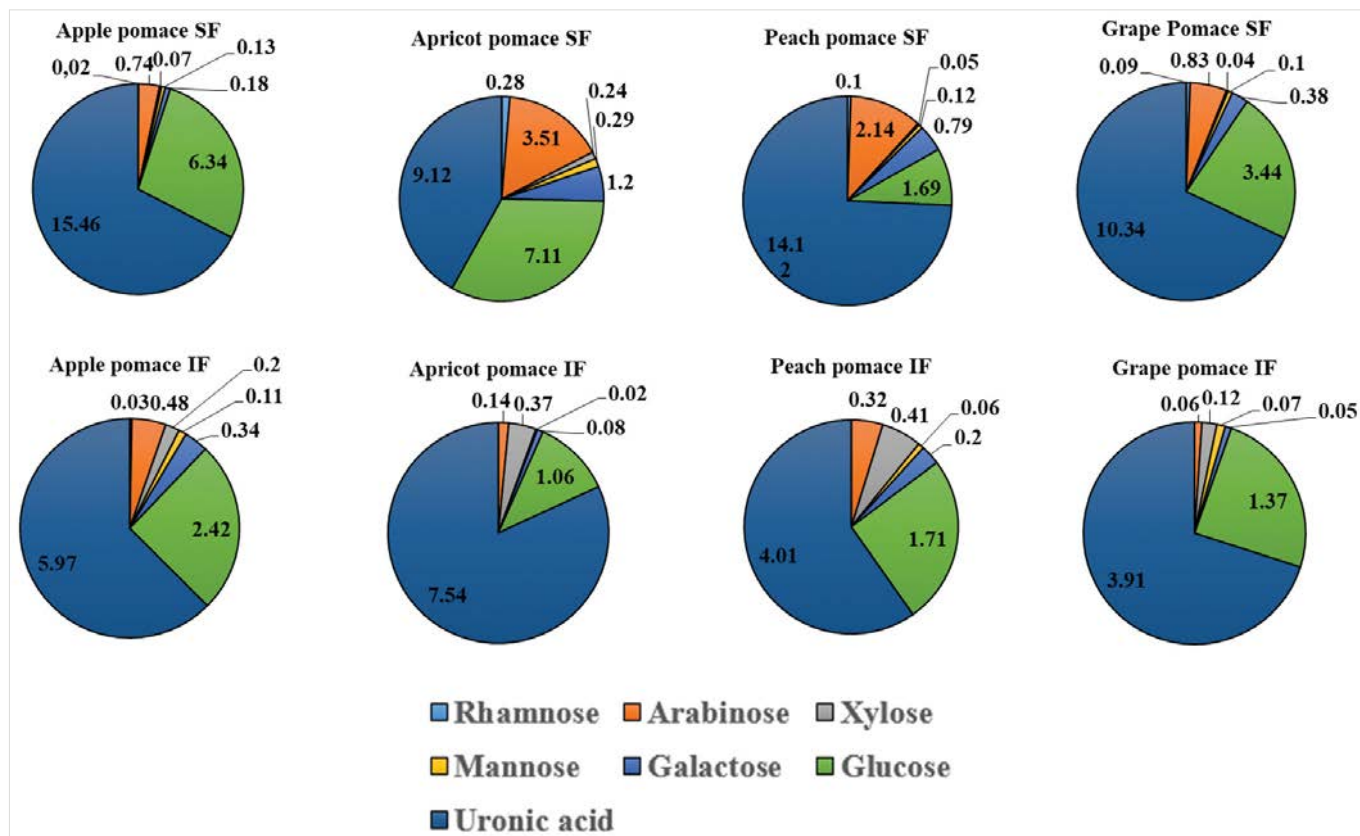
Components	Content (% dry weight basis)			
	Apple pomace	Apricot pomace	Peach pomace	Grape pomace
SF	14.98	16.60	18.71	6.55
IF	74.37	52.22	42.74	74.06

Abbreviations: SF: Soluble fraction; IF: Insoluble fraction

According to Larrauri (1999), foods containing more than 50 g of fiber per 100 g are classified as good dietary fiber sources. Variations in fiber content may arise from differences in plant varieties, species, ripening stages, and extraction methods.

Figure 1 shows the monosaccharide composition and the amount of uronic acid content in the water-soluble and insoluble fractions from fruit pomaces. Understanding these profiles is important for evaluating how structural features of dietary fibers influence microbial fermentation and SCFA production, as supported by previous studies (Wang et al., 2015; Zheng et al., 2018; Tang et al., 2024).

Monosaccharide composition varied among the soluble (SF) and insoluble (IF) fractions of the four pomaces. Glucose was the predominant monosaccharide in SF fractions, especially in apricot and apple, while peach had the lowest glucose level. Arabinose and galactose were significantly higher in apricot pomace ( $P < 0.05$ ). Xylose and mannose were moderately present with minimal variation. Fucose was not detected in any sample.



Abbreviations: SF: Soluble fraction; IF: Insoluble fraction

**Figure 1.** Monosaccharide composition (expressed as percentage of total monosaccharides) of SF and IF fractions obtained from fruit pomaces (100 mg sample)

Glucose and xylose are major components of plant cell wall polysaccharides such as cellulose and hemicelluloses (e.g., xyloglucans, heteromannans,  $\beta$ -glucans), likely explaining their presence in pomace samples. Similar monosaccharide profiles have been reported in other fruit by-products, such as citrus peel and dragon fruit pectin (Coşkuner et al., 2025; Wang et al., 2015; Muhammad et al., 2014).

In the hydrolyzed insoluble fiber (IF) fraction, glucose was again the most abundant monosaccharide, followed by arabinose, galactose, mannose, rhamnose, and xylose, with no significant differences among pomace types. The presence of rhamnose, arabinose, galactose, and uronic acid indicates pectic polysaccharides integrated into the cell wall matrix (de Souza et al., 2019).

Overall, SF fractions were richer in soluble pectic polysaccharides such as arabinogalactans and accessible glucans, while IF fractions predominantly consisted

of structural carbohydrates like cellulose and tightly bound hemicelluloses. This structural heterogeneity likely influences their physicochemical properties and fermentability (Carlsen and Pajari, 2023).

Figure 1 shows that acid hydrolysis released higher levels of uronic acid (UA) from the soluble fiber (SF) fractions compared to the insoluble ones (IF). Among the samples, apple and peach pomaces exhibited the highest UA levels, whereas apricot and grape had lower values. In the IF fractions, apricot and apple showed greater UA content than peach and grape.

Uronic acids—primarily galacturonic (GalA) and glucuronic (GlcA) acids—are key indicators of pectic polysaccharide content within the fiber matrix. Their distribution varied considerably across pomace types and fiber fractions. High UA levels in the SF fractions of apple and peach suggest a greater abundance of soluble pectins. Conversely, the high UA content in apricot's IF

fraction points to a significant presence of tightly bound or less extractable pectin components.

These findings indicate that uronic acids are differentially partitioned between soluble and insoluble matrices depending on fruit type. Soluble fractions tend to contain more accessible pectins, while certain pomaces—such as apricot—retain substantial amounts in the insoluble matrix. This distribution likely affects hydration capacity, gel-forming ability, and fermentability of the fibers, which are critical for their functional and prebiotic properties (Carlsen and Pajari, 2023; Karim et al., 2024).

Supporting literature shows similar trends. Dou et al. (2015) reported that glucuronic and galacturonic acids in apple-derived polysaccharides comprised 2.51–7.47% of the total sugar content, while pectin represents about 10–15% of apple pomace dry matter (Wang et al., 2007). The detection of uronic acids in the IF fractions underscores the integration of pectic substances within the cell wall matrix, where they may resist solubilization.

Overall, the structural features of dietary fibers—including solubility, degree of polymerization, and branching—play key roles in determining their fermentability and effects on microbial fermentation. Differences in uronic acid and monosaccharide composition among pomaces reflect underlying variations in cell wall polysaccharide architecture, ultimately shaping their functional properties (Coste et al., 2015).

#### ***Production of colonic microbial metabolites (SCFA) from fruit pomace dietary fibres during in-vitro fecal fermentation***

SCFA production (acetate, butyrate, propionate) from dietary fiber fractions obtained from fruit pomaces is presented in Figures 2 and 3. Significant and gradual increases in acetate, butyrate, and propionate concentrations were observed for all dietary fibers during microbial fermentation ( $P < 0.05$ ). Population studies indicate that fecal SCFA production typically follows the molar ratio acetate > propionate > butyrate, approximately 60:20:20 (Cummings et al., 1979). Despite potential

variations due to diet, this ratio is generally stable. Similarly, in this study, SCFA formation followed the order acetate > propionate > butyrate, with molar ratios around 65:20:15.

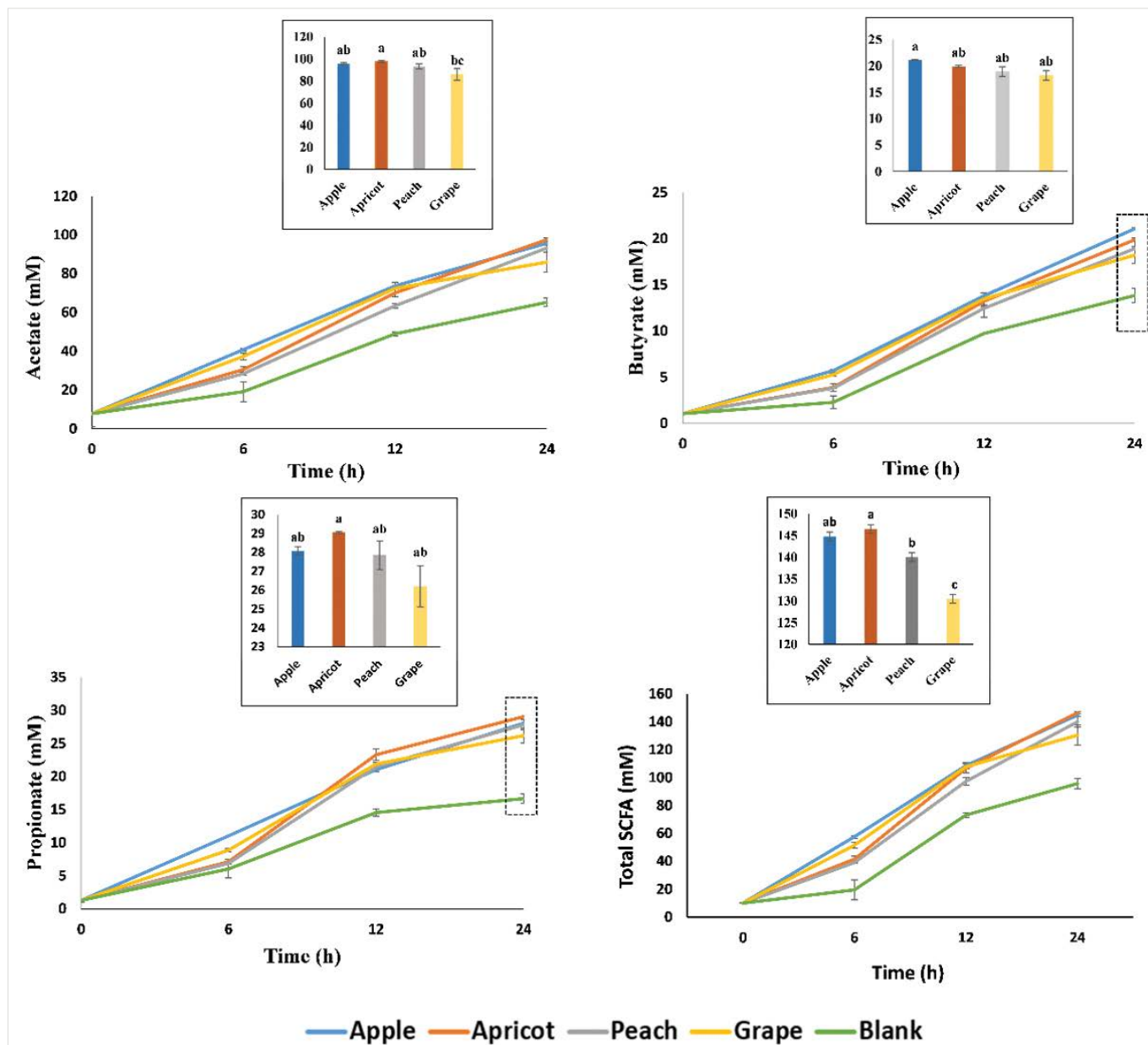
At 6 hours of fermentation, after lactulose, the soluble fractions (SF) of grape and apple pomaces produced higher acetate levels compared to apricot and peach, while the negative control had the lowest acetate. The most pronounced increases in acetate production occurred between 6 and 12 hours. SF from apple pomace generated the highest acetate levels.

Butyrate concentrations also increased significantly during fermentation across all dietary fibers ( $P < 0.001$ ), with apple pomace SF and insoluble fractions (IF) showing the highest production at 6 hours.

Propionate production peaked in apple SF at 6 hours, whereas apricot, peach, and grape pomaces produced similar but lower amounts. No significant differences in propionate levels among fiber types were found at the end of fermentation ( $P > 0.05$ ).

These findings highlight that microbial fermentation activity is strongly influenced by the type of substrate reaching the colon. Different dietary fibers can alter microbial metabolic outputs, including SCFA profiles, depending on their structural properties (de Souza et al., 2019). SCFA production is commonly used as an indicator of microbial fermentation; these metabolites are associated with maintaining intestinal barrier function and microbial ecosystem balance in model systems (Cui et al., 2019).

In this study, grape pomace exhibited the highest phenolic content and antioxidant capacity, while other pomace types showed similar values. Since antioxidant phytochemicals can be bound to dietary fiber matrices, they may influence microbial fermentation dynamics. Although soluble fiber (SF) is generally more readily fermented than insoluble fiber (IF), SCFA production also depends on fiber composition and physicochemical characteristics. Grape pomace, despite its high antioxidant content, resulted in lower SCFA levels, likely due to its low soluble fiber content (Koh et al., 2016; Herrera-Cazares



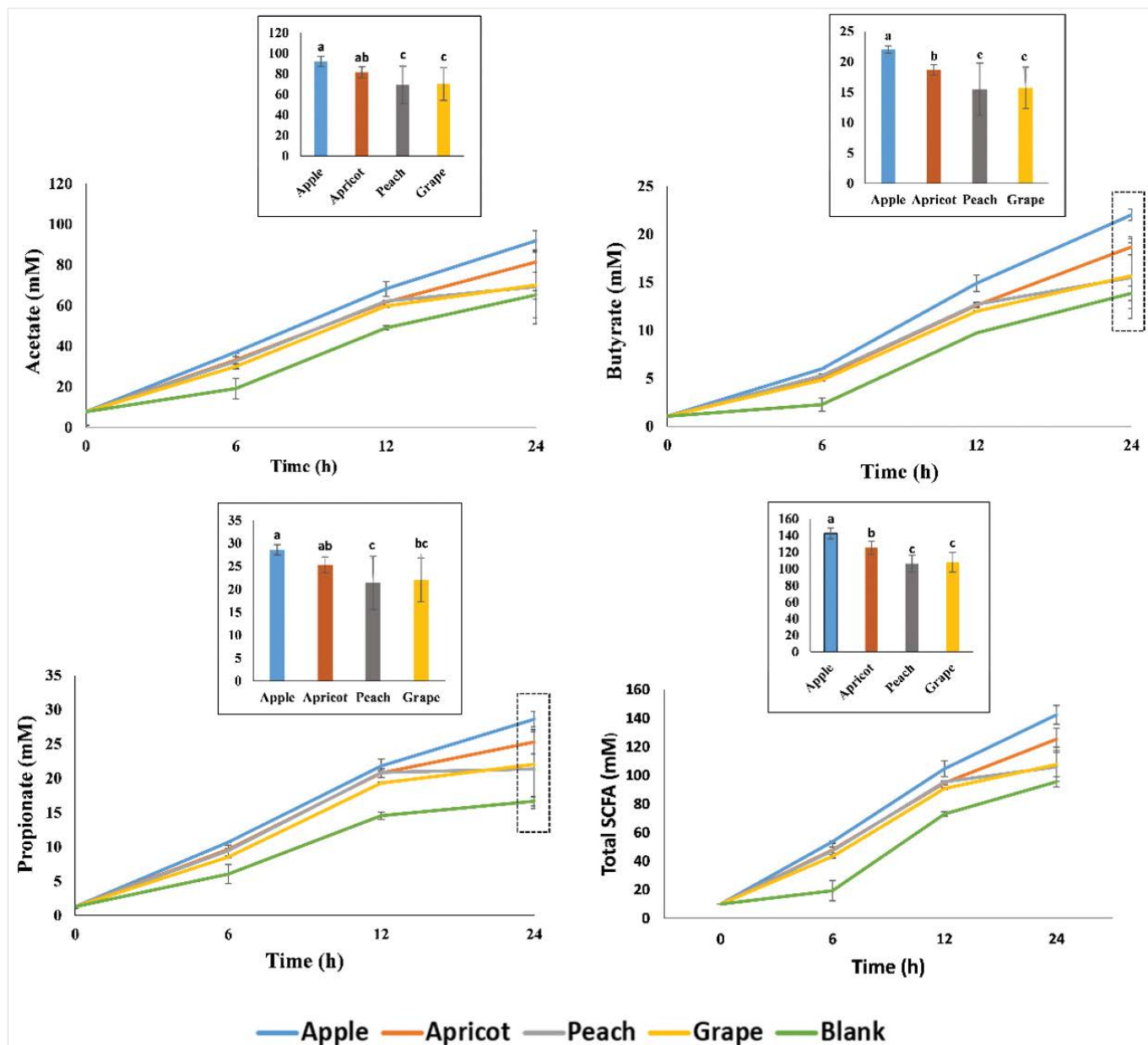
**Figure 2.** Short-chain fatty acid (SCFA) formation from fruit pomace SF during a 24-hour colonic *in-vitro* fecal fermentation. Bar graphs indicate the concentrations of SCFAs (acetate, propionate, and butyrate), expressed in millimoles per liter (mM), which are combined to provide the overall SCFA value. Tukey's test ( $P < 0.05$ ) shows that bars with the same letters are not significantly different, while bars with different letters indicate statistically significant differences. SF: Soluble fraction

et al., 2019). These observations suggest that SCFA production is influenced by multiple substrate factors beyond fiber solubility alone.

Various components in fruit pomace—such as pectin, hemicellulose, oligosaccharides, insoluble fibers, and polyphenols—have been shown to modulate microbial fermentation *in vitro*. These compounds may contribute to increased SCFA production and support microbial

stability under controlled conditions. The fermentability of fruit pomace components highlights their potential as functional ingredients in non-digestible carbohydrate research.

Moreover, the source and composition of dietary fiber are key factors influencing fermentation kinetics and fiber behavior during gastrointestinal passage (Cui et al., 2019; Xu et al., 2022).



**Figure 3.** Short-chain fatty acid (SCFA) formation from fruit pomace IF during colonic *in-vitro* fecal fermentation. Bar graphs indicate the concentrations of SCFAs (acetate, propionate, and butyrate), expressed in millimoles per liter (mM), which are combined to provide the overall SCFA value. Tukey's test ( $P < 0.05$ ) shows that bars with the same letters are not significantly different, while bars with different letters indicate statistically significant differences. IF: Insoluble fraction

## CONCLUSION

This study provides a comparative assessment of apple, apricot, peach, and grape pomaces, focusing on their physicochemical characteristics and SCFA production during *in vitro* fecal fermentation. All pomaces demonstrated high dietary fiber contents, with varying ratios of soluble and insoluble fractions that influenced their metabolic outputs as a result of fecal fermentation. Glucose and uronic acid were among the dominant

monosaccharide components, indicating a strong presence of structural polysaccharides such as cellulose and pectins. *In vitro* fermentation revealed significant SCFA production from all pomaces, particularly in the soluble fractions. Apple pomace exhibited the highest overall SCFA yield, while grape pomace—with high phenolic content but low soluble fiber—showed comparatively lower fermentability. These findings

underline the role of fiber composition and solubility in shaping fermentation outcomes. While this study did not directly assess gut microbiota composition or host physiological responses, the observed SCFA profiles highlight the potential functional value of fruit pomace fibers. Future work may focus on characterizing individual fiber components and evaluating their effects through *in vivo* or simulated digestion models.

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