

Harnessing apple pomace as a bioactive bread ingredient: Influence of exogenous soluble polysaccharides on water dynamics and structure-dependent polyphenol stability

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ABSTRACT

Apple pomace is composed predominantly of cell walls enriched with polyphenols. Its incorporation as a fortifying hydrocolloid in bread-making poses two principal challenges: its elevated water-binding capacity, which interferes with the hydration dynamics of gluten and starch, thereby impairing dough structure and bread quality; and the susceptibility of pomace-derived polyphenols to degradation under the thermal and oxidative stresses encountered during bread-making, potentially diminishing their bioactivity. This study investigates the chemical stability of pomace polyphenols (at subclass and compound levels via UPLC-ESI-QTOF-MS/MS) during bread-making and evaluates the efficacy of co-formulated soluble polysaccharides for modulating plasticizing water availability and protecting polyphenols against degradation. Wheat breads were prepared with 5 % pomace (W:POM), with and without the co-addition of psyllium (W:POM:PSY) or pectin (W:POM:P). W:POM showed lower volume, double the hardness and reduced proportion of available water compared to wheat bread (W). Adding psyllium or pectin to W:POM improved dough/crumb hydration, balanced water dynamics and resulted in similar volume and texture to W. Incorporating 5 % apple pomace into dough doubled the total polyphenol content, with bread-making primarily affecting dihydrochalcones and monomeric flavanols, while HBAs, HCAs, and oligomeric flavanols showed minimal changes. Significant variability in the degradation patterns of individual flavonols suggested that discrete polyphenol structures, rather than core subclass structures, influence their stability during baking. Psyllium and pectin generally prevented the loss of dihydrochalcones and flavanols, with pectin enhancing HBAs, HCAs, and oligomeric flavanol recovery. Hierarchical clustering indicated that polyphenol stability is influenced by both their chemical makeup and the molecular structure of the polysaccharides.

1. Introduction

While dietary fibre has traditionally been recognized for its positive metabolic effects – attributed to its inherent physical properties, such as increasing solution viscosity and providing insoluble particles, as well as to its fermentation by the gut microbiome (Grundy et al., 2016) – its role as a carrier of polyphenols has emerged as an additional key function. Notably, combinations of dietary fibre and polyphenols have demonstrated greater metabolic benefits than either compound alone (Aprikian

et al., 2003; Bechtner et al., 2025; Kristek et al., 2019). This function is particularly relevant for fruit and vegetable by-products, such as pomace, which primarily consist of plant cell walls and adsorbed cell contents such as polyphenols. Therefore, incorporating fruit and vegetable pomaces into staple foods like bread offers a promising strategy to enhance their nutritional value while simultaneously reducing waste and improving resource efficiency. This approach aligns with the zero-waste concept and circular economy principles (Gómez & Martínez, 2018) and supports the food use hierarchy, which prioritizes reuse

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over recovery and disposal (Teigiserova et al., 2020).

Global production of apple pomace, a major byproduct of the apple juice and cider industries, reached approximately 4 million tons in 2020 (Food and Agriculture Organization of the United Nations, 2020), accounting for 25–30 % of the processed apple weight (Antonic et al., 2020). Apple pomace represents a promising and sustainable source of plant cell walls impregnated with polyphenols (Arnous & Meyer, 2009; Lu & Foo, 1997; Sánchez-Rabaneda et al., 2004; Widaningrum et al., 2024). Apple pomace is particularly rich in dietary fibre, which accounts for 45–51 % of its dry weight. This fraction comprises cellulose (7–44 %), pectin (5–10 %), hemicellulose (4–25 %), and lignin (15–25 %) with ratios depending on the apple cultivar. The remaining components include simple sugars, lipids, proteins, and ash (Kausar et al., 2024; Lyu et al., 2020; Pascoalino et al., 2025). In terms of polyphenols, apples have been reported to contain 24 phenolic acids - six hydroxybenzoic acids (HBAs) and 18 hydroxycinnamic acids (HCAs) (Lopez-Rodulfo, Stentoft, & Martinez, 2025) - as well as 33 flavonoids, primarily flavanols, dihydrochalcones, and flavonols (Lopez-Rodulfo et al., 2024). The same authors reported that apple pomace retained 33 % of the total polyphenol content of the whole apple, equivalent to 23.14 ± 0.66 mg of polyphenols per 100 g of apple (fresh weight). Apple pomace was particularly rich in flavonols and flavanols (degree of polymerization 5–7), accounting for 92 % and 79 % of their total apple content, respectively (Lopez-Rodulfo et al., 2024). Hence, given the potential of apple pomace as a source of dietary fiber and different polyphenol subclasses, its incorporation into baked goods has been thoroughly studied during the past decades (Chen et al., 1988; Lu & Foo, 1997; Masoodi & Chauhan, 1998; Sánchez-Rabaneda et al., 2004; Vasantha Rupasinghe & Kean, 2008).

The successful incorporation of apple pomace, like other pomaces, into food products presents the major technological challenge in maintaining consumer acceptability. Similar to other insoluble fibres, pomace has a high water absorption capacity (~ 9 – 10 g of water per gram of dry matter, as reported by (Widaningrum et al., 2024), leading to competition with gluten and starch for available moisture. Consequently, incorporating pomace into bread formulations may result in insufficient hydration of these key structuring components, as observed with other insoluble fibers (Leys et al., 2020). As a result, the dough becomes stiffer and less extensible, which disrupts the formation of a strong and elastic gluten network - an essential factor for the structure and volume of baked goods (Hemdane et al., 2017). This aligns with reports of the detrimental effects of apple pomace inclusion in bread systems (Gumul et al., 2021; Masoodi et al., 2001).

The chemical instability of polyphenols is an additional limiting factor in the use of polyphenol-rich ingredients in processed food systems. Precisely, thermal and enzymatic stressors can degrade polyphenols, compromising their bioactive potential. Reported losses vary depending on processing intensity, with free phenolic reductions of 15–60 % during fruit juice pasteurization (Yang et al., 2019), and 60–90 % in more severe thermal treatments such as baking and extrusion (Abdel-Aal & Rabalski, 2013; Pico et al., 2019). During bread-making, polyphenols undergo multiple degradation pathways, including oxidation, epimerization, and polymerization (Song et al., 2015; Takahama & Hirota, 2000; Debelo et al., 2020). Despite the importance of polyphenol stability in real food systems, studies remain scarce. Most research has relied on isolated extracts, pure standards, or limited polyphenol profiling using traditional techniques such as High-Performance Liquid Chromatography with Diode-Array Detection (HPLC-DAD), and often focusing on aglycones (Sivam et al., 2011; Hayta et al., 2014; Szawara-Nowak et al., 2016; Guo et al., 2019; Lin and Zhou, 2018). In this context, a comprehensive omics-based approach could provide detailed insights into the stability of pomace polyphenols during bread-making at both individual and subgroup levels.

To address these challenges, we hypothesized that the co-integration of supportive polysaccharide ingredients with apple pomace could serve a dual function: mitigating the negative water dynamics provided by

pomace while providing a protective matrix against polyphenol degradation. On one hand, soluble polysaccharides such as psyllium have been shown to increase the water demand required for optimal dough hydration, leading to greater intergranular starch water content and enhanced gluten plasticization throughout the bread-making process (Franco et al., 2024). Similarly, the addition of pectin to wheat bread has been reported to improve physical properties, including increased loaf volume and a softer texture (Scappaticci et al., 2024). On the other hand, polysaccharides can interact with polyphenols through non-covalent mechanisms, primarily involving van der Waals forces, hydrogen bonding, and hydrophobic interactions. The extent of these interactions is influenced by the chemical composition and intrinsic solubility of both components (Liu, Le Bourvellec, & Renard, 2020). While these interactions may enhance the stability of polyphenols against thermal degradation, the underlying effects and mechanisms remain poorly understood, highlighting a significant gap in current knowledge. A deeper understanding of the molecular mechanisms governing polysaccharide-polyphenol interactions in real food systems subjected to hydrothermal processing could help bridge food processing with the metabolic potential of polyphenol-enriched baked goods. Ultimately, this knowledge could inform the development of new guidelines for designing healthier and more nutritious food products.

This study aims to investigate the chemical stability of apple pomace polyphenols during bread-making (from dough to bread) in a real bread system and explores the dual potential of exogenous soluble polysaccharides to counteract the negative water dynamics induced by pomace while providing a protective platform for polyphenol degradation. To achieve this, wheat breads were prepared with the integration of 5 % apple pomace, with and without the co-addition of two distinct soluble polysaccharides: psyllium husk (a mucilage composed of arabinoxylans with a comb-like, highly branched structure featuring a β -(1,4)-linked D-Xyl p backbone and side chains nearly exclusively made up of arabinose and xylose residues) and high methoxy pectin (a negatively charged polyuronide backbone that is partially methyl-esterified, with side chains mainly composed of L-arabinose and D-galactose). Using a non-targeted screening approach and semi-quantification of polyphenols via UPLC-ESI-QTOF-MS/MS, the stability of polyphenols in all formulations was assessed at both the subclass and individual polyphenol levels to understand the relationships between polyphenol structure and stability during the bread-making process, as well as the role of psyllium and pectin in stabilizing polyphenols. Bread moisture, proton relaxometry via Low Field-NMR, and texture were analysed to assess the impact of added ingredients on water dynamics and bread physical properties.

2. Materials and methods

2.1. Materials and chemicals

Wheat flour (14 % moisture, 0.55 % ash and 380 s falling number) was provided by Molinos del Duero - Carbajo Hermanos S.A. (Zamora, Spain). Psyllium seed husk powder (P95, Vitalcel®) was donated by J. Rettenmaier & Söhne GmbH & Co. KG (JRS, Rosenberg, Germany). This concentrate consists of 95 % pure milled psyllium husks, with an average particle size of 250 μ m and approximately 80 % dietary fibre content (Fratelli et al., 2018). Apple pectin was purchased from Sigma-Aldrich (St. Louis, MO, USA, ref 9000-69-5). This pectin ingredient has been reported to present a degree of esterification of 56.7 (Konrade et al. (2023)). Instant yeast (Dosu Maya Mayacilik A.S, Istanbul, Turkey), salt (Salina Salt, Mariager, Denmark), and tap water were used for bread-making. Jonagold apples (*Malus domestica* var. Jonagold) were purchased from Ørskov Frugt® (Denmark). Chemicals used for analysis, including acetonitrile (MeCN), methanol, and formic acid, were of LC-MS analytical grade (HiPerSolv CHROMANORM®) from VWR™ Chemicals (Søborg, Denmark). Polyphenol standards (+)-catechin, 5-caffeoylquinic acid, 4-hydroxybenzoic acid, (–)-epicatechin,

p-coumaric acid, trans-ferulic acid, quercetin 3-o-rhamnoside, and phloridzin dihydrate, were obtained from Sigma-Aldrich (St. Louis, MO, USA). Quercetin 3-glucoside was purchased from PhytoLab® (Vestenbergsgreuth, Germany). Additionally, Amicon Ultra-0.5 Centrifugal Filters with a 10 kDa MWCO were obtained from Merck (Darmstadt, Germany), and ultrapure water (18.2 MΩ) from MilliQ, Millipore (Darmstadt, Germany) was used for the experiments.

2.2. Preparation of apple pomace by conventional cold pressing

Jonagold apples (*Malus domestica* var. Jonagold), cultivated in Denmark and harvested in July 2023, were sourced in bulk from Ørskov Frugt® (Denmark), washed, and stored at -18°C for subsequent processing and analysis, to avoid polyphenol degradation during mechanical processing and storage (Lopez-Rodulfo et al., 2024). Frozen apple slices were subjected to cold pressing using an Enerpac® hydraulic pump model N° 808091 (Nidec Leroy-Somer, Angoulême, France) at a pressure of 5000 psi. The resulting pomace was immediately frozen at -70°C , followed by freeze-drying using a Delta 2–24 LSC plus freeze dryer (Martin Christ, Osterode am Harz, Germany), and subsequently ground into powder. All samples were stored in airtight, light-proof containers at -18°C until further analysis.

2.3. Bread-making

Breads were prepared using the straight dough method 10-10.03 (AACC International, 2015) with wheat flour, water (with a concentration adjusted to the absorption values calculated by a Farinograph device for each formulation, according to the AACC method 54-21 (AACC International, 2015), instant dry yeast (2 % flour basis, f.b.), and salt (1.8 % f.b.). A controlled methodological design including six different formulations was developed with different ratios of the solid ingredient according to previous studies (Correa et al., 2012; Franco et al., 2024; Franco & Gómez, 2022; Sivam et al., 2011): 100 % (f.b.) wheat bread (W), W with 5 % wheat flour replaced by psyllium powder (W:PSY) or by apple pectin (W:P), and W, PSY or P with the addition of 5 % pomace powder (W:POM), labelled as W + POM, W:POM:PSY and W:POM:P, respectively. The inclusion level of apple pomace was selected based on the negative impact observed at additions greater than 5 % (de Eguino et al., 2025). The water temperature for all preparations was adjusted to achieve a final dough temperature of 25°C . All ingredients were initially mixed using a spiral beater in a Kitchen Aid 5KSM150 mixer (Kitchen Aid, Michigan, USA) for a duration of 2 min at speed 2 (Franco & Gómez, 2022). Subsequently, water was added, and the mixture was kneaded for 13 min at speed 3. The dough was then divided into 150 g portions, rounded and let it rest for 10 min. Subsequently, it was shaped into loaves and placed into aluminium moulds ($232 \times 60 \times 108$ mm). After fermenting at 30°C and 75 % relative humidity for 60 min, the loaves were baked at 215°C for 15 min in an electric convection oven. After baking, breads were demoulded, allowed to cool for 2 h (until crumb temperature reached 20°C), and immediately analysed for texture, moisture content and proton relaxometry or sealed in polyethylene bags at room temperature, frozen (-70°C), freeze-dried, grinded and storage at -20°C for polyphenol analyses.

2.4. Crumb texture, moisture content and water partitioning

Texture, moisture content and water partitioning of bread crumb were measured 2 h after baking. Crumb texture was measured using a TA-XT2 texture analyser (Stable Microsystems, Surrey, UK) equipped with the “Texture Expert” software. A 25 mm diameter cylindrical aluminium probe was used in a “Texture Profile Analysis” (TPA) double-compression test to penetrate up to 50 % of the sample depth at a test speed of 60 mm/s, with a 30 s delay between the two compressions. Hardness, cohesiveness, and resilience were calculated from the TPA curve. Texture analyses were performed on two cylindrical samples,

each measuring 25 mm in width and 15 mm in diameter, taken from the central slices of at least two different loaves per batch, with measurements performed in triplicate across two independent production batches. Moisture content was analysed in 30 mm crumb cylinders obtained from the central slice of one loaf per bread according to approved method 44–15.02 (AACC International, 2015). Analyses were conducted in duplicate across two different production batches. The water distribution of doughs and breads was investigated by the measurement of the transverse relaxation time (T_2) in a Bruker minispec mq20 NMR analyzer (Bruker Optik GmbH, Ettlingen, Germany) with a magnetic field strength of 0.47 T and a resonance frequency for the protons of 20 MHz. The samples (approx. 2 cm of sample carefully packed) were placed into 18 mm diameter glass tubes and inserted into the NMR probe. The spin-spin relaxation time, T_2 , was measured using the Carr-Purcell-Meiboom-Gill pulse sequence (CPMG). The time delay between the 90° pulse and the 180° pulse (τ value) was 300 μs . Data from 6000 echoes were obtained in 16 scan repetitions. All relaxation measurements were performed at 20°C . The CONTIN fit procedure was used to determine the water mobility distribution in each sample. Doughs were also measured for water partitioning following the same procedure. All low field-NMR (LF-NMR) experiments were performed by triplicate.

2.5. Extraction of polyphenols with acidified methanol

Polyphenol extracts from apple pomace, dough and bread were obtained using an adapted method from a previous study (Kammerer et al., 2004). Briefly, 3.15 mL of an ice-cold methanol-formic acid-water solution (80:1:19) and 0.8 mL of miliQ water were added to 50 mg of freeze-dried pomace or 1g of freeze-dried dough or bread samples and homogenised by vortexing. Samples were then agitated at 1000 rpm using an orbital shaker (VXR basic Vibrax, Ika®, Germany) for 2 h in a dark, cold room at 4°C , then subjected to centrifugation at 4800g at 4°C for 15 min (Megafuge ST4R Plus, Thermo Scientific, Germany). This extraction process was performed three times.

2.6. Untargeted screening for the semi-quantification of polyphenols

2.6.1. Ultra-high-performance liquid chromatography electrospray ionization quadrupole time-of-flight mass spectrometry (UHPLC-ESI-QTOF-MS/MS) analysis

Polyphenol profiling was conducted through ultra-high performance liquid chromatography (UHPLC) mass spectrometry according to our previous protocol (Lopez-Rodulfo et al., 2024). Prior to injection, polyphenol extracts were filtered using a 0.22 μm syringe filter (Phenomenex, Germany). Polyphenols were chromatographically separated employing an Agilent 1290 Infinity UHPLC system coupled to a to a 6560 electrospray-ionization quadrupole time-of-flight mass spectrometer UHPLC-ESI-QTOF-MS/MS (Agilent, Germany) and a Luna Omega C₁₈ column (100 $\times$ 2.1 mm, 1.6 μm particle size, Phenomenex, Germany) protected by a Phenyl precolumn (2.1 $\times$ 5 mm, 1.7 μm particle size; VanGuard, Waters, USA). Columns were maintained at 30°C and a linear elution gradient was applied, using a mobile phase consisting of 0.1 % of formic acid in water (A) and 0.1 % formic acid in acetonitrile (B) as follows: 5–10 % B, 0–5 min; 10–12 % B, 5–8 min; 12–15 % B, 8–10 min; 15 % B, 10–15 min; 15–20 % B, 15–18 min; 20–25 % B, 18–20 min; 25–55 % B, 20–23 min; 55–90 % B, 23–25 min; 90–5 % B, 25–30 min. The column was re-equilibrated between injections for 3 min under the initial mobile phase conditions. Flow rate was 300 $\mu\text{L}/\text{min}$, and the injection volume was 5 μL . The UHPLC system autosampler was thermostated at 10°C to prevent degradation reactions during analysis.

For comprehensive polyphenol identification, total ion spectra were collected in negative mode over the mass range of m/z 100–1500 at an acquisition rate of 3.0 spectra/s. A capillary voltage of 4 kV was applied. Subsequently, MS/MS analyses were executed in all-ion mode with varying collision energies (10, 20 and 30 eV) for fragmentation. The

ionization source was operating at 325 °C, using high purity nitrogen (99.998 %) at a pressure of 35 psi and at a flow rate of 12 L/min. Octupole RF and fragment of voltage were −750 V and 350 V, respectively.

Data collection and analysis were conducted using Agilent Mass Hunter Qualitative Software V. 10.0 (Agilent Technologies, USA). Compounds were identified by matching precise MS and MS/MS spectral data against the Personal Compound Data Base Library (PCDL), ensuring a score above 80 % and a mass error below 5 ppm. The MS spectral analysis and fragmentation patterns were validated in silico using ACD/Spectrus Processor software (ACD Labs, Toronto, Canada) and corroborated with fragmentation patterns previously documented in the literature (Li et al., 2021; Lopez-Rodulfo et al., 2024, 2025).

2.6.2. Semi-quantification of polyphenols

Semi-quantification of the compounds was carried out using corresponding external standards by examining peaks from extracted ion chromatograms (EIC) in negative mode, with standard calibration curves ranging from 0.16 to 40 mg/L. The semi-quantification was based on the structural similarity of the selected phenolic compounds to the chosen external standards. Specifically, 4-hydroxybenzoic acid and gallic acid were used as representatives of hydroxybenzoic acids (HBAs); caffeic acid, ferulic acid, p-coumaric acid, and 5-caffeoylquinic acid as hydroxycinnamic acids (HCAs); epicatechin and catechin as flavanols (monomers and oligomers); quercetin-3-glucoside and quercetin-3-rhamnoside as flavonols; and phloridzin as dihydrochalcone. The quantities were expressed as standard equivalents for each compound. Since the pomace-containing breads were prepared with a 5 % pomace addition, the polyphenol content (mg) was determined in both 5 g of POM and in 100 g (dry weight, d.w.) of dough or bread for all samples (including those with and without pomace). The results were ultimately expressed as mg of polyphenol subclass per 100 g of bread or dough (d. w.). The semi-quantification method was validated by comparing the area under the curve in the EIC of three structurally similar polyphenols: quercetin, quercetin-3-O-glucoside, and quercetin-3-O-rhamnoside.

2.7. Statistical analysis

All the experiments were performed in two independent batches, each in triplicate ($n = 3$) and the data was expressed in mean $\pm$ standard deviation. To ensure the validity of parametric analyses, data were first tested for normality using the Shapiro–Wilk test and for homogeneity of variance using Levene's test. Analysis of variance (on-way ANOVA) was used to study the differences among results. Fisher's least significant difference (LSD) was used to describe means with 95 % confidence intervals. The statistical analysis was performed using Statgraphics Centurion 18 V18.1.13 software (StatPoint Technologies, Warrenton, VA, USA). Statistical analysis of differences in polyphenol concentration (%) between dough and bread abundance data was performed using ANOVA with Holm-Sidak All Pairwise Multiple Comparison Procedures and paired *t*-test implemented in OriginPro® 2024 (OriginLab, USA). Hierarchical clustering, heatmaps and principal component analysis (PCA) were prepared in OriginPro® 2024 (OriginLab, USA). Although the number of samples was limited ($n = 9$), the PCA was conducted using six polyphenol subclasses as variables with the sole purpose of providing a descriptive visualization of the relationships among samples. The results should therefore be interpreted cautiously and not as confirmatory evidence.

3. Results and discussion

3.1. Effect of fibre inclusion on bread physical quality and water dynamics

Bread is an ideal system for incorporating fruit and vegetable by-products rich in dietary fibre and plant secondary metabolites, such as

fruit pomaces (Gómez & Martínez, 2018). In this study, apple pomace was added at a 5 % substitution level for wheat flour (W:POM), which has been reported as a good compromise for introducing bioactive compounds while minimizing sensory acceptability loss (Anil, 2007; Gómez & Martínez, 2018; Gumul et al., 2021).

The addition of pomace resulted in the widely recognized negative impact on the physical properties of wheat bread (W) (Chen et al., 1988; Masoodi et al., 2001). An overall reduction in loaf volume (Fig. 1a), a two-fold increase in hardness (Fig. 1b–Table S1) and a loss of cohesiveness (Table S1) was observed. Notably, despite having similar dough hydration level (57.0 %) and moisture content (42.7 %) as W, W:POM exhibited a significantly different water distribution, suggesting negative changes in water and biopolymer dynamics. Specifically, the proportion of population $T_{2(3)}$, representing mobile OH protons of water, starch, and gluten in the gel network, was dramatically lower ($A_{2(3)}$) and appeared at shorter relaxation times ($t_{2(3)}$) (Table 1). These results indicate a lower proportion of available free mobile water within the matrix, likely limiting starch swelling and gluten plasticization during baking (Franco et al., 2024; Hemdane et al., 2017; Nawrocka et al., 2017), the latter being a key determinant of dough elasticity and strength. Furthermore, the decreased oven spring was likely due to the stiffer gluten network in pomace-rich dough, which may have reduced dough extensibility compared to the control. Similar reductions in relaxation times and the area of $T_{2(3)}$ have been observed with the addition of wheat bran, emphasizing the negative impact of insoluble fibers on water dynamics (Hemdane et al., 2017).

The negative effects of incorporating pomace observed in W:POM contrasted with the positive effects of adding the soluble fibres psyllium and pectin instead. The inclusion of psyllium (W:PSY) increased the water demand to achieve optimal dough hydration (75.8 %) and led to a significant reduction in hardness (by half), along with a slight increase in cohesiveness, without affecting loaf volume compared to W (Fig. 1a and b, Table S1). The W:PSY crumb also exhibited twice the moisture content as W (Fig. 1), consistent with the higher dough hydration and partial moisture retention during baking (Franco et al., 2024). Moreover, the integration of psyllium resulted in a greater $T_{2(3)}$ area and relaxation time (Fig. 1c–Table 1) compared to W bread, aligning with psyllium's ability to retain water without competing with starch and gluten (Franco et al., 2024). These findings are consistent with Franco et al. (2024), suggesting that the additional water was primarily located in the intergranular space, forming part of a continuous network of gluten, starch (likely leached amylose), and arabinoxylan molecules from psyllium. This implies that psyllium does not deplete plasticizing water from starch and gluten but instead provides additional mobile water layers within the dough matrix (Ruan & Chen, 1997; Franco et al., 2024). Similar to psyllium, the addition of pectin in W:P significantly increased dough hydration (82.3 %), loaf volume, and cohesiveness, while reducing hardness by 90.5 % compared to W (Fig. 1b–Table S1). These results align with those from Correa et al. (2012), who reported enhanced specific bread volume with 4 % apple pectin addition. However, unlike W:PSY, W:P exhibited a lower $T_{2(3)}$ area and relaxation time than W, indicating a decrease in free mobile water. This could be attributed to high-methoxy pectin forming a viscous network in the dough, reducing water mobility and potentially withdrawing water from gluten (Nawaz et al., 2021). This aligns with the reported dehydration of the gluten matrix due to water competition between gluten proteins and pectin (Nawrocka et al., 2018). Additionally, the dominance of an intergranular pectin network may physically interfere with gluten, not only by sequestering water but also by preventing glutenin polymers from aligning properly and forming strong disulfide bonds. Whether pectin affected gluten structure solely through hydration competition or also via steric hindrance, these effects did not hinder proper dough development, as evidenced by the production of high-volume breads with a soft, cohesive crumb. Further studies are needed to determine whether hydration competition (and possibly steric hindrance) was insufficient to cause detrimental effects or if these were counterbalanced

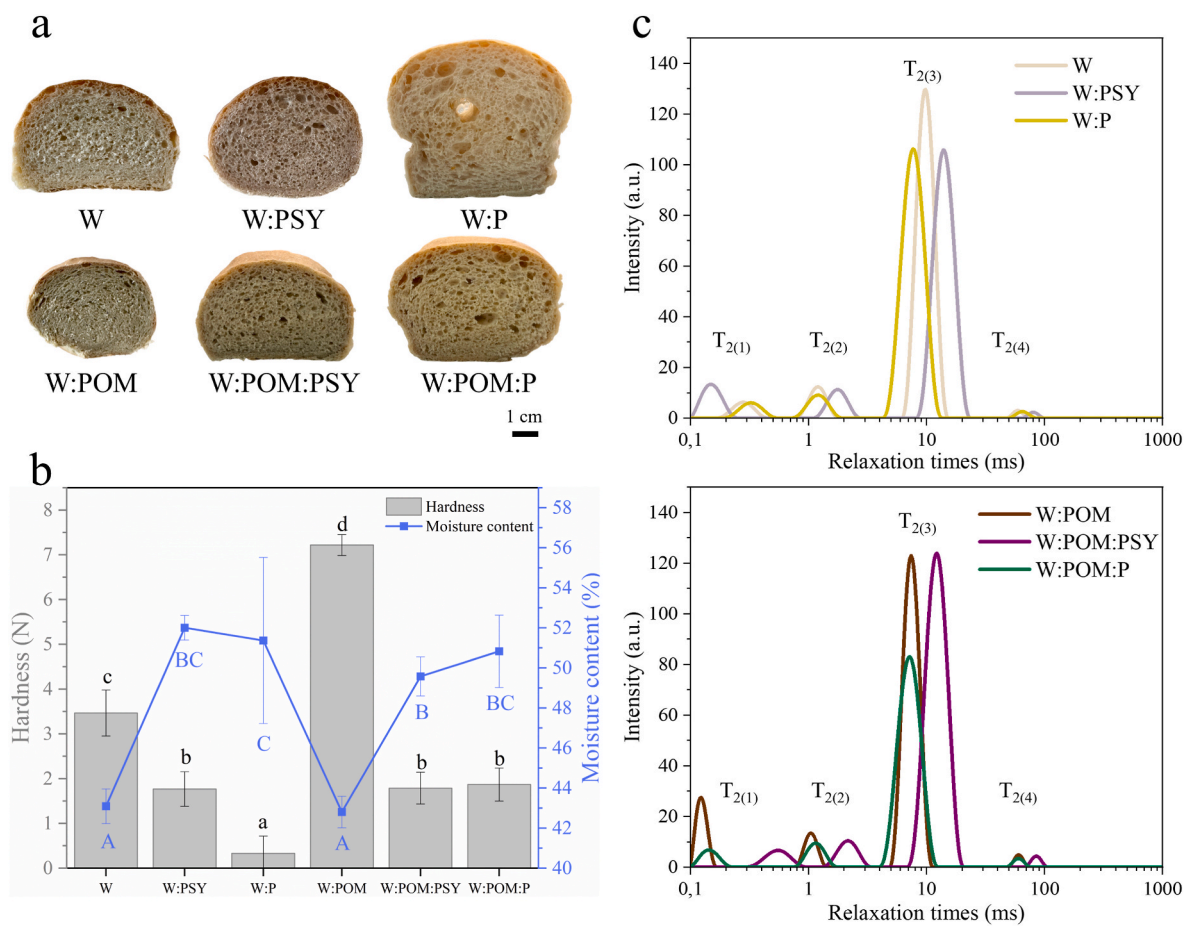


Fig. 1. (a) Digital pictures of breads made with 100 % wheat flour (W), or with 5 % addition of apple pomace (W:POM), or W or W:POM with 5 % of the wheat flour replaced by psyllium (W:PSY or W:POM:PSY, respectively) or pectin (W:P or W:POM:P, respectively). (b) Moisture content and hardness of bread crumb 2 h after baking. (c) Distribution of multi-exponentially fitted relaxation time (T_2) curves of bread crumb obtained 2 h after baking.

Table 1

Water absorption determined by farinograph analysis and relaxation times (t) and absolute areas (A) from the Carr-Purcell-Meiboom-Gill proton distributions of crumb bread as determined by time-domain ^1H NMR relaxometry.

Sample	Water absorption (g/100 g)	T2(1)		T2(2)		T2(3)		T2(4)	
		t (ms)	A (au)	t (ms)	A (au)	t (ms)	A (au)	t (ms)	A (au)
W	57.0	0.3 ± 0.2 b	1.5 ± 1.1 cd	1.3 ± 0.4 a	6.5 ± 0.5 a	9.6 ± 0.1 b	615.9 ± 59.0 b	62.3 ± 6.8 a	61.8 ± 13.7 b
W:PSY	75.8	0.1 ± 0.1 ab	0.7 ± 0.3 ab	1.4 ± 0.3 ab	6.7 ± 2.1 a	15.2 ± 2.9 d	719.3 ± 13.03 bc	80.0 ± 10.0 b	43.3 ± 19.7 a
W:P	82.3	0.3 ± 0.1 b	0.7 ± 0.3 ab	1.1 ± 0.1 a	5.5 ± 0.9 a	7.8 ± 0.2 a	435.1 ± 15.6 a	69.0 ± 2.3 a	69.0 ± 2.3 a
W:POM	57.0	0.1 ± 0.0 a	1.0 ± 0.1 bc	1.4 ± 0.5 a	4.4 ± 1.1 a	7.5 ± 0.1 a	466.5 ± 66.4 a	63.7 ± 2.9 a	63.7 ± 2.9 a
W:POM:PSY	75.8	0.6 ± 0.2 c	2.0 ± 0.3 e	1.9 ± 0.2 b	10.0 ± 0.6 b	11.8 ± 0.1 c	796.3 ± 37.9 c	82.7 ± 2.5 b	82.7 ± 2.5 b
W:POM:P	82.3	0.1 ± 0.1 a	0.3 ± 0.3 a	1.1 ± 0.2 a	6.7 ± 3.0 a	7.0 ± 0.6 a	390.4 ± 64.1 a	67.0 ± 1.0 a	67.5 ± 8.8 a

Bread made with 100 % wheat flour (W) or with 5 % addition of apple pomace (W:POM), or W or W:POM with 5 % of the wheat flour replaced by psyllium (W:PSY or W:POM:PSY, respectively) or pectin (W:P or W:POM:P, respectively). Values with the same letter in the same parameter do not present significant differences ($p < 0.05$).

by increased dough hydration or potential electrostatic interactions between gluten and the carboxyl ($-\text{COO}^-$) groups of the non-methylated segments of pectin, which may have contributed to a tighter gluten network. In conclusion, at the concentrations investigated in this study, both psyllium and pectin significantly increased dough hydration, crumb moisture, bread volume, and crumb softness, with psyllium particularly reinforcing hydration layers around gluten.

Given the contrasting effects of pomace and soluble fibers, the co-integration of psyllium or pectin in breads containing pomace was explored to balance hydration effects and unlock the successful integration of pomace as a bioactive ingredient in breads. When psyllium was co-added with pomace (W:POM:PSY), it mitigated the negative

effects of pomace on volume and hardness, resulting in a similar loaf volume and even softer crumbs than W. Additionally, cohesiveness improved beyond that of W:POM alone (Table S1). W:POM:PSY also exhibited a significantly higher moisture content than W:POM (Fig. 1b), along with a greater $T_{2(3)}$ area and relaxation time (Table 1). This clearly demonstrates that psyllium mitigates the adverse effects of pomace by increasing dough hydration, thereby providing more available water for starch and gluten and reinforcing hydration layers around them. Similarly, the co-addition of pectin in pomace bread (W:P:POM) counteracted the negative effects of pomace, resulting in a softer, more cohesive crumb and a greater loaf volume than W:POM (Fig. 1b; Table S1). As with psyllium, incorporating pectin into pomace-enriched bread

Table 2

UHPLC-ESI-QTOF MS/MS identification of polyphenols in apple pomace from the cultivar 'Jonagold' and dough and bread samples.

Proposed compounds	Molecular Formula	RT (min)	Molecular Weight	Theoretical (m/z)	Observed (m/z)	Mass Error (ppm)	MS/MS Product Ions	Source
Hydroxybenzoic acids								
Gallic acid-4-O-glucoside	C ₁₃ H ₁₆ O ₁₀	2.50	332.074	331.0665	331.0676	3.32	169, 125	Wheat, pomace
Protocatechuic acid-4-O-glucoside	C ₁₃ H ₁₆ O ₉	3.46	316.0794	315.0721	315.072	−0.32	153, 109	Wheat, pomace, psyllium
4-Hydroxybenzoic acid-4-O-glucoside	C ₁₃ H ₁₆ O ₈	4.31	300.845	299.0772	299.0781	3.01	179,137	Pomace
Hydroxybenzoic acid ^b	C ₇ H ₆ O ₃	6.725	138.0316	137.0237	137.0243	4.37	93, 137	Wheat, psyllium
Syringic acid-4-O-glucoside	C ₁₅ H ₁₈ O ₈	4.93	360.1056	359.0978	359.0995	4.73	197, 153	Wheat, pomace, psyllium
Vanillic acid-4-O-glucoside	C ₁₄ H ₁₈ O ₉	7.96	330.0950	329.0873	329.0862	4.73	167	Pomace
Hydroxycinnamic acids								
Caffeoyl glucose	C ₁₆ H ₂₀ O ₉	7.38	342.0951	341.0878	341.0873	−1.47	179,135	Pomace
Caffeic acid ^b	C ₉ H ₈ O ₄	8.887	180.0422	179.0343	179.0353	4.47	162, 180	Wheat, pomace
Trans- <i>p</i> -Coumaric acid-4-O-glucoside	C ₁₆ H ₂₀ O ₉	8.032	326.1002	325.0929	325.0914	−4.61	145, 163	Pomace
Ferulic acid-4-O-glucoside	C ₁₃ H ₁₆ O ₁₀	9.421	356.1107	355.1034	355.103	−1.13	134, 175, 193	Pomace
5-Caffeoylquinic acid	C ₁₆ H ₁₈ O ₉	7.949	354.095	353.0878	353.0869	−2.55	163	Pomace
3- <i>p</i> -Coumaroylquinic acid	C ₁₆ H ₁₈ O ₈	9.46	338.1002	337.0929	337.0925	−1.19	191, 173, 163	Pomace
5- <i>p</i> -Coumaroylquinic acid	C ₁₆ H ₁₈ O ₈	10.794	338.1002	337.0929	337.0927	−0.59	191, 173, 163	Pomace
Feruloyl glucose	C ₁₃ H ₁₆ O ₁₀	11.439	356.1107	355.1034	355.1044	2.82	134, 175, 193	Pomace
Ferulic acid ^b	C ₁₀ H ₁₀ O ₄	14.548	194.0579	193.0501	193.0510	4.66	176, 177	Wheat
Flavanols								
Catechin	C ₁₅ H ₁₄ O ₆	7.773	290.079	289.0717	289.0728	−3.11	245, 205, 179	Wheat, pomace
Epicatechin	C ₁₅ H ₁₄ O ₆	10.662	290.079	289.0717	289.0716	−0.35	245, 205, 179	Pomace
Procyanidin B1	C ₃₀ H ₂₆ O ₁₂	6.891	578.1424	577.1351	577.1358	1.21	289, 125	Pomace
Procyanidin B2	C ₃₀ H ₂₆ O ₁₂	9.923	578.1424	577.1351	577.1364	2.25	425, 289, 125	Pomace
Procyanidin trimer C1	C ₄₅ H ₃₈ O ₁₈	10.04	866.2058	865.1985	865.1964	−2.43	739, 713, 695	Pomace
Procyanidin trimer C2	C ₄₅ H ₃₈ O ₁₈	12.47	866.2058	865.1980	865.2044	2.77	739, 713, 695	Pomace
Procyanidin tetramer 1	C ₆₀ H ₅₀ O ₂₄	10.811	1154.2692	1153.261	1153.2614	0.35	577, 289, 125	Pomace
Procyanidin tetramer 2	C ₆₀ H ₅₀ O ₂₄	11.119	1154.2692	1153.261	1153.2614	0.35	577, 289, 125	Pomace
Procyanidin tetramer 3	C ₆₀ H ₅₀ O ₂₄	13.595	1154.2692	1153.261	1153.2665	4.77	577, 289, 125	Pomace
Procyanidin pentamer 1 ^a	C ₇₅ H ₆₂ O ₃₀	12.112	1442.3325	720.1548	720.1565	2.36	289, 125	Pomace
Procyanidin pentamer 2 ^a	C ₇₅ H ₆₂ O ₃₀	14.074	1442.3325	720.1548	720.1573	3.47	289, 125	Pomace
Procyanidin pentamer 3 ^a	C ₇₅ H ₆₂ O ₃₀	15.116	1442.3325	720.1548	720.1582	4.72	289, 125	Pomace
Procyanidin hexamer 1 ^a	C ₉₀ H ₇₄ O ₃₆	12.244	1730.3959	864.1902	864.192	2.08	289, 125	Pomace
Procyanidin hexamer 2 ^a	C ₉₀ H ₇₄ O ₃₆	13.589	1730.3959	864.1902	864.1903	0.11	289, 125	Pomace
Procyanidin hexamer 3 ^a	C ₉₀ H ₇₄ O ₃₆	15.227	1730.3959	864.1902	864.193	3.24	289, 125	Pomace
Procyanidin heptamer 3 ^a	C ₁₀₅ H ₈₆ O ₄₂	17.708	2018.4594	1008.2220	1008.2268	4.76	289, 125	Pomace
Flavonols								
Kaempferol-7-O-glucoside	C ₂₁ H ₂₀ O ₁₁	8.467	448.1005	447.0933	447.0917	−3.58	285	Pomace
Quercetin-3-O-galactoside (Hyperoside)	C ₂₁ H ₂₀ O ₁₂	18.16	464.0955	463.0882	463.0896	3.02	300, 271, 255	Pomace
Quercetin-3-O-glucoside (Isoquercetin)	C ₂₁ H ₂₀ O ₁₂	18.712	464.0955	463.0882	463.0897	3.24	300, 271, 255	Pomace
Quercetin 3-O- α -arabinofuranoside (Avicularin)	C ₂₀ H ₁₈ O ₁₁	19.638	434.0849	433.0776	433.0789	3.00	301	Pomace
Quercetin-3-O-xyloside	C ₂₀ H ₁₈ O ₁₁	20.498	434.0849	433.0776	433.078	0.92	301	Pomace
Quercetin-3-O-arabinopyranoside	C ₂₀ H ₁₈ O ₁₁	20.707	434.0849	433.0776	433.0779	0.69	301, 416	Pomace
Quercetin-3-O-rhamnoside (Quercitrin)	C ₂₁ H ₂₀ O ₁₁	20.911	448.1005	447.0928	447.0984	3.58	300, 301, 271	Pomace
Isorhamnetin-pentoside 1	C ₂₁ H ₂₀ O ₁₁	22.036	448.1005	447.0928	447.0939	2.46	243, 271, 315	Pomace
Isorhamnetin-pentoside 2	C ₂₁ H ₂₀ O ₁₁	22.24	448.1005	447.0928	447.0935	1.57	243, 271, 315	Pomace
Quercetin 3-O-rhamnosyl-glucoside (Rutin)	C ₂₇ H ₃₀ O ₁₆	17.961	610.1534	609.1456	609.1480	3.94	611	Pomace
Quercetin ^b	C ₁₅ H ₁₀ O ₇	23.109	302.0426	301.0348	301.0342	−1.99	257, 285	Pomace
Flavonone								
Hesperetin 7-(2,6-dirhamnosylglucoside)	C ₃₄ H ₄₄ O ₁₉	18.11	756.2404	755.2476	755.2453	−3.04	593, 447, 161	Psyllium
Homohesperetin 7-rutinoside	C ₁₇ H ₁₆ O ₆	19.32	624.2054	623.1946	623.1935	−1.76	461, 315, 161	Psyllium
Dihydrochalcones								
3-Hydroxyphloretin-2-O-xylosyl-glucoside	C ₂₆ H ₃₂ O ₁₅	17.757	584.1741	583.1665	583.1689	4.11	289, 271, 125	Pomace
3-Hydroxyphloretin-2-O-glucoside	C ₂₁ H ₂₄ O ₁₁	19.864	452.1319	451.1246	451.1258	2.66	289, 271, 167	Pomace
Phloretin-pentosyl-hexoside 1	C ₂₆ H ₃₂ O ₁₄	20.498	568.1792	567.1719	567.1711	−1.41	23, 167, 485	Pomace
Phloretin-pentosyl-hexoside 2	C ₂₆ H ₃₂ O ₁₄	20.829	568.1792	567.1719	567.1739	3.53	273, 167, 485	Pomace
Phloretin-2'-O-glucoside (Phloridzin)	C ₂₁ H ₂₄ O ₁₀	21.821	436.1369	435.1286	435.1298	2.76	273, 167, 125	Pomace
Phloretin ^b	C ₁₅ H ₁₄ O ₅	23.609	274.084	273.0761	273.0758	−1.09	142, 143, 62	Pomace

^a Compounds found double charged. RT stands for "retention time."^b Aglycones.

increased dough hydration (82.3 %) and final crumb moisture (52.50 %). However, in this case, no significant differences were observed in the $T_{2(3)}$ area or relaxation time between W:P and W:POM:P, reflecting the distinct impact of pectin compared to psyllium on water dynamics, as discussed in the previous paragraph.

In summary, the results indicate that psyllium and pectin can counteract the negative effects of apple pomace on water dynamics in a wheat bread system by significantly increasing dough and crumb hydration. Additionally, psyllium further reinforces hydration layers around starch and gluten. These mechanisms result in pomace-rich breads with loaf volume and crumb texture comparable to or superior to those of control wheat bread without pomace. Given that the integration of apple pomace into a bread matrix was successfully achieved with the co-addition of psyllium and pectin, and considering the propensity of polysaccharides to interact with the abundant polyphenols found in pomace (Lopez-Rodulfo et al., 2024), the chemical stability of pomace polyphenols during bread-making and the role of psyllium and pectin in stabilizing them are addressed in the following sections.

3.2. Identification and semi-quantification of polyphenols in raw materials and dough and bread systems

A comprehensive screening of polyphenols sourced from apple pomace or from dough or bread formulations was performed using ultra-high-performance liquid chromatography coupled with electrospray ionization quadrupole time-of-flight tandem mass spectrometry (UHPLC-ESI-QTOF-MS/MS), adapting a previously reported method by Lopez-Rodulfo et al. (2024). To ensure comparability between pomace and dough/bread samples, extracts from 5 g of apple pomace (corresponding to its actual inclusion in bread formulations) and 100 g (d.w.) of dough and bread were analysed. A total of 51 polyphenols were detected, with 46 derived from apple pomace, three exclusive to wheat flour (detected in all dough/bread samples), and two identified in psyllium (only detected in formulations containing psyllium). Formulations with pectin did not depict any extra phenolic compound. These compounds were further classified into six major groups: hydroxybenzoic acids (HBAs), hydroxycinnamic acids (HCAs), flavonols, flavanols (with a degree of polymerization ranging from 1 to 4 [DP 1–4] or 5 to 7 [DP 5–7]), flavanones, and dihydrochalcones. Notably, flavanones were only detected in psyllium-enriched dough and breads formulations, indicating that this subclass is sourced exclusively from psyllium husk. The two flavanones initially identified by their mass-to-charge ratio (m/z) matches with the PCDL library were further confirmed by MS/MS analysis. Hesperetin 7-(2,6-dirhamnosylglucoside) (m/z 755.2476) was confirmed by the product ions m/z 593, resulting from the loss of a glucose moiety, and m/z 447, resulting from the loss of both glucose and rhamnose. Notably, the hesperetin aglycone form (expected m/z 301) was not produced, suggesting that higher collision energy is required for its formation. On the other hand, homohesperetin 7-rutinoside (m/z 623.1946) was confirmed by the production of the homohesperetin aglycone form (m/z 315), also known as hesperetin 3'-methyl ether, and m/z 461, derived from the loss of glucose. It is worth noting that the present study elucidated and reported, for the first time, these flavanones sourced from psyllium husk. Polyphenols from this dietary fiber have not been deeply investigated in the literature, with most studies relying on colorimetric methods without compound profiling (Talukder, Talapatra, Ghoshal, & Sen Raychaudhuri, 2016). As a result, psyllium husk has not been previously identified as a flavanone source (Hädrugä & Hädrugä, 2023). These findings provide novel insights into the polyphenolic composition of psyllium husk, emphasizing the utility of high-resolution mass spectrometry in expanding knowledge of its bioactive constituents.

A semi-quantitative analysis of all identified polyphenols was conducted using standards analytically comparable to the detected compounds (Table 3, Table S2). The total polyphenol content in apple pomace was found to be 26.81 ± 4.82 mg per 5 g pomace, with flavonols

(11.08 ± 1.43 mg/5 g pomace) and HBAs (6.58 ± 2.41 mg/5 g pomace) being the predominant groups, followed by flavanols (3.24 ± 0.29 mg/5 g pomace), HCAs (2.72 ± 0.41 mg/5 g pomace), and dihydrochalcones (1.80 ± 0.15 mg/5 g pomace) (Table 3). These findings are consistent with previous research from our group (Lopez-Rodulfo et al., 2024). In control wheat bread (W), a greater presence of HBA compared to pomace was observed, attributed to wheat-derived compounds such as gallic acid-4-O-glucoside, protocatechuic acid-4-O-glucoside, and syringic acid-4-O-glucoside. This contributed to a total HBA content of 21.58 ± 3.19 mg per 100 g (d.w.) from the 22.00 ± 3.28 mg per 100 g of the total polyphenol content of W bread (Table S2). Although flavonoids and phenolic acids are the primary bioactive components in whole wheat (Liu, Le Bourvellec, & Renard, 2020), the negligible content of flavonoids in refined wheat flour might be due to the removal of the outer layers of the kernel where most flavonoids are retained (Parenti et al., 2020). Finally, the presence of wheat-derived polyphenols and psyllium-sourced flavanones and protocatechuic acid-4-O-glucoside and syringic acid-4-O-glucoside in breads containing psyllium (W:PSY), contributed to a total polyphenol content of 46.70 ± 2.29 mg per 100 g of bread. Contrarily, no additional polyphenols were detected in pectin-containing samples, with W:P breads reaching 19.93 ± 5.95 mg per 100 g.

3.3. Chemical stability of polyphenol subclasses during bread-making (from dough to bread)

The incorporation of pomace resulted in wheat doughs with 54.77 ± 4.59 mg/100 g total polyphenols, which demonstrated a two-fold increase of total polyphenol content from the control wheat bread to 25.20 ± 5.02 mg total polyphenols/100 g (Table S2). The content of individual polyphenols of control doughs and breads, is also shown in Table S2. Polyphenols in W:POM consisted mostly of HBAs (34.89 ± 4.18 mg/100 g), flavonols (13.07 ± 0.28 mg/100 g), flavanols DP 1–4 (3.33 ± 0.05 mg/100 g), and dihydrochalcones (2.63 ± 0.02 mg/100 g), followed by HCAs (1.21 ± 0.02 mg/100 g) and flavanols DP 5–7 (0.82 ± 0.00 mg/100 g), as reported in Table 3 together with the content of individual polyphenols within each subclass. Nevertheless, polyphenols within bread systems are exposed to a range of acidity, heat, light and enzymatic reactions during bread-making that impact polyphenol profiles in the final product, with polyphenols broadly reported to have modest to high sensitivity, mainly attributed to thermal stress (Debelo et al., 2020). In cases where breads are formulated with additional ingredients, interactions between macromolecules and polyphenols may also impact polyphenol stability. In this regard, the changes in polyphenol concentration (Δ %) from dough to bread were calculated in all samples to assess the impact of bread-making on polyphenol stability as well as the role of psyllium and pectin in stabilizing polyphenols. The differential change in polyphenol concentration was calculated as:

$$\Delta \text{ Polyphenols (\%)} = \left(\frac{\text{Dough} - \text{bread}}{\text{dough}} \right) * 100$$

Data was presented as the difference in concentration for each polyphenol subclass between dough and bread (Fig. 2). A Δ value of 0 indicates no degradation or alteration in polyphenol concentration between dough and bread, suggesting no loss of polyphenols during the bread-making process. A positive Δ value indicates that polyphenol concentrations were higher in the dough than in the final bread, indicating degradation or chemical transformation during bread-making. In contrast, a negative Δ value indicates a higher concentration of polyphenols in the bread compared to the dough, suggesting retention or protection during bread-making and improved release from the bread matrix. The content of the 51 polyphenols identified in this study in pomace-enriched dough and bread formulations is shown in Table 3.

Table 3

Polyphenol content (mg) in 5 g of apple pomace (POM) and doughs or breads (mg/100g dry basis^a) made with 100 % wheat flour (W), or with 5 % addition of apple pomace (W:POM), or W or W:POM with 5 % of the wheat flour replaced by psyllium (W:PSY or W:POM:PSY, respectively) or pectin (W:P or W:POM:P, respectively).

Phenolic Compounds	Pomace	W:POM Dough	W:POM Bread	W:POM:PSY Dough	W:POM:PSY Bread	W:POM:P Dough	W:POM:P Bread
Gallic acid-4-O-glucoside	0.47 ± 0.13 a	14.27 ± 0.75 c	12.14 ± 1.21 b	14.62 ± 3.21	12.19 ± 2.01	12.27 ± 0.61	15.41 ± 1.57
Protocatechuic acid-4-O-glucoside	3.14 ± 1.03 a	5.51 ± 0.79 c	5.07 ± 0.44 ab	12.26 ± 1.46	4.71 ± 0.48	4.31 ± 0.34	10.28 ± 1.27
4-Hydroxybenzoic acid-4-O-glucoside	0.22 ± 0.01 a	0.68 ± 0.06 c	0.55 ± 0.04 b	1.22 ± 0.26	0.63 ± 0.04	0.42 ± 0.3	1.51 ± 0.22
Hydroxybenzoic acid ^b	0.00 ± 0.00 a	7.25 ± 1.42 c	5.74 ± 1.70 b	7.14 ± 2.75	9.24 ± 2.46	3.83 ± 0.14	7.44 ± 0.80
Vanillic acid-4-O-glucoside	1.20 ± 0.77 a	1.22 ± 0.05 a	2.22 ± 0.26 b	1.82 ± 0.60	2.45 ± 0.48	2.26 ± 0.08	1.48 ± 0.18
Syringic acid-4-O-glucoside	1.54 ± 0.55 a	5.96 ± 1.25 b	5.20 ± 0.6 b	6.82 ± 1.05	3.85 ± 0.44	4.05 ± 0.26	7.83 ± 1.84
Total Hydroxybenzoic acids (HBAs)	6.58 ± 2.41 a	34.89 ± 4.18 b	30.93 ± 4.21 b	43.87 ± 8.20	33.08 ± 5.81	23.32 ± 0.41	43.96 ± 3.81
Caffeoyl glucose	0.24 ± 0.03 c	0.15 ± 0.00 a	0.19 ± 0.00 b	0.19 ± 0.02	0.17 ± 0.00	0.19 ± 0.01	0.19 ± 0.01
Caffeic acid ^b	0.11 ± 0.00 a	0.11 ± 0.00 a	0.10 ± 0.00 a	0.11 ± 0.00	0.10 ± 0.00	0.10 ± 0.00	0.11 ± 0.00
Trans- <i>p</i> -Coumaric acid 4-O-glucoside	0.33 ± 0.05 b	0.00 ± 0.00 a	0.00 ± 0.00 a	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Ferulic acid ^b	0.00 ± 0.00 a	0.15 ± 0.01 c	0.09 ± 0.00 b	0.02 ± 0.05	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Ferulic acid 4-O-glucoside	1.15 ± 0.17 b	0.00 ± 0.00 a	0.04 ± 0.04 a	0.14 ± 0.04	0.03 ± 0.04	0.04 ± 0.01	0.21 ± 0.02
Feruloyl glucose	0.25 ± 0.05 a	0.25 ± 0.01 a	0.27 ± 0.05 a	0.41 ± 0.11	0.29 ± 0.05	0.29 ± 0.01	0.51 ± 0.06
5-Caffeoylquinic acid	0.52 ± 0.07 a	0.23 ± 0.01 b	0.11 ± 0.02 c	0.41 ± 0.11	0.28 ± 0.03	0.19 ± 0.01	0.67 ± 0.08
3- <i>p</i> -Coumaroylquinic acid	0.01 ± 0.01 a	0.01 ± 0.00 a	0.01 ± 0.00 a	0.03 ± 0.02	0.01 ± 0.01	0.01 ± 0.00	0.02 ± 0.00
5- <i>p</i> -Coumaroylquinic acid	0.23 ± 0.04 a	0.31 ± 0.00 b	0.28 ± 0.02 ab	0.35 ± 0.07	0.34 ± 0.04	0.33 ± 0.02	0.37 ± 0.03
Total Hydroxycinnamic acids (HCAs)	2.83 ± 0.41 b	1.21 ± 0.02 a	1.10 ± 0.07 a	1.48 ± 0.36	0.98 ± 0.16	0.87 ± 0.08	1.89 ± 0.22
(+) Catechin	0.20 ± 0.04 a	0.23 ± 0.01 a	0.19 ± 0.02 a	0.20 ± 0.02	0.23 ± 0.03	0.18 ± 0.00	0.25 ± 0.01
(-) Epicatechin	0.90 ± 0.10 b	1.01 ± 0.02 b	0.73 ± 0.05 a	0.85 ± 0.12	1.15 ± 0.13	0.92 ± 0.02	1.12 ± 0.09
Procyanidin B1	0.19 ± 0.01 a	0.20 ± 0.00 a	0.19 ± 0.00 a	0.19 ± 0.01	0.20 ± 0.01	0.18 ± 0.00	0.20 ± 0.01
Procyanidin B2	0.75 ± 0.09 a	0.94 ± 0.03 b	0.86 ± 0.05 ab	0.83 ± 0.13	0.97 ± 0.11	0.81 ± 0.03	0.95 ± 0.07
Procyanidin trimer C1	0.19 ± 0.01 b	0.17 ± 0.00 a	0.17 ± 0.00 a	0.16 ± 0.00	0.17 ± 0.00	0.16 ± 0.00	0.19 ± 0.00
Procyanidin trimer C2	0.53 ± 0.05 b	0.41 ± 0.00 a	0.41 ± 0.01 a	0.39 ± 0.01	0.40 ± 0.01	0.36 ± 0.01	0.49 ± 0.02
Procyanidin tetramer 1	0.13 ± 0.00 b	0.12 ± 0.00 a	0.12 ± 0.00 a	0.12 ± 0.00	0.12 ± 0.00	0.12 ± 0.00	0.12 ± 0.00
Procyanidin tetramer 2	0.14 ± 0.00 b	0.12 ± 0.00 a	0.12 ± 0.00 a	0.12 ± 0.00	0.12 ± 0.00	0.12 ± 0.00	0.13 ± 0.00
Procyanidin tetramer 3	0.21 ± 0.01 b	0.13 ± 0.00 a	0.13 ± 0.00 a	0.13 ± 0.00	0.13 ± 0.00	0.13 ± 0.00	0.16 ± 0.00
Total Flavanols (DP 1–4)	3.24 ± 0.29 a	3.33 ± 0.05 a	2.9 ± 0.13 a	2.99 ± 0.27	3.48 ± 0.28	2.96 ± 0.05	3.61 ± 0.19
Procyanidin pentamer 1 ^a	0.18 ± 0.01 b	0.12 ± 0.00 a	0.12 ± 0.00 a	0.12 ± 0.00	0.12 ± 0.00	0.12 ± 0.00	0.13 ± 0.00
Procyanidin pentamer 2 ^a	0.19 ± 0.03 b	0.12 ± 0.00 a	0.12 ± 0.00 a	0.12 ± 0.00	0.12 ± 0.00	0.12 ± 0.00	0.13 ± 0.01
Procyanidin pentamer 3 ^a	0.23 ± 0.03 b	0.12 ± 0.00 a	0.12 ± 0.00 a	0.12 ± 0.00	0.12 ± 0.00	0.12 ± 0.00	0.13 ± 0.01
Procyanidin hexamer 1 ^a	0.15 ± 0.01 b	0.12 ± 0.00 a	0.12 ± 0.00 a	0.12 ± 0.00	0.12 ± 0.00	0.12 ± 0.00	0.12 ± 0.00
Procyanidin hexamer 2 ^a	0.12 ± 0.00 b	0.12 ± 0.00 a	0.12 ± 0.00 a	0.12 ± 0.00	0.12 ± 0.00	0.12 ± 0.00	0.12 ± 0.00
Procyanidin hexamer 3 ^a	0.17 ± 0.03 a	0.12 ± 0.00 a	0.12 ± 0.00 a	0.12 ± 0.00	0.12 ± 0.00	0.12 ± 0.00	0.12 ± 0.00
Procyanidin heptamer 3 ^a	0.14 ± 0.03 a	0.12 ± 0.00 a	0.12 ± 0.00 a	0.12 ± 0.00	0.12 ± 0.00	0.12 ± 0.00	0.12 ± 0.00
Total Flavanols (DP 5–7)	1.19 ± 0.13 b	0.82 ± 0.00 a	0.82 ± 0.00 a	0.82 ± 0.00	0.82 ± 0.00	0.81 ± 0.00	0.86 ± 0.02
Kaempferol 7-O-glucoside	0.20 ± 0.04 b	0.02 ± 0.00 a	0.17 ± 0.03 b	0.08 ± 0.03	0.01 ± 0.01	0.09 ± 0.00	0.01 ± 0.01
Quercetin ^b	0.10 ± 0.00 b	0.46 ± 0.03 c	0.02 ± 0.00 a	0.13 ± 0.04	0.31 ± 0.01	0.06 ± 0.01	0.45 ± 0.01
Quercetin-3-o-galactoside (Hyperoside)	3.07 ± 0.40 b	2.28 ± 0.12 a	2.02 ± 0.20 a	2.21 ± 0.67	1.92 ± 0.42	1.80 ± 0.10	2.62 ± 0.32
Quercetin-3-O-glucoside (Isoquercetin)	0.72 ± 0.25 b	0.00 ± 0.05 a	0.43 ± 0.05 b	0.03 ± 0.06	0.22 ± 0.07	0.31 ± 0.01	0.00 ± 0.01
Quercetin-3-O- α -arabinofuranoside	1.97 ± 0.79 a	3.28 ± 0.05 b	3.49 ± 0.25 b	3.45 ± 0.85	2.47 ± 0.33	2.82 ± 0.08	3.39 ± 0.34
Quercetin 3-O-xyloside	2.18 ± 0.28 a	2.89 ± 0.04 b	2.49 ± 0.11 ab	2.59 ± 0.46	2.37 ± 0.25	2.33 ± 0.03	2.99 ± 0.19
Quercetin-3-O-arabinopyranoside	0.20 ± 0.04 a	0.40 ± 0.01 c	0.31 ± 0.02 b	0.32 ± 0.03	0.29 ± 0.05	0.29 ± 0.03	0.42 ± 0.04
Quercetin-3-O-rhamnoside (Quercitrin)	2.50 ± 0.32 a	3.5 ± 0.09 b	3.21 ± 0.21 b	3.38 ± 0.63	3.10 ± 0.38	3.14 ± 0.05	3.73 ± 0.20
Isorhamnetin-pentoside 1	0.07 ± 0.02 a	0.08 ± 0.00 a	0.06 ± 0.01 a	0.05 ± 0.02	0.04 ± 0.01	0.04 ± 0.00	0.07 ± 0.01
Isorhamnetin-pentoside 2	0.06 ± 0.01 a	0.06 ± 0.00 a	0.06 ± 0.00 a	0.07 ± 0.03	0.04 ± 0.01	0.05 ± 0.00	0.03 ± 0.00
Quercetin 3-O-rhamnosyl-glucoside (Rutin)	0.12 ± 0.02 a	0.15 ± 0.00 a	0.13 ± 0.02 a	0.66 ± 0.15	0.16 ± 0.02	0.14 ± 0.01	0.70 ± 0.09
Total Flavonols	11.08 ± 1.43 a	13.07 ± 0.28 a	12.39 ± 0.85 a	12.82 ± 2.97	10.93 ± 1.50	11.07 ± 0.29	14.32 ± 1.20
Hesperetin 7–2,6 - dirhamnosyl glucoside	0.00 ± 0.00 a	0.00 ± 0.00 a	0.00 ± 0.00 a	0.3 ± 0.09	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Homohesperetin 7 rutinoside	0.00 ± 0.00 a	0.00 ± 0.00 a	0.00 ± 0.00 a	0.92 ± 0.26	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Total Flavonone	0.00 ± 0.00 a	0.00 ± 0.00 a	0.00 ± 0.00 a	1.22 ± 0.35	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
3-Hydroxyphloretin-2-O-xylosyl-glucoside	0.20 ± 0.01 b	0.11 ± 0.00 a	0.11 ± 0.00 a	0.12 ± 0.00	0.13 ± 0.00	0.12 ± 0.00	0.13 ± 0.00
3-Hydroxyphloretin-2-O-glucoside	0.14 ± 0.01 b	0.11 ± 0.00 a	0.11 ± 0.00 a	0.55 ± 0.08	0.28 ± 0.01	0.27 ± 0.00	0.68 ± 0.02
Phloretin ^b	0.10 ± 0.00 a	0.62 ± 0.00 c	0.26 ± 0.01 b	0.11 ± 0.00	0.12 ± 0.00	0.11 ± 0.00	0.12 ± 0.00
Phloretin-pentosyl-hexoside 1	0.37 ± 0.04 a	0.52 ± 0.01 b	0.50 ± 0.02 b	0.54 ± 0.06	0.59 ± 0.04	0.62 ± 0.02	0.53 ± 0.05
Phloretin-pentosyl-hexoside 2	0.13 ± 0.00 a	0.18 ± 0.00 c	0.17 ± 0.00 b	0.17 ± 0.01	0.18 ± 0.01	0.19 ± 0.00	0.19 ± 0.02
Phloretin-2'-O-glucoside (Phloridzin)	0.86 ± 0.09 b	1.08 ± 0.02 c	0.55 ± 0.02 a	0.55 ± 0.02	1.12 ± 0.08	0.82 ± 0.02	1.01 ± 0.03
TOTAL Dihydrochalcones	1.80 ± 0.15 b	2.63 ± 0.02 c	1.70 ± 0.04 a	2.05 ± 0.17	2.42 ± 0.14	2.13 ± 0.04	2.66 ± 0.12
TOTAL POLYPHENOLS	26.81 ± 4.82 a	54.77 ± 4.59 b	49.83 ± 5.30 b	65.39 ± 12.04	51.73 ± 7.91	45.10 ± 1.02	67.40 ± 5.54

^a To ensure comparability of the results between pomace and doughs and breads, extracts from 5 g of apple pomace (corresponding to its actual inclusion in the bread formulations) and 100 g (d.w.) of dough and bread were analysed.

^b Aglycones.

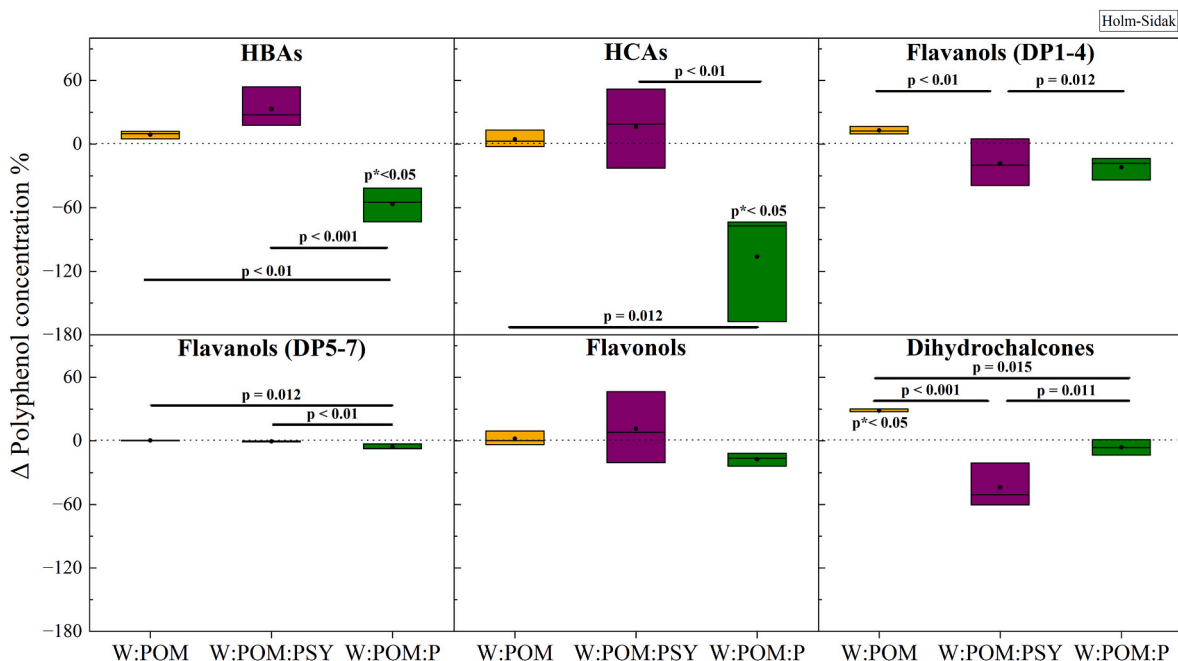


Fig. 2. Differences in the concentration (%) of six polyphenol subclasses between dough and bread samples made with 100 % wheat flour (W), or with 5 % addition of apple pomace (W:POM), or W or W:POM with 5 % of the wheat flour replaced by psyllium (W:PSY or W:POM:PSY, respectively) or pectin (W:P or W:POM:P, respectively). Box plots show median, maximum and minimum percentiles. Dots represent means of the individual samples that were run in triplicates or duplicates as stated in the method section. Differences between all samples were determined using One-Way Anova with Holm-Sidak All Pairwise Multiple Comparison Procedures; differences between dough and bread were also tested with paired *t*-test. *indicates the *p*-value derived from the paired *t*-test. HCA and HBA refer to hydroxycinnamic acid and hydroxybenzoic acid derivatives, respectively.

3.3.1. Polyphenol stability in pomace-containing wheat bread

Initially, the stability of the six polyphenols subclasses (sharing common core structures) in a wheat bread system enriched with apple pomace (W:POM) during bread-making (i.e., proofing and baking) was investigated (Fig. 2, yellow). The bread-making process most affected the dihydrochalcone subclass, resulting in a significant degradation of approximately 30 %, primarily due to the breakdown of phloretin-2'-O-glucoside (phloridzin). Table 3 highlighted that the considerable decrease of dihydrochalcones resulted mainly from the high thermal sensitivity of phlorizin and phloretin, whose concentration decreased approximately two-fold during bread-making. In the case of dihydrochalcones, they possess a more flexible, open-chain structure and a saturated carbonyl feature lacking the double bond linked to stabilizing the phenoxy radical or semiquinone (Li et al., 2018). This occurrence might increase their vulnerability to molecular rearrangements or breakdown during thermal processing, as observed by other authors (Human et al., 2021; Li et al., 2020). Likewise, flavanols (DP1-4) exhibited a slight loss during bread-making, attributed to the decrease of catechin and (–)-epicatechin. This aligns with previous reports highlighting catechin degradation during thermal processing (Pico et al., 2019; Soto-Vaca et al., 2012). Multiple degradative pathways during processing such as bread making have been proposed for flavanols, including oxidation, epimerization, and polymerization (Debelo et al., 2020; Song et al., 2015). Heat accelerates these processes, breaking down the compounds more quickly than it does for other polyphenols, which may not have as many susceptible reactive sites or as much tendency for these reactions. Thus, careful consideration must be given when enriching bread systems with dihydrochalcones, flavanols, or ingredients containing them.

Interestingly, during dough preparation, higher levels of phloretin-

pentosyl-hexoside 1 and 2, and phlorizin, originated from pomace, were detected compared to their original concentrations in pomace (Table 3). This suggests that the kneading or fermentation stages may enhance the release of these polyphenols more effectively than conventional methanol extraction, and that they are particularly stable during mixing and kneading processes occurring at room temperature.

HBAs, HCAs, flavanols (DP5-7), and flavonols exhibited minimal changes in concentration after baking, with Δ values greater than or equal to 0, indicating no significant degradation. The heat stability of HCAs and HBAs has been well reported during heating (Debelo et al., 2020; Huang et al., 2025). Being the simplest polyphenol subclasses analysed, the lack of highly reactive functional groups that are susceptible to heat-induced degradation could play a role on their thermal stability. The observed increase in recovery after baking could be attributed to their release from the polysaccharide matrix of the kernel by cleaving covalent or non-covalent bonds, releasing free and extractable forms of phenolic acids (Abdel-Aal & Rabalski, 2022). The greater recovery after baking could also be explained by the degradation of more complex polyphenols such as chlorogenic acid into HBA or HCA quantified structures. For example, heat treatment of chlorogenic acid at 100–200 °C in aqueous solution has shown to release hydrolysis products such as caffeic acid (Dawidowicz & Typek, 2017).

Flavanols (DP5-7) remained particularly stable, likely due to their high molecular weight, ensuring low volatility and strong association with polysaccharides, such as those from the pomace primary cell wall (PCW), as observed by Lopez-Rodulfo et al. (2024).

As for flavonols, despite their lower molecular weight than flavanols (DP5-7), they have a more extended and conjugated aromatic ring system compared to the thermolabile dihydrochalcones and catechins (Ou, 2021). Moreover, the combination of structural features in

flavonols plays a crucial role in electron delocalization and radical stabilization, enhancing resistance to oxidation. This stability is primarily granted by the double bond between C2 and C3, the hydroxyl group at C3, and the carbonyl group at C4 in the C-ring, which together create a conjugated system. Given their thermal stability observed in the present study, this extended conjugation might be a key factor in stabilizing flavonols not only against oxidation but also against heat-induced changes, offering greater thermal resilience compared to other subclasses such as flavanols and dihydrochalcones, whose conjugation systems are less complex. Moreover, flavonols can form strong hydrogen bonds with surrounding molecules or can be glycosylated, which can enhance their stability during cooking or baking by reducing the extent of thermal degradation due to the blockage of the 3-hydroxyl group in the C-ring by a sugar moiety. This might be due to the fact that the formation of the carbanion was blocked in the presence of a sugar moiety on the position of 3-hydroxyl group in the C-ring (Buchner et al., 2006). Nevertheless, significant variability was observed in the degradation patterns of individual flavonols, with differences in release and degradation behaviors illustrated in Table 3. This highlights the major role of polyphenol discrete structures at individual level, rather than the common core structures featuring the subclasses, at determining their stability during bread-making. For example, hydroxylation, especially on the B-ring of flavonoids, can reduce stability, making these compounds more prone to degradation (Zhang et al., 2022; Cao et al., 2021; Xiao et al., 2015), whereas glycosylation (Xiao, 2017), methylation (Koirala et al., 2016), and acylation (Ishihara & Nakajima, 2003) enhance thermal stability by increasing hydrophilicity or introducing steric hindrance. Studies have shown that quercetin and rutin (a glycosylated form of quercetin) rapidly degrade in aqueous environments at 100 °C (Makris & Rossiter, 2000) and under subcritical water conditions (120–220 °C) (Ravber et al., 2016), whereas glycosylated flavonoids such as rutin and luteolin-7-O-glucoside generally exhibit greater thermal stability compared to their aglycone counterparts. However, exceptions exist; for example, rutin (quercetin-3-O-rutinoside) is more heat-sensitive than naringin (Chaaban et al., 2017). Bednříček et al. (2020) reported that in breads supplemented with onion peel, a polyphenol-rich ingredient, quercetin glycosides - including dimers and trimers - decomposed during baking, releasing free quercetin. In contrast, this decomposition was not observed in aglycone forms, suggesting that glycosylation increases susceptibility to heat-induced degradation. Overall, hydrophobic flavonols, in particular, appeared to be more susceptible to thermal degradation, following a degradation pattern similar to that of dihydrochalcones. This suggests that the structural characteristics of polyphenols, including their hydrophobicity and the presence of specific functional groups, play a pivotal role in determining their stability during bread-making. These observations underscore the importance of conducting compound-level analyses to better understand how individual polyphenols behave during bread-making.

3.3.2. Effect of the co-addition of psyllium on the stability of polyphenol subclasses in pomace-containing wheat bread

The co-addition of psyllium generally halted the loss of the most labile polyphenol subgroups, dihydrochalcones and flavanols (DP 1–4), showing Δ values not statistically different from 0 (Fig. 2). Psyllium husk powder, a hydrocolloid rich in mucilage arabinoxylans, has the potential to elevate the demand of water to reach optimum dough hydration, dramatically increasing the moisture content of the system and specially of the intergranular space as part of the continuous network formed by gluten, starch and arabinoxylan molecules (Franco et al., 2024). This much greater moisture may reduce the exposure of polyphenols to baking-induced thermal stress, thereby minimizing their degradation. In addition, psyllium's ability to self-assemble may play a crucial role in protecting polyphenols. According to Yu et al. (2017), psyllium arabinoxylans form a three-dimensional network through hydrogen bonding, potentially encapsulating polyphenols and shielding

them from degradation during bread-making. It is also noted that the greater volume of loaves upon the addition of psyllium results in lower surface area and potentially lower exposure of the central crumb fraction to the surface heat. Therefore, the role of a greater volume at protecting the most labile polyphenols should not be ruled out.

It is noted however that gel entrapment could also hinder polyphenol interactions with other biopolymers such as starch, possibly affecting their stability. Thus, while encapsulation by psyllium arabinoxylans appears to protect certain polyphenols, it may simultaneously restrict the beneficial binding interactions with other matrix components, contributing to variability in degradation patterns observed across different polyphenol subclasses.

The polyphenol subclasses that displayed a negligible loss during bread-making without the incorporation of psyllium (W:POM), that is HBAs, HCAs, flavanols (DP 5–7) and flavonols, again presented a mean Δ value not statistically different from 0. HBAs seemed to display a mean Δ value above zero, although not statistically significant. While the standard deviation and mean Δ values for flavanols (DP 5–7) remained narrow and identical to W:POM (highlighting a remarkable resistance of oligomeric flavanols to the bread-making process), HBAs, and specially HCAs and flavonols, presented a much greater variability of Δ values in W:POM:PSY than in W:POM (Fig. 2), which was attributed to a different degree of loss among the different individual polyphenols within each of these subclasses (Table 2). This suggests that while some individual polyphenols were more degraded in psyllium breads, others were stable and better released from the matrix after the baking process in psyllium breads, contributing to the observed differences. Although further research is warranted to clarify the mechanisms behind this increased variability, given the complexity of the matrix, the softer crumb achieved with the co-addition of psyllium may enhance the release of polyphenols that are more resistant to the bread-making process while exacerbating the degradation of those sensitive to water, thereby increasing the variability of Δ values.

3.3.3. Effect of the co-addition of pectin on the stability of polyphenol subclasses in pomace-containing wheat bread

The incorporation of pectin into the bread matrix had a similar protective effect on the most thermolabile polyphenol subclasses (flavanols DP 1–4 and dihydrochalcones) as psyllium. Interestingly, the co-addition of pectin resulted in significantly lower Δ values for HBAs, HCAs, and flavanols DP 5–7 compared to the co-addition of psyllium. This suggests that pectin more effectively minimized the degradation of these polyphenol subclasses while enhancing their release from the bread matrix. These observations point towards pectin's dual role in both protecting and modulating polyphenol extractability during bread-making.

The mechanisms underlying pectin's behavior are likely linked to its structural properties, which determine its ability to form complexes with polyphenols or, at a macroscopic level, its capacity to reduce the surface area of bread loaves (Fig. 1). Firstly, polyphenols could interact with pectin through hydrogen bonding (Lohani & Muthukumaran, 2017; Liu et al., 2020), since pectin is not only rich in hydroxyl (–OH) groups capable of forming hydrogen bonds with the hydroxyl groups of polyphenols, but also in carboxyl (COOH) providing binding sites for polyphenols. Secondly, pectin, a complex polysaccharide, contains negatively charged galacturonic acid units, enabling electrostatic interactions with polyphenols, particularly at the relatively neutral pH of standard wheat dough (pH 5.5–6.2), where most carboxyl groups are deprotonated (Lin et al., 2021; Xue et al., 2024; Hu et al., 2025). Thirdly, pectin consists of α -(1 → 4)-linked D-galacturonic acid units, and these linkages provide greater chain flexibility than other polysaccharides. This flexibility is especially pronounced in high methoxyl pectin (such as the one used in this study), where weaker pectin-pectin interactions allow for a more dynamic conformation, facilitating better accommodation and binding of polyphenols (Fernandes et al., 2020; Assifaoui et al., 2024). Moreover, pectin's flexible and hydrated gel-like structure

enhances surface contact for polyphenol adsorption. Its swelling and water retention properties increase the availability of binding sites, stabilizing polyphenols in aqueous environments and preventing their degradation (Zdunek et al., 2021). This interaction is particularly relevant in food matrices where polyphenols are prone to oxidation or precipitation, as pectin can encapsulate polyphenols, protecting them from enzymatic degradation and improving their functional efficacy (Lin et al., 2021).

Besides protection to the bread-making process, pectin resulted in greater values of HCAs and HBAs in bread than in dough, with Δ values statistically lower than 0. This effect was not observed for psyllium and highlights the effect of pectin at facilitating the recovery of polyphenols after the baking process. This event could be attributed to weak gel structure formed by the high methoxy pectin, which could be easily disrupted during the baking process or became weaker and easy to break down during polyphenol extraction prior to analysis. This would result in HCAs and HBAs easier to be liberated from the crumb. It is noted that W:POM:PSY and W:POM:P breads exhibited similar hardness. Therefore, crumb softness is unlikely to explain the significant recovery of HBAs and HCAs in these breads.

3.4. Stability of polyphenols at individual level under different formulations

A hierarchical clustered heatmap of the differential concentration ($\Delta\%$) of individual polyphenols across the three different pomace-containing bread matrices is depicted in Fig. 3a, providing a detailed

classification of polyphenol behavior at individual level based on statistically clustered trends. The hierarchical clustered heatmap grouped polyphenols based on their behavior during bread-making in the three food matrices, as well as grouped the matrices according to their influence on polyphenol profiles.

Analyzing the behavior of individual polyphenols within these food matrices, three primary categories emerged. **Cluster 1 (blue branch)** included polyphenols that showed lower concentrations after baking in W:POM:P but higher extractability in W:POM and W:POM:PSY. In other words, this group clustered those polyphenols whose stability to the bread-making process (or extractability from the bread matrix) was negatively affected by the co-addition of pectin, which included phloretin pentosyl hexoside, kaempferol 7-O-glucoside, caffeoyl glucose, isorhamnetin-pentoside 1, vanillic acid 4-O-glucoside, homohesperetin 7-rutinoside, hesperetin 7-2,6-dirhamnosyl glucoside, 3-Hydroxyphloretin 2-O-glucoside, procyanidin heptamer 3, phloretin pentosyl hexoside 2, phloridzin, procyanidin B2, 3-Hydroxyphloretin 2-O-xylosyl-glucoside, quercetin-3-O-glucoside (isoquercetin), epicatechin, procyanidin B1, 5-p-Coumaroylquinic acid, avicularin, procyanidin hexamer 3, procyanidin pentamer 2, procyanidin pentamer 1, procyanidin tetramer, procyanidin trimer C2, procyanidin trimer C1, procyanidin tetramer 3, 3-p-Coumaroylquinic acid, procyanidin hexamer 2, procyanidin hexamer 1, procyanidin pentamer 3, chlorogenic acid, procyanidin tetramer 2, feruloyl glucose, quercetin, quercetin 3-O-rhamnosyl-glucoside, 4-Hydroxybenzoic acid-4-O-glucoside, syringic acid-4-O-glucoside, protocatechuic acid-4-O-glucoside, phloretin, isorhamnetin-pentoside 1, catechin, quercetin 3-O-arabinopyranoside, quercetin-O-glucoside, quercetin 3-O-xyloside, quercetin 3-O-rhamnoside/arabinofuranoside, quercetin-3-O-galactoside (Hyperoside), gallic acid-4-O-glucoside.

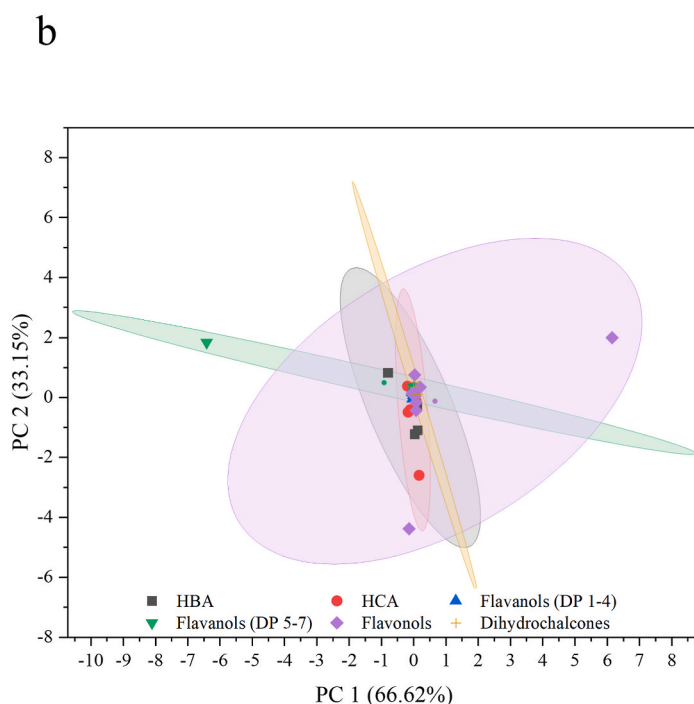
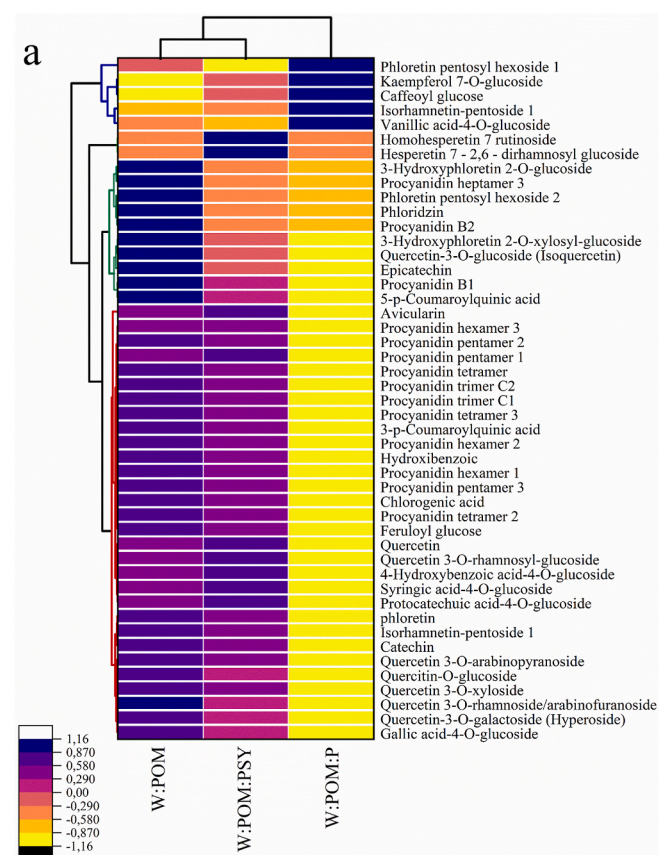


Fig. 3. (a) Hierarchical clustered heatmap differences in polyphenol concentration (%) between dough and bread made with 100 % wheat flour (W), or with 5 % addition of apple pomace (W:POM), or W or W:POM with 5 % of the wheat flour replaced by psyllium (W:PSY or W:POM:PSY, respectively) or pectin (W:P or W:POM:P, respectively). Hierarchical clustering was performed based on Euclidean distances. (b) Principal Component Analysis (PCA) was conducted on all polyphenols, with scores representing individual polyphenols and loadings reflecting the differences in polyphenol concentration (%) between dough and bread made with 100 % wheat flour (W), or with 5 % addition of apple pomace (W:POM), or W or W:POM with 5 % of the wheat flour replaced by psyllium (W:PSY or W:POM:PSY, respectively) or pectin (W:P or W:POM:P, respectively).

polyphenols that exhibited degradation in both W:POM and W:POM:PSY + POM, but not in W:POM:P, and even showed greater concentration in bread than in dough when including pectin. The addition of pectin seems to facilitate the protection and release of polyphenols after the baking process, which was attributed in the previous sections to multiple discrete structure of pectin molecules, such as high hydrophilicity, abundance of carboxyl groups (presumably partially deprotonated), methyl esters, and chain flexibility. Overall, hierarchical clustering analysis indicated that the grouping of polyphenols is primarily influenced by both the polyphenol chemical makeup and the molecular structure and colloidal properties of co-added supportive soluble polysaccharides, rather than by the polyphenol chemical makeup alone. This is better observed in the PCA reported in Fig. 3b, which did not show any grouping based on core structures or polyphenol subclasses. This highlights the importance of supportive ingredients and food structuring, underscoring the significant role of matrix composition in modulating polyphenol stability during food processing.

W:POM and W:POM:PSY were clustered together, exhibiting a similar trend in polyphenol behavior. In contrast, W:POM:P showed a distinct pattern, characterized by greater overall protection of most individual polyphenols during bread-making, aligning with the previous discussion at the polyphenol subgroup level (section 3.3.3).

4. Conclusions

Psyllium and pectin effectively counteracted the negative effects of apple pomace on water dynamics in wheat bread, significantly increasing dough and crumb hydration. Moreover, psyllium further reinforced hydration layers around starch and gluten. These mechanisms resulted in pomace-rich breads with loaf volume and crumb texture comparable to or superior to those of control wheat bread without pomace. Given that the integration of apple pomace into a bread matrix was successfully achieved with the co-addition of psyllium and pectin, and considering the propensity of polysaccharides to interact with the abundant polyphenols found in pomace, the chemical stability of pomace polyphenols during bread-making and the role of psyllium and pectin in stabilizing them were addressed via UPLC-ESI-QTOF-MS/MS.

Incorporating just 5 % apple pomace into dough doubled the total polyphenol content, with bread-making primarily affecting dihydrochalcones and monomeric flavanols, while HBAs, HCAs, and oligomeric flavanols showed minimal changes. Significant variability in the degradation patterns of individual flavonols suggested that discrete polyphenol structures, rather than core subclass structures, influence their stability during baking. These findings highlight the importance of compound-level analyses to better understand individual polyphenol behavior during the baking process.

The co-addition of psyllium and pectin generally prevented the loss of dihydrochalcones and flavanols, with pectin showing significantly greater recovery of HBAs, HCAs, and oligomeric flavanols. This suggests that pectin better minimized the degradation of these polyphenol subclasses and enhanced their release from the bread matrix. Hierarchical clustering analysis revealed that polyphenol stability during baking is influenced by both their chemical makeup and the molecular structure of the co-added polysaccharides, rather than by polyphenol composition alone.

Given the successful incorporation of unrefined apple pomace and the dual benefits of its dietary fiber and polyphenol fractions, this study demonstrates its unique potential as a sustainable functional ingredient in baked goods. It also highlights the need for more efficient management of the large volumes of apple pomace and other fruit and vegetable by-products produced globally. Nevertheless, future research is warranted to determine whether these findings can be extrapolated to all types of pomaces, or only to those closely resembling our pomace system in terms of composition and colloidal properties.

CRediT authorship contribution statement

Maria Franco: Writing – original draft, Visualization, Methodology, Investigation. **Ivan M. Lopez-Rodulfo:** Visualization, Methodology, Investigation. **Federico Bianchi:** Investigation. **Manuel Gomez:** Investigation. **Mario M. Martinez:** Writing – review & editing, Visualization, Supervision, Methodology, Funding acquisition, Conceptualization.

Declaration of competing interest

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

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.foodhyd.2026.112444>.

Data availability

Data will be made available on request.

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