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ORIGINAL PAPER

Quince and persimmon vinegars as functional foods: phenolic characterization and bioactivity assessment

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Abstract
The present study aimed to characterize the phenolic composition and in vitro biological potential of quince and persimmon vinegars in comparison with their unfermented ethanolic extracts. The highest total phenolic and total flavonoid contents were recorded as 102.18 ± 1.86 µg PEs/mg in Quince Vinegar 2 and 171.36 ± 1.45 µg/mg in Persimmon Vinegar 3, respectively, whereas the corresponding alcoholic extracts contained markedly lower phenolic levels (e.g., 5.70 µg PEs/mg in Q Extract 1). LC–MS/MS analysis identified quinic, caffeic, chlorogenic and gallic acids, together with quercetin and catechins, as major organic acids and phenolic constituents. Antioxidant assays revealed a strong inverse relationship between phenolic content and IC_{50} values, with fermented vinegar samples consistently exhibiting higher activity than their unfermented counterparts. Both quince and persimmon vinegars showed pronounced inhibition of α-glucosidase, while additional in vitro inhibitory effects were observed against cholinesterase and urease enzymes. Moderate inhibition of tyrosinase, elastase and collagenase was also detected. Overall, fermented vinegars displayed broader phenolic diversity and stronger multifunctional bioactivities compared to non-fermented extracts, indicating that fermentation-related matrix effects enhance phenolic availability and biological response. These findings provide a compositional and functional basis for further investigation of fermented fruit vinegars as functional food matrices, with potential applications pending validation through in vivo and clinical studies.

Keywords *Cydonia oblonga* · *Diospyros kaki* · Antioxidant capacity · Flavonoid content · Antidiabetic activity · Anti-aging activity

Abbreviations

α-TOC	α-tocopherol
ABTS	2,2′-Azino-bis (3-ethylbenzthiazoline-6-sulfonic acid)
AChE	Acetylcholinesterase
BChE	Butyrylcholinesterase
BHT	Butylated hydroxytoluene
CUPRAC	Cupric ion reducing antioxidant capacity
DPPH	2,2-diphenyl-1-picrylhydrazyl

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IC_{50}	half-maximal inhibitory concentration
LC–MS/MS	Liquid chromatography with tandem mass spectrometry
P extract 1	Alcoholic persimmon extract
P extract 2	Hydroalcoholic persimmon extract
PC1	Principal component 1
PC2	Principal component 2
PCA	Principal component analysis
Persimmon vinegar 1	Commercial persimmon vinegar 1
Persimmon vinegar 2	Commercial persimmon vinegar 2
Persimmon vinegar 3	Commercial persimmon vinegar 3
Q extract 1	Alcoholic quince extract
Q extract 2	Hydroalcoholic quince extract
Quince vinegar 1	Commercial quince vinegar 1
Quince vinegar 2	Commercial quince vinegar 2
Quince vinegar 3	Commercial quince vinegar 3

Introduction

Vinegar is a traditional fermented food produced through sequential alcoholic and acetic acid fermentations and is widely consumed as a culinary ingredient across different cultures. Beyond its gastronomic use, vinegar has attracted scientific interest due to its complex chemical composition, which is shaped by raw material characteristics, fermentation processes and post-fermentation handling [1]. Vinegar production involves a two-step bioprocess, comprising alcoholic fermentation of sugars followed by acetic acid fermentation mediated by *Acetobacter* spp., and can be derived from a wide range of carbohydrate-rich substrates, including fruits, cereals and other plant sources [2].

Fruit vinegars are chemically distinct from non-fermented plant products due to the combined effects of microbial metabolism, acidic conditions and matrix transformation during fermentation. These processes influence the extractability, stability and chemical form of organic acids and phenolic compounds, which are considered major contributors to the chemical diversity of vinegars [2, 3]. Consequently, fruit vinegars have been investigated primarily as complex food matrices in which fermentation-associated changes may alter compositional profiles and in vitro biological responses, rather than as isolated sources of single bioactive compounds.

Previous studies have reported that fruit vinegars contain a wide range of organic acids, phenolic acids, flavonoids and fermentation-derived metabolites, and that these constituents may collectively contribute to antioxidant capacity and enzyme inhibitory activities under in vitro conditions [2–5]. While in vivo and clinical studies have suggested associations between vinegar consumption and metabolic outcomes, such findings require cautious interpretation, as clinical validation represents a subsequent research stage beyond compositional and in vitro bioactivity assessments [1, 4]. Accordingly, in vitro studies remain essential for elucidating how fermentation-related matrix effects influence chemical composition and biological response patterns.

Cydonia oblonga Mill. (quince) is a member of the Rosaceae family and is characterized by a diverse phytochemical profile, including phenolic acids, flavonoids, tannins and organic acids [6-9]. Recent studies have indicated that quince-derived fermented products may exhibit altered phenolic profiles and enhanced in vitro antioxidant and enzyme inhibitory activities compared to non-fermented forms, highlighting the potential impact of fermentation on matrix composition [10, 11]. Similarly, *Diospyros kaki* L. (persimmon) is rich in phenolic compounds, proanthocyanidins, carotenoids and dietary fiber, and has been increasingly utilized in vinegar production as a means of valorizing both edible portions and processing by-products [12–15]. Fermentation of persimmon substrates has been shown to modify phenolic composition, organic acid profiles and in vitro bioactivities, although reported effects vary depending on processing conditions and raw material characteristics [14–16].

Despite growing interest in fruit vinegars, integrated studies that simultaneously compare the phenolic composition and multifunctional in vitro bioactivities of fermented vinegars with their corresponding unfermented extracts remain limited, particularly for quince and persimmon. Moreover, the extent to which fermentation modulates specific classes of phenolic compounds and how these compositional changes are reflected in antioxidant and enzyme inhibitory responses at the matrix level is not fully elucidated.

Therefore, the aim of the present study was to comprehensively evaluate the organic acid and phenolic profiles and key in vitro biological activities of commercial quince and persimmon vinegars in comparison with their alcoholic and hydroalcoholic extracts. We hypothesized that fermentation modulates phenolic composition by enhancing the extractability and diversity of phenolic acids and flavonoids, and that these changes are associated with altered antioxidant and enzyme inhibitory activities at the extract and vinegar matrix level. The novelty of this research lies in its integrated assessment of multiple commercial vinegars and corresponding extracts through detailed LC–MS/MS-based chemical profiling combined with comprehensive in vitro bioactivity evaluation, providing a comparative and hypothesis-generating framework for future mechanistic and controlled fermentation studies.

Materials and methods

Chemicals

All solvents used for extraction and analysis were of analytical or LC–MS grade. Ethanol (≥99.8%, Merck, Darmstadt, Germany), methanol (LC–MS grade, Merck, Darmstadt, Germany) and formic acid (≥98%, Merck, Darmstadt, Germany) were used for sample preparation and chromatographic analysis. Folin–Ciocalteu reagent (Merck, Darmstadt, Germany), sodium carbonate (Merck, Darmstadt, Germany), aluminum chloride (Merck, Darmstadt, Germany), DPPH (2,2-diphenyl-1-picrylhydrazyl; Merck, Darmstadt, Germany), ABTS (2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid); Merck, Darmstadt, Germany) and CUPRAC reagents were used for antioxidant assays.

Enzymes and substrates used in enzyme inhibition assays included acetylcholinesterase (AChE), butyrylcholinesterase (BChE), urease, α-glucosidase, α-amylase, tyrosinase, elastase and collagenase, together with their respective substrates, all purchased from Merck (Darmstadt, Germany). Galanthamine, thiourea, acarbose, kojic acid, oleanolic acid and epicatechin gallate were used as reference inhibitors.

Authentic phenolic and organic acid standards used for LC–MS/MS (Shimadzu, LCMS-8040 model, Tokyo, Japan) identification and quantification-including phenolic acids, flavonoids and organic acids—were purchased as certified reference materials from Merck (Darmstadt, Germany). Detailed information on individual compounds is provided in Table S1. Deuterated internal standards (ferulic acid-D_3, rutin-D_3 and quercetin-D_3) were obtained from Merck (Darmstadt, Germany).

Plant material, extraction procedure and vinegars

Fresh fruits of quince and persimmon were purchased from a local market in Istanbul, Türkiye. The fruits were washed, cut into small pieces, and dried in a laboratory drying oven (Nuve, Türkiye). The dried samples were then ground into a coarse powder using an electric grinder (Retsch GmbH, 1 mm diameter).

Extraction procedure for quince followed the method of Persic et al. [17] with modifications while persimmon extraction was adapted from Kawase et al. [18]. Two solvent systems of ethanol (EtOH) (alcoholic) and ethanol: water (70:30%, v/v) (hydroalcoholic) were used in the experiments. For each extraction, 840 g of powdered sample was extracted with either EtOH or EtOH: water for 24 h at room temperature and repeated three times. The extraction period of 24 h was selected to allow sufficient solvent diffusion and phenolic release under mild, room-temperature conditions, thereby avoiding thermal or oxidative degradation associated with accelerated extraction techniques. Repeating the extraction three times ensured exhaustive recovery of phenolic compounds and improved analytical reproducibility. Following filtration, combined extracts were concentrated under reduced pressure and freeze-dried at −50 °C. This process yielded 143 g alcoholic extract (Q extract 1) and 64 g hydroalcoholic extract (Q extract 2) from quince, and 93 g crude alcoholic extract (P extract 1) and 80 g hydroalcoholic extract (P extract 2) from persimmon. Extraction yield (%) was calculated with Eq. (1).

$$Yield\,(\%) = \frac{Finalweight - initialweight}{initialweight} * 100 \qquad (1)$$

Vinegar samples were freeze-dried at −50 °C under vacuum (≤0.05 mbar) for 48 h prior to extraction and analysis. Dried extracts were stored at −18 °C for further analysis of their biological activity. Commercial quince and persimmon vinegars from three different brands, complying with the Turkish Food Codex and ISO quality standards, were included. For comparative purposes, vinegar samples from a widely available brand were used as reference products. Brand names were omitted to avoid potential legal implications. Stock solutions (4.000 µg/mL) were prepared from crude extracts with dilutions made according to Akdeniz et al. [19].

LC-MS/MS analysis for organic acids and phenolic compounds

All phenolic concentrations determined by LC–MS/MS are expressed as mg/g of freeze-dried sample. Vinegar samples were freeze-dried prior to analysis to allow direct comparison with extract samples and to minimize matrix-related variability. Therefore, absolute concentration values should not be directly compared with liquid-based vinegar data reported in the literature.

Organic acids and phenolic compounds were determined by a validated LC-MS/MS method in the extracts and vinegars [19]. A Shimadzu Nexera UHPLC system connected to a triple quadrupole LC-MS/MS-8040 (Shimadzu, Japan) with the electrospray ionization source in positive and negative modes was used for analysis. Chromatographic separation was performed on a Poroshell 120 EC-C18 column (150 × 2.1 mm, 2.7 µm) at 40 °C with the mobile phase consisting of eluent A (water containing 5 mM ammonium formate and 0.1% formic acid) and eluent B (methanol containing 5 mM ammonium formate and 0.1% formic acid) using gradient elution. For SCE analysis, gradient program employed was 20–100% B (0–25 min), 100% B (25–35 min), and 20% B (35–45 min) with a flow rate of 0.5 mL/min and an injection volume of 5 µL. Quantification

was carried by MRM mode, which included optimum precursor product ion transitions and collision energies of the analytes. Instrument parameters were as follows: drying gas (N_2) flow at 15 L/min, nebulizing gas flow at 3 L/min, DL temperature at 250 °C, interface temperature at 350 °C and heat block temperature at 400 °C. Data were collected and processed by LabSolutions software (Shimadzu).

Compound identification was based on the comparison of retention times, multiple reaction monitoring (MRM) transitions, and ion ratios with those of authentic standards analysed under identical conditions.

Antioxidant activities

Antioxidant potential of the samples was assessed using three complementary assays: ABTS cation radical scavenging assay [20], DPPH free radical scavenging assay [21] and CUPRAC assay [22]. These methods provide a broad evaluation by targeting different radical species and redox mechanisms. α-Tocopherol and BHT were included as positive controls for validation. For each assay, half-maximal inhibitory concentration (IC_{50}) values were calculated from sample dilutions at concentrations of 100, 50, 25, 10 and 1 µg/mL with Microsoft Excel.

Total phenolic and flavonoid contents

Total phenolic and flavonoid contents were determined using the methods of Moreno et al. [23] and Slinkard and Singleton [24], respectively. Results were expressed as pyrocatechol equivalents (PEs) for phenolics and quercetin equivalents (QEs) for flavonoids.

Anticholinesterase activity

Acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) inhibitory activities were determined using the spectrophotometric method of Ellman et al. [25]. AChE and BChE inhibitory activities were determined according to the spectrophotometric Ellman method. Briefly, the reaction mixture contained phosphate buffer (pH 8.0), enzyme solution and sample extract, followed by pre-incubation at room temperature. The reaction was initiated by the addition of acetylthiocholine iodide or butyrylthiocholine chloride as substrates together with 5,5′-dithiobis-(2-nitrobenzoic acid) (DTNB). The formation of the yellow-colored 5-thio-2-nitrobenzoate anion was monitored at 412 nm. Galanthamine was used as the reference inhibitor, and percent inhibition was calculated relative to the control. The final concentrations of AChE and BChE were 100 U/mL and 2.28 U/mL, and acetylthiocholine iodide and butyrylthiocholine chloride were used as substrates at a final concentration of 7.085 mM and 0.785 mM. Galanthamine was tested at a final concentration of 100 µg/mL. Percent inhibition was measured at a final sample concentration of 100 µg/mL. Ethanol was used as the solvent control. The concentration of 100 µg/mL was selected as an initial screening dose, as it is widely used in in vitro enzyme inhibition studies involving plant extracts and complex matrices. This concentration allows for effective comparison of inhibitory potential across samples while minimizing non-specific effects and facilitating subsequent IC_{50} determination. In addition, analyses of all enzyme activities were repeated three times.

Antiurease activity

Urease inhibitory activity was evaluated using the method of Hina et al. [26]. Urease inhibitory activity was evaluated using a colorimetric method based on ammonia production from urea hydrolysis. Reaction mixtures containing urease enzyme, phosphate buffer and test samples were pre-incubated prior to the addition of urea substrate. After incubation, ammonia formation was quantified using a phenol–hypochlorite reaction, and absorbance was measured spectrophotometrically. Thiourea was employed as the reference inhibitor, and inhibition percentages were calculated by comparison with the control reaction. Urease was used at a final concentration of 21 U/mL, with urea as the substrate at 100 mM. Thiourea was employed as the reference inhibitor at 100 μg/mL.

Antidiabetic activity

α-Glucosidase and α-amylase inhibitory activities were determined as described by Lazarova et al. [27]. The α-amylase assay was adapted from the Caraway-Somogyi iodine/potassium iodide method to improve sensitivity and reproducibility. α-Glucosidase inhibitory activity was assessed using p-nitrophenyl-α-D-glucopyranoside as the substrate. Samples were pre-incubated with the enzyme solution, and the reaction was initiated by substrate addition. The release of p-nitrophenol was measured spectrophotometrically. α-Amylase inhibitory activity was determined using the Caraway–Somogyi iodine/potassium iodide method, which quantifies residual starch following enzymatic hydrolysis. Acarbose was used as the positive control in both assays, and inhibition percentages were calculated relative to the control. For the α-glucosidase assay, p-nitrophenyl-α-D-glucopyranoside was used as the substrate at 2.51 mM, and the enzyme concentration was 0.2 U/mL. In the α-amylase assay, soluble starch was used at 100 mg/mL with α-amylase at 10.0 U/mL.

Anti-aging activity

Anti-aging potential was assessed by measuring inhibitory activities of tyrosinase [28], elastase [29] and collagenase [30]. Anti-aging potential was evaluated by determining inhibitory activities against tyrosinase, elastase and collagenase enzymes. Tyrosinase inhibition was measured using L-DOPA as the substrate by monitoring dopachrome formation spectrophotometrically. Elastase inhibitory activity was assessed using N-succinyl-Ala-Ala-Ala-p-nitroanilide as the substrate, while collagenase inhibition was determined using FALGPA as the substrate. Kojic acid, oleanolic acid and epicatechin gallate were used as reference inhibitors for tyrosinase, elastase and collagenase assays, respectively. Enzyme inhibition percentages were calculated relative to control reactions. Percentage inhibition was calculated with Eq. (2) for all enzyme assays.

$$Inhibition\,(\%) = 100 - \left(\frac{ODtestwell}{ODcontrol}\right) * 100 \qquad (2)$$

Tyrosinase, elastase and collagenase were used at final concentrations of 17.1 KU/mL, 0.5 U/mL and 0.8 U/mL, respectively. Substrate concentrations were 8.50 mM for L-DOPA (tyrosinase), 1.015 mM mM for N-succinyl-Ala-Ala-Ala-p-nitroanilide (elastase) and 2.0 mM for FALGPA (collagenase). Kojic acid, oleanolic acid and epicatechin gallate were employed as the reference inhibitor at 100 µg/mL.

All enzyme inhibition assays were initially performed at a sample concentration of 100 µg/mL as a preliminary screening dose. Samples exhibiting inhibition greater than 50% were subsequently subjected to IC_{50} determination using serial dilutions. Substrate and enzyme concentrations for each assay were selected based on established literature protocols and are detailed below. All experiments were conducted in triplicate as technical replicates and repeated independently on three separate days to ensure biological reproducibility.

Statistical analysis

All experiments were performed in triplicate. Results are reported as mean±standard deviation. Statistically significant differences among the independent variables were determined using analysis of variance (ANOVA) with a significance level of $p \leq 0.05$. Duncan's multiple range test was employed to conduct mean comparisons in instances where a significant effect was detected. Statistical computations and principal component analysis (PCA) were used to characterize variations in phenolic compounds and performed with IBM SPSS Statistics 27.0.1 software. Data visualization was conducted using SR Plot [31].

Results and discussion

Extraction yield results

Extraction yields of quince and persimmon samples differed significantly among extraction solvents and fruit types. The highest yield was obtained from Q Extract 1 (39.7±2.1%), followed by Q Extract 2 (17.7±0.9%), P Extract 1 (11.0±0.6%) and P Extract 2 (9.5±0.5%). Statistical analysis revealed that extraction yields differed significantly between samples ($p < 0.05$), reflecting the influence of solvent polarity and plant matrix characteristics. All values are expressed as mean±standard deviation ($n = 3$).

LC-MS/MS analysis of organic acids and phenolic contents

Figure 1(A), Table S1 and Table S2 present the organic acids and phenolic composition of quince and persimmon samples showing clear differences in extraction solvents and fermentation products. In total, 53 organic acids and phenolic compounds and 3 internal standards were analysed (Fig. 2(A), Fig. 3(A), Fig. 4(A)). In quince samples, Q extract 1 (Fig. 2(B)) contained 9 organic acids and phenolic compounds while Q extract 2 (Fig. 2(C)) comprised 8 compounds. In vinegar preparations, 15 compounds were detected in Quince Vinegar 1 as shown in Fig. 3(B), 16 compounds in Quince Vinegar 2 as can be seen in Fig. 3(C) and 15 compounds in Quince Vinegar 3 as demonstrated in Fig. 3(D). The highest diversity organic acid and phenolic content was recorded in Quince Vinegar 2, followed by Quince Vinegar 3, Q Extract 2, Q Extract 1 and Quince Vinegar 1. These results are consistent with previous studies reporting higher extractable organic acids and phenolic levels in fermented fruit products, suggesting that fermentation-related matrices are associated with enhanced phenolic availability [32, 33].

Among quince samples, epicatechin represented the most abundant flavonoid detected in Quince Vinegar 2, followed by lower levels of protocatechuic acid, catechin, chlorogenic acid and caffeic acid. These findings are consistent with previous reports identifying quince as a source of hydroxycinnamic acids and flavonoids [7, 31]. The relatively high absolute concentrations observed should be interpreted in the context of dry weight–based quantification following freeze-drying, which yields higher numerical values than liquid-based vinegar expressions commonly reported in the literature.

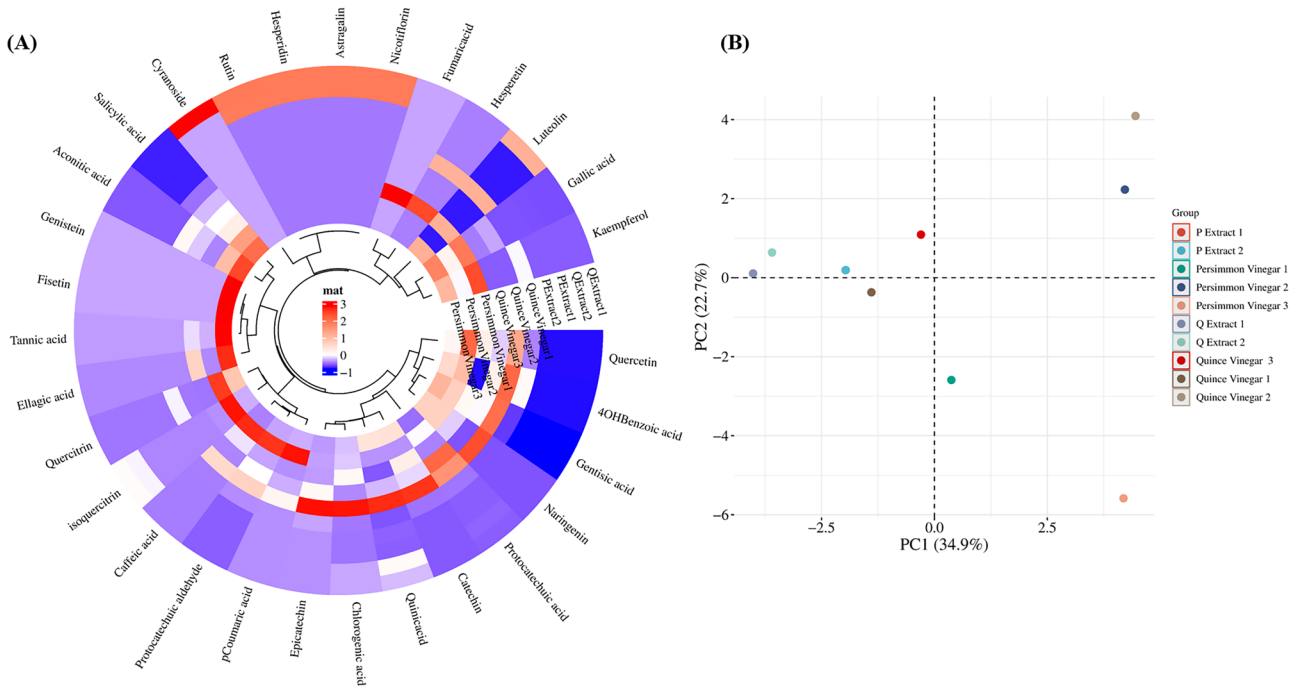


Fig. 1 **A** Heatmap, and **B** Biplot from PCA of phenolic compounds determined in Q extract 1, Q extract 2, P extract 1, P extract 2, Quince vinegar 1, Quince vinegar 2, Quince vinegar 3, Persimmon vinegar 1, Persimmon vinegar 2, and Persimmon vinegar 3 by LC-MS/MS. Q extract 1: Alcoholic quince extract, Q extract 2: Hydroalcoholic quince extract, P extract 1: Alcoholic persimmon extract, P extract 2: Hydroalcoholic persimmon extract, Quince vinegar 1: Commercial quince vinegar 1, Quince vinegar 2: Commercial quince vinegar 2, Quince vinegar 3: Commercial quince vinegar 3, Persimmon vinegar 1: Commercial persimmon vinegar 1, Persimmon vinegar 2: Commercial persimmon vinegar 2, and Persimmon vinegar 3: Commercial persimmon vinegar 3

In persimmon samples, both P extract 1 (Fig. 2(D)) and P extract 2 (Fig. 2(E)) contained only gallic acid as the phenolic component and quinic acid as the predominant organic acid, whereas vinegar samples displayed greater diversity of both organic acids and phenolic compounds. In contrast, vinegar samples showed greater organic acids and phenolic diversity, with 20 compounds identified in Persimmon Vinegar 1 (Fig. 4(B)), 19 compounds in Persimmon Vinegar 2 (Fig. 4(C)) and 23 compounds in Persimmon Vinegar 3 (Fig. 4D). Gallic acid, catechin and epicatechin were among the major phenolics detected in persimmon vinegars, while quinic acid constituted the dominant organic acid. Such compositional differences are consistent with previous studies reporting increased extractable organic acids and phenolic diversity in fermented fruit matrices.

The significant rise in phenolic concentrations associated with fermentation-related matrices agrees with prior studies showing that acetic acid bacteria and yeast not only mediate acetification but also convert complex phenolics into smaller and more bioavailable molecules [14–16]. It should be emphasized that quinic acid, although abundant in vinegar samples, is classified as an organic acid rather than a phenolic compound. Its elevated levels likely reflect fermentation- and acid-related matrix effects, as well as dry weight normalization, rather than direct enrichment of phenolic antioxidants. Quinic acid concentrations were much higher in vinegar samples especially in Quince Vinegar 2 compared to alcoholic extracts. This finding was consistent with earlier research reporting total malic and quinic acid levels ranging from 6.725 to 14.058 mg/kg in *C. oblonga* pulp and from 7.566 to 13.728 mg/kg in the peel suggesting a substantial presence of quinic acid [34, 35]. Studies further confirm that acidic or fermentation-like processing facilitates hydrolysis of bound phenolics including caffeoylquinic derivatives and thus increasing their detectability [36].

Similar trends were observed in persimmon samples. Quinic acid was repeatedly identified as one of the dominant organic acids across different cultivars and maturity stages where often matching or surpassing citric acid depending on genotype and harvest timing. Both fruits exhibited higher phenolic levels in the peels compared to pulps. This explains the elevated concentrations in vinegars prepared from whole fruits [15, 18]. In persimmon, hydrolysable tannins undergo depolymerization during fermentation and this generates smaller phenolics with potentially greater biological activity [12]. Figures 1(B) and S1 display PCA of phenolic compounds identified in both extract and vinegar samples of quince and persimmon. The first two principal components, PC1 and PC2, accounted for 34.9% and 22.7%

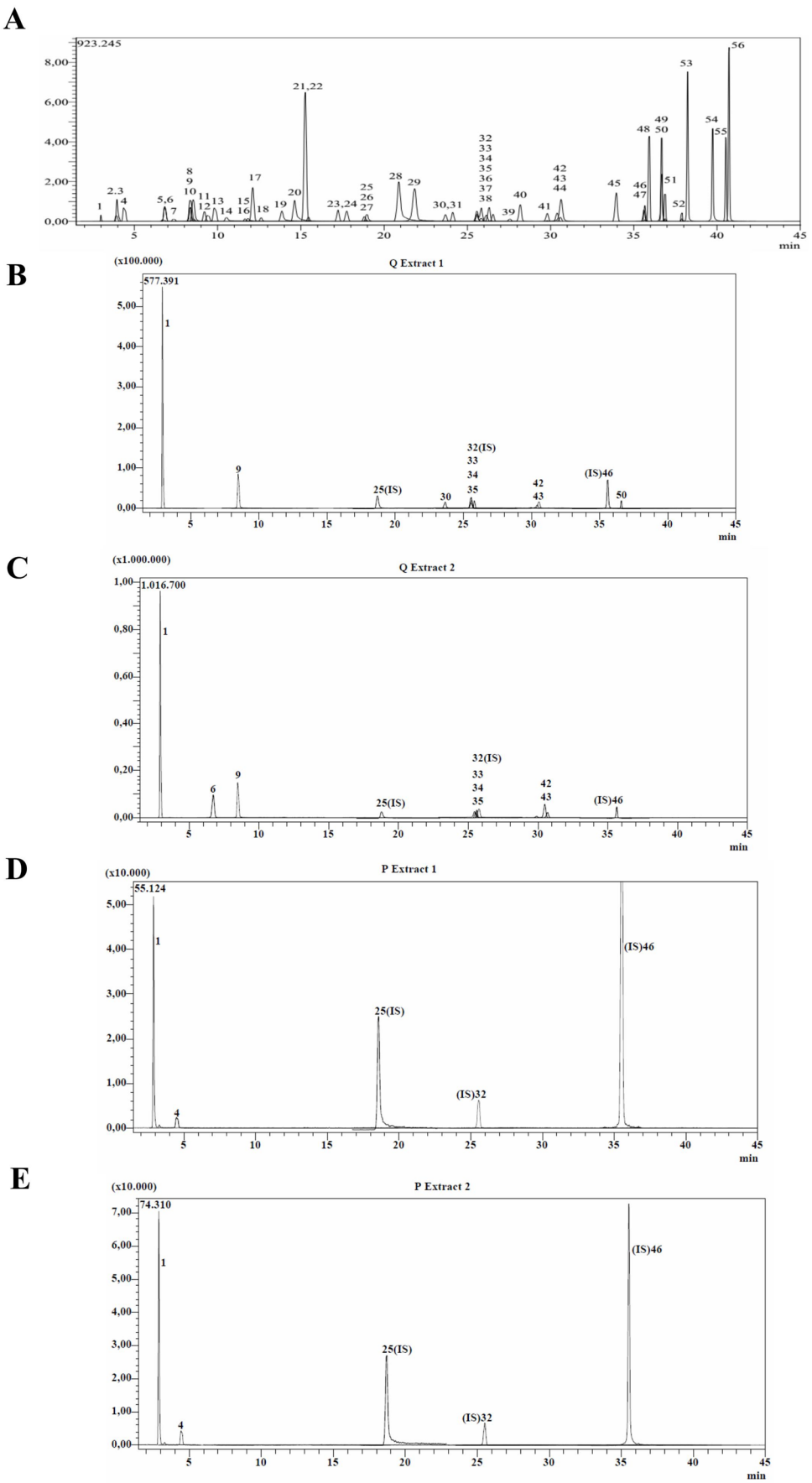


Fig. 2 LC-MS/MS chromatograms **A** TIC chromatogram of standard chemicals analysed by LC-MS/MS method. 1: Quinic acid. 2: Fumaric acid. 3: Aconitic acid. 4: Gallic acid. 5: Epigallocatechin. 6: Protocatechuic acid. 7: Catechin. 8: Gentisic acid. 9: Chlorogenic acid. 10: Protocatechuic aldehyde. 11: Tannic acid. 12: Epigallocatechin gallate. 13: 1,5-dicaffeoylquinic acid. 14: 4-OH Benzoic acid. 15: Epicatechin. 16: Vanilic acid. 17: Caffeic acid. 18: Syringic acid. 19: Vanillin. 20: Syringic aldehyde. 21: Daidzin. 22: Epicatechin gallate. 23: Piceid. 24: p-Coumaric acid. 26: Ferulic acid. 27: Sinapic acid. 28: Coumarin. 29: Salicylic acid. 30: Cynaroside. 31: Miquelianin. 32: Rutin. 34: isoquercitrin. 35: Hesperidin. 36: o-Coumaric acid. 37: Genistin. 38: Rosmarinic acid. 39: Ellagic acid. 40: Cosmosiin. 41: Quercitrin. 42: Astragalin. 43: Nicotiflorin. 44: Fisetin. 45: Daidzein. 47: Quercetin. 48: Naringenin. 49: Hesperetin. 50: Luteolin. 51: Genistein. 52: Kaempferol. 53: Apigenin. 54: Amentoflavone. 55: Chrysin. 56: Acacetin; **B** LC-MS/MS chromatogram of the Q extract 1; **C** LC-MS/MS chromatogram of the Q extract 2; **D** LC-MS/MS chromatogram of the P extract 1; **E** LC-MS/MS chromatogram of the P extract 2

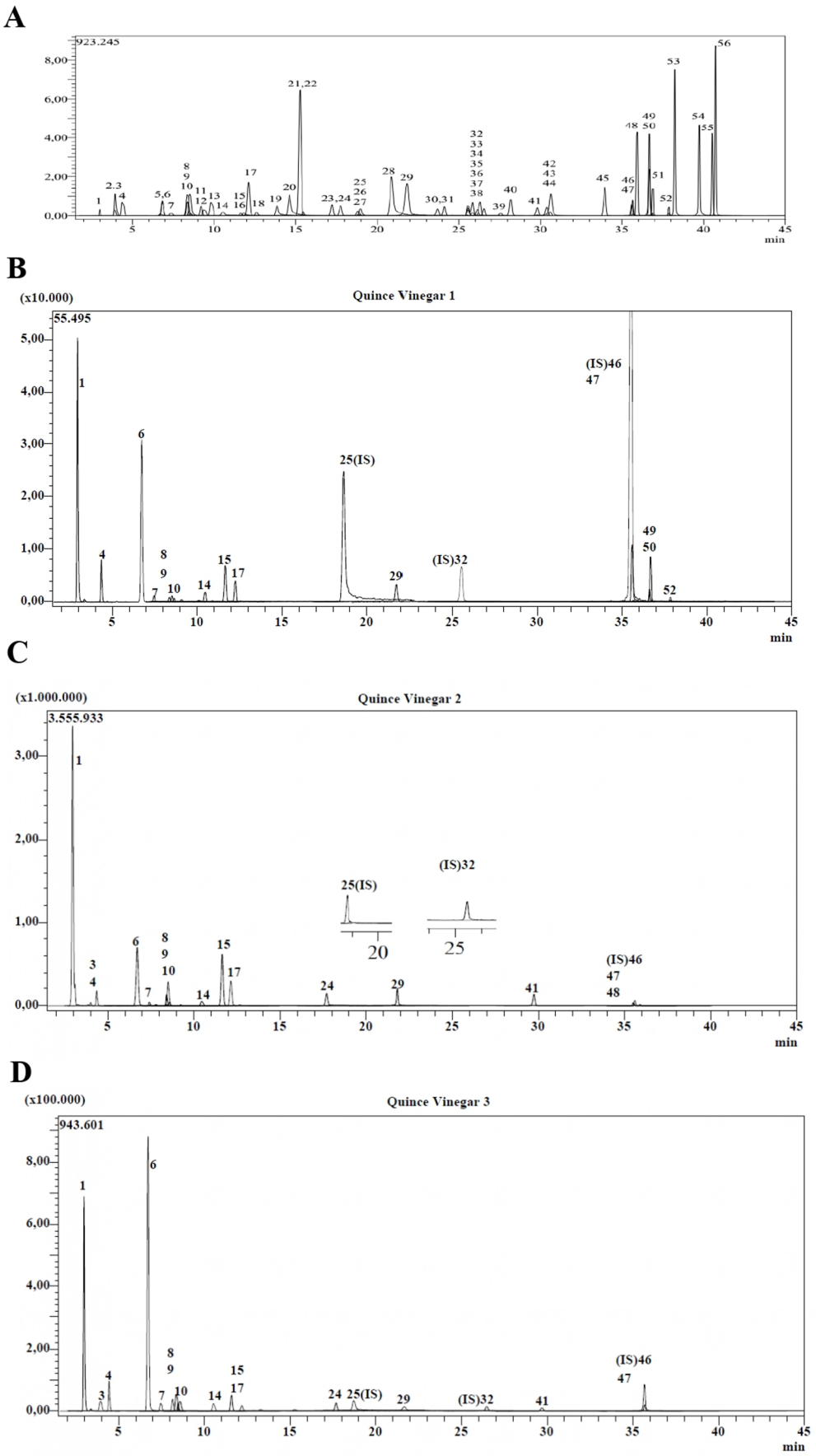

A
923.245
B
(x10.000)
Quince Vinegar 1
55.495
(IS)46
47
25(IS)
(IS)32
C
(x1.000.000)
Quince Vinegar 2
3.555.933
D
(x100.000)
Quince Vinegar 3
943.601
min

Fig. 3 LC-MS/MS chromatograms **A** TIC chromatogram of standard chemicals analysed by LC MS/MS method. 1: Quinic acid. 2: Fumaric acid. 3: Aconitic acid. 4: Gallic acid. 5: Epigallocatechin. 6: Protocatechuic acid. 7: Catechin. 8: Gentisic acid. 9: Chlorogenic acid. 10: Protocatechuic aldehyde. 11: Tannic acid. 12: Epigallocatechin gallate. 13: 1,5-dicaffeoylquinic acid. 14: 4-OH Benzoic acid. 15: Epicatechin. 16: Vanilic acid. 17: Caffeic acid. 18: Syringic acid. 19: Vanillin. 20: Syringic aldehyde. 21: Daidzin. 22: Epicatechin gallate. 23: Piceid. 24: p-Coumaric acid. 26: Ferulic acid. 27: Sinapic acid. 28: Coumarin. 29: Salicylic acid. 30: Cynaroside. 31: Miquelianin. 32: Rutin. 34: isoquercitrin. 35: Hesperidin. 36: o-Coumaric acid. 37: Genistin. 38: Rosmarinic acid. 39: Ellagic acid. 40: Cosmosiin. 41: Quercitrin. 42: Astragalin. 43: Nicotiflorin. 44: Fisetin. 45: Daidzein. 47: Quercetin. 48: Naringenin. 49: Hesperetin. 50: Luteolin. 51: Genistein. 52: Kaempferol. 53: Apigenin. 54: Amentoflavone. 55: Chrysin. 56: Acacetin; **B** LC-MS/MS chromatogram of the Quince vinegar 1; **C** LC-MS/MS chromatogram of the Quince vinegar 2; **D** LC-MS/MS chromatogram of the Quince vinegar 3

of the relative anrichment variance, respectively. Quince Vinegar 2 and Persimmon Vinegar 3 had the highest loadings according to PC1 and PC2, respectively. PCA plot provided further evidence for the distinct phenolic compound profiles of Quince Vinegar 2 and Persimmon Vinegar 3.

Overall, these findings indicate that fermentation-related matrices are associated with increased diversity and extractability of organic acids and phenolic compounds in quince and persimmon vinegars, while the extent and drivers of these changes require confirmation through controlled fermentation studies [13].

Total phenolic and flavonoid contents and antioxidant activities

Figure 5 and Table S3 reveal significant differences in total phenolic content, total flavonoids and antioxidant activity among quince and persimmon extracts and vinegars. The highest total phenolic content was found in Persimmon Vinegar 3 (171.36 µg PEs/mg) followed by Quince Vinegar 2 (102.18 µg PEs/mg). On the other hand, alcoholic and hydroalcoholic extracts (particularly from quince) contained significantly lower amounts. For instance, Q Extract 1 had a value of 5.70 µg PEs/mg. This clearly illustrates that phenolic content is greatly enhanced after fermentation probably due to the acid and enzyme-induced hydrolysis of bound phenolics.

Flavonoid contents also showed a similar trend (Fig. 5). Persimmon Vinegar 2 had the highest level (27.88 µg QEs/mg), followed by Persimmon Vinegar 1 (12.01 µg QEs/mg) and Quince Vinegar 3 (9.23 ± 0.04 µg QEs/mg). Q Extract 1 and 2 contained negligible or undetectable amounts suggesting that fermentation either releases bound flavonoids or converts them into more bioactive forms.

Antioxidant activities evaluated via DPPH, ABTS and CUPRAC assays showed a strong inverse relationship between IC_{50} values and phenolic concentrations (Fig. 5). Antioxidant assays (DPPH, ABTS and CUPRAC) were used as complementary screening methods to compare the redox-related capacity of extracts and vinegars within the same experimental system. The lowest IC_{50} values (indicating the highest antioxidant capacity) were observed in Persimmon Vinegar 3 and Quince Vinegar 2, in some cases surpassing the standard antioxidants BHT and α-tocopherol as can be seen in Fig. 5 and Table S3. These results align with earlier studies reporting that acidic and microbial fermentation not only enhances the release of caffeic, chlorogenic, and gallic acids but also liberates flavonoids such as quercetin and catechins, thereby improving the radical scavenging and reducing capacities of fruit-derived products [13, 14, 32, 33]. Given the complex matrix nature of vinegars and extracts, antioxidant responses should be interpreted at the sample level rather than attributed to individual compounds. These assays provide a relative comparison of antioxidant-related properties among samples but do not directly translate into functional or health-related outcomes. Accordingly, antioxidant assay results should be interpreted as comparative indicators of redox-related capacity rather than predictors of in vivo efficacy. Therefore, these findings highlight the functional food potential of fermented vinegars as a natural source of phenolic antioxidants.

Enzyme inhibition activity results

The results shown in Fig. 6 and Table S4 reveal significant differences in anticholinesterase, antiurease, antidiabetic and anti-aging activities in the quince and persimmon samples. At tested concentration Persimmon Vinegar 2 showed the strongest inhibition against acetylcholinesterase (67.81%) and butyrylcholinesterase (23.40%) although both remained below the reference inhibitor galanthamine (89.98% and 77.93%, respectively). In general, vinegar samples exhibited higher inhibitory activity than raw extracts which indicates that fermented vinegar matrices are associated with higher availability of organic acids and phenolic constituents with enzyme inhibitory potential. This finding aligns with the study of Dereli et al. [37] where a link between polyphenol-rich fermented foods and neuroprotective effects through the inhibition of cholinesterase was demonstrated.

All vinegar samples also showed a strong urease inhibition, particularly Persimmon Vinegar 1 (93.12%) and Persimmon Vinegar 2 (92.18%), approaching the activity of thiourea (96.56%). Since urease-producing bacteria (such as *Helicobacter pylori*) are associated with peptic ulcer disease and an increased risk of gastric cancer. Mentioned inhibition observed here suggests a potential in vitro relevance in the context of urease inhibition, which warrants further investigation under controlled conditions. This effect is likely driven by fermentation-mediated release of phenolics with

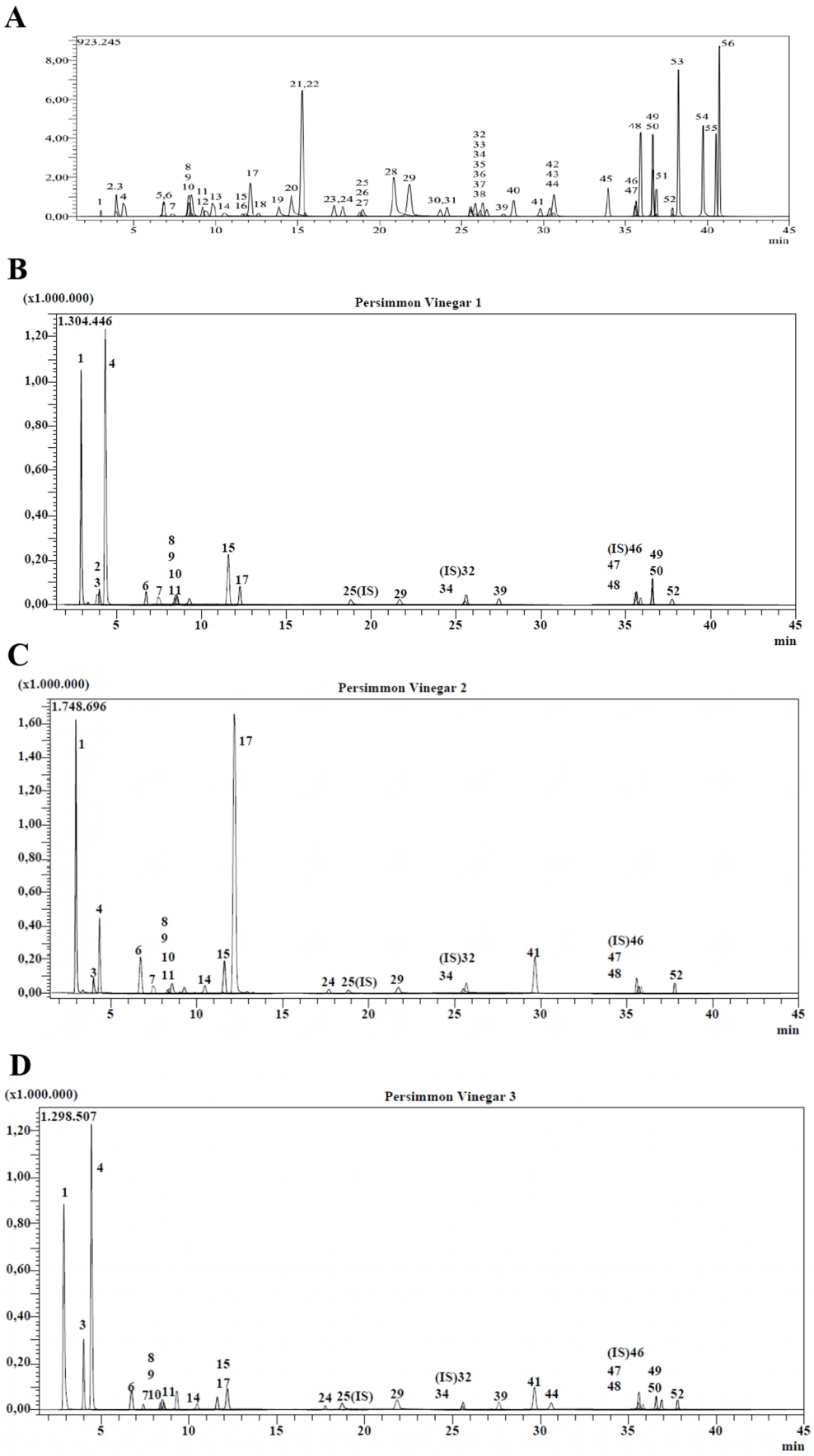

A
923.245
B
(x1.000.000)
Persimmon Vinegar 1
1.304.446
C
(x1.000.000)
Persimmon Vinegar 2
1.748.696
D
(x1.000.000)
Persimmon Vinegar 3
1.298.507
min

Fig. 4 LC-MS/MS chromatograms **A** TIC chromatogram of standard chemicals analysed by LC-MS/MS method. 1: Quinic acid. 2: Fumaric acid. 3: Aconitic acid. 4: Gallic acid. 5: Epigallocatechin. 6: Protocatechuic acid. 7: Catechin. 8: Gentisic acid. 9: Chlorogenic acid. 10: Protocatechuic aldehyde. 11: Tannic acid. 12: Epigallocatechin gallate. 13: 1,5-dicaffeoylquinic acid. 14: 4-OH Benzoic acid. 15: Epicatechin. 16: Vanilic acid. 17: Caffeic acid. 18: Syringic acid. 19: Vanillin. 20: Syringic aldehyde. 21: Daidzin. 22: Epicatechin gallate. 23: Piceid. 24: p-Coumaric acid. 26: Ferulic acid. 27: Sinapic acid. 28: Coumarin. 29: Salicylic acid. 30: Cynaroside. 31: Miquelianin. 32: Rutin. 34: isoquercitrin. 35: Hesperidin. 36: o-Coumaric acid. 37: Genistin. 38: Rosmarinic acid. 39: Ellagic acid. 40: Cosmosiin. 41: Quercitrin. 42: Astragalin. 43: Nicotiflorin. 44: Fisetin. 45: Daidzein. 47: Quercetin. 48: Naringenin. 49: Hesperetin. 50: Luteolin. 51: Genistein. 52: Kaempferol. 53: Apigenin. 54: Amentoflavone. 55: Chrysin. 56: Acacetin; **B** LC-MS/MS chromatogram of the Persimmon vinegar 1; **C** LC-MS/MS chromatogram of the Persimmon vinegar 2; **D** LC-MS/MS chromatogram of the Persimmon vinegar 3

antimicrobial and anti-inflammatory properties. This is consistent with previous findings on polyphenol-rich vinegars [14].

The highest tyrosinase inhibition was recorded for Quince Vinegar 3 (62.30%) and Persimmon Vinegar 2 (55.20%), however, both remained below kojic acid (83.22%). Given tyrosinase's central role in melanin biosynthesis and its link to hyperpigmentation disorders, these results support the cosmeceutical potential of quince and persimmon vinegars. Previous studies have confirmed that phenolic acids and flavonoids (abundant in fermented plant products) exhibit skin-lightening and antioxidant properties [38].

For anti-aging activity, collagenase inhibition was modest and observed only in Persimmon Vinegar 2 (3.54%). Although there was low and even partial suppression of collagenase and elastase both were involved in extracellular matrix degradation and may delay skin aging. Phenolics such as quercetin, catechins and tannins present in these vinegars have previously been reported to show protective effects against skin structural damage [14, 39].

All vinegar samples demonstrated strong inhibition of carbohydrate-hydrolysing enzymes with the most pronounced effects against α-glucosidase. Persimmon Vinegar 2 achieved the highest inhibition (99.10%) far exceeding acarbose (36.79%). The α-amylase inhibition was moderate and peaked in Quince Vinegar 2 (60.84%) and Persimmon Vinegar 2 (48.27%). The combined inhibition of both enzymes suggests a synergistic antidiabetic effect as α-glucosidase inhibition delays glucose absorption and α-amylase inhibition reduces starch hydrolysis. This contributes to the postprandial glycemic control. These results are consistent with studies showing that tannin- and flavonoid-rich extracts modulate carbohydrate metabolism by suppressing digestive enzyme activity [15, 16].

In the present study, dose–effect relationships were evaluated at the extract and vinegar matrix level rather than at the level of individual phytochemicals. Given that the biological assays were performed using complex mixtures, the observed activities reflect the cumulative and synergistic effects of multiple phenolic constituents, as evidenced by the consistent association between higher organic acid and phenolic content and stronger bioactivity.

The considerable variability observed in phenolic composition and bioactivity among vinegars derived from the same fruit type can be attributed to multiple interconnected factors inherent to fermented food systems. Differences in fruit cultivar and harvest maturity are known to influence the initial phenolic profile, while variations in fermentation-associated microorganisms may lead to distinct biotransformation pathways and metabolite formation. In addition, acetic acid concentration and fermentation dynamics can modulate phenolic stability and enzyme inhibitory activity. Post-fermentation factors, including aging duration, storage conditions and processing practices, may further contribute to compositional and functional differences among commercial vinegar products. Collectively, these factors highlight that the observed variability reflects the intrinsic complexity of vinegar production rather than experimental inconsistency, underscoring the importance of standardized processing conditions for achieving reproducible phenolic profiles and bioactivities.

The enzyme inhibition trends observed in this study are consistent with the LC–MS/MS organic acids and phenolic profiles of the samples. These findings support the hypothesis that fermentation-induced modulation of phenolic classes, rather than changes in individual compounds alone, plays a key role in shaping the observed bioactivity patterns. Vinegar samples exhibiting higher inhibition of α-glucosidase, urease and cholinesterases were characterized by elevated levels of including organic acids (notably quinic acid) and phenolic acids and flavonoids such as chlorogenic acid, caffeic acid and catechins. Although these certain organic acids and phenolic compounds were quantitatively dominant in some extracts and all vinegars, the observed biological activities are better explained by synergistic interactions among multiple phenolics enhanced through fermentation, rather than by the action of a single dominant compound.

Fermentation-associated enrichment of phenolic compounds may be reflected in altered bioactivity patterns, potentially contributing to the stronger inhibitory responses observed in vinegar samples compared to their corresponding extracts. However, direct causality cannot be established due to the complex and heterogeneous nature of the sample matrices. Therefore, the observed differences should be interpreted at the matrix level, as fermented vinegars and ethanolic extracts differ fundamentally in acidity, solvent composition and compound solubility. Within this context, the concordance between phenolic enrichment trends and

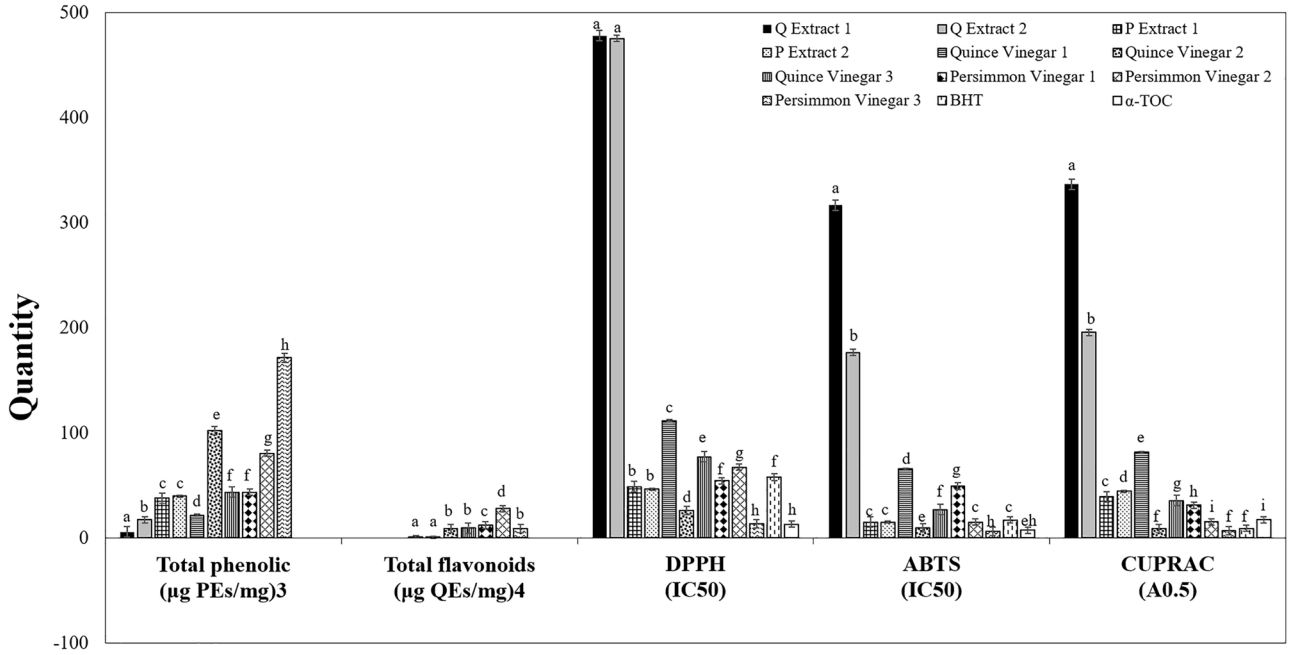


Fig. 5 Total phenolic-flavonoid contents, and antioxidant activities of the samples. Q extract 1: Alcoholic quince extract, Q extract 2: Hydroalcoholic quince extract, P extract 1: Alcoholic persimmon extract, P extract 2: Hydroalcoholic persimmon extract, Quince vinegar 1: Commercial quince vinegar 1, Quince vinegar 2: Commercial quince vinegar 2, Quince vinegar 3: Commercial quince vinegar 3, Persimmon vinegar 1: Commercial persimmon vinegar 1, Persimmon vinegar 2: Commercial persimmon vinegar 2, and Persimmon vinegar 3: Commercial persimmon vinegar 3. Samples with common lowercase letters are not significantly different ($p > 0.05$)

enzyme inhibition profiles should be regarded as comparative and hypothesis-generating rather than mechanistic.

A major limitation of this study is the use of commercially available vinegars, for which detailed information on fermentation conditions, microbial consortia, acetic acid content, pH and maturation time is not accessible. Consequently, the observed differences among vinegar samples cannot be mechanistically attributed to specific fermentation parameters, raw materials or processing variables. Due to the complex and multicomponent nature of the matrices analysed, compound-specific structure–activity relationships were beyond the scope of the present study and will require future investigations using isolated compounds and controlled systems. In addition, comparisons between ethanolic extracts and vinegars should be interpreted cautiously, as these matrices differ substantially in acidity, solvent composition and compound solubility. Therefore, the present findings should be regarded as comparative and hypothesis-generating, highlighting the need for future studies employing controlled fermentation systems.

Conclusions

This study provides a comprehensive evaluation of the phenolic profile and multifunctional biological properties of quince and persimmon vinegars in comparison with their alcoholic and hydroalcoholic extracts. LC–MS/MS analysis demonstrated that fermentation significantly enhanced total phenolic and flavonoid levels, with Quince Vinegar 2 and Persimmon Vinegar 3 exhibiting the highest enrichment. These findings suggest that acid and microbial fermentation facilitate the release of bound organic acids (such as quinic acid) and phenolic compounds, including caffeic, chlorogenic and gallic acids, and promote their conversion into more bioavailable forms.

Biological assays revealed that vinegar samples exhibited stronger antioxidant, anticholinesterase, antiurease and antidiabetic activities, along with moderate anti-aging potential, compared to non-fermented extracts. In particular, the pronounced α-glucosidase and urease inhibition observed in persimmon vinegars, together with the strong antioxidant and cholinesterase inhibitory activities detected in both fruit vinegars, highlight their relevance as functional food matrices under in vitro conditions. Consistent with the chemical profiling results, vinegar samples exhibiting stronger enzyme inhibition were also characterized by higher organic acid and phenolic diversity and abundance, supporting a composition–activity relationship at the extract and vinegar matrix level.

Overall, the findings of this study support the initial hypothesis that fermentation enhances phenolic bioavailability and leads to stronger antioxidant and enzyme inhibitory activities in quince and persimmon vinegars compared

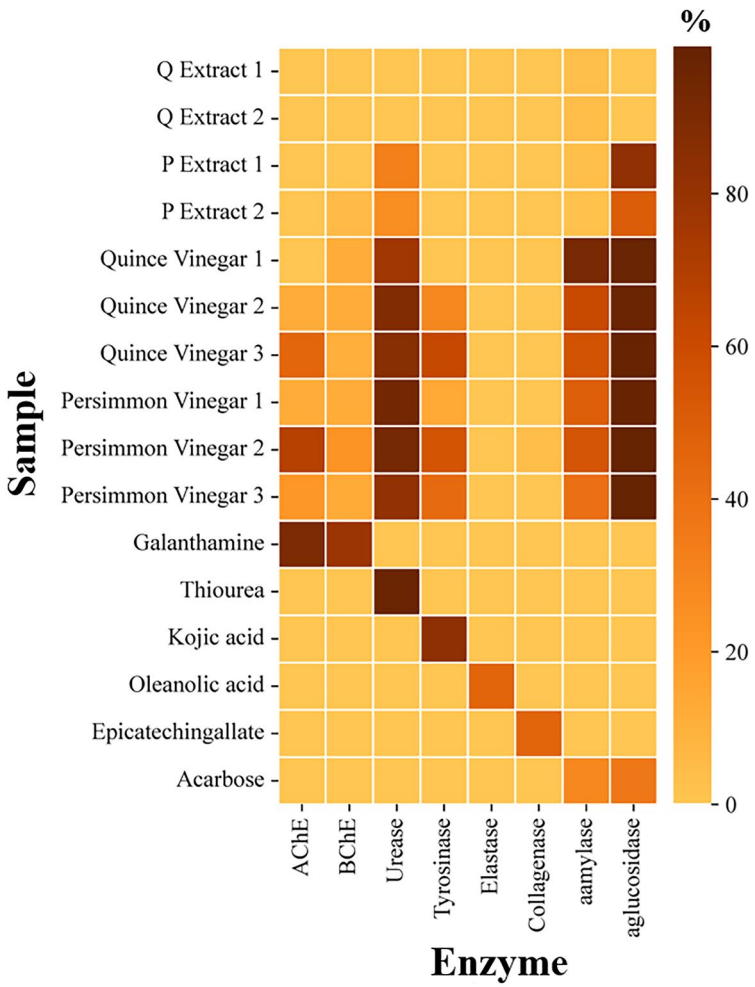


Fig. 6 Heatmap demonstration of enzyme inhibition activities (%) of samples at 100 µg/mL. Q extract 1: Alcoholic quince extract, Q extract 2: Hydroalcoholic quince extract, P extract 1: Alcoholic persimmon extract, P extract 2: Hydroalcoholic persimmon extract, Quince vinegar 1: Commercial quince vinegar 1, Quince vinegar 2: Commercial quince vinegar 2, Quince vinegar 3: Commercial quince vinegar 3, Persimmon vinegar 1: Commercial persimmon vinegar 1, Persimmon vinegar 2: Commercial persimmon vinegar 2, Persimmon vinegar 3: Commercial persimmon vinegar 3, AChE: acetylcholinesterase, and BChE: butyrylcholinesterase

to their corresponding non-fermented extracts. Variations observed among vinegars derived from the same fruit types are likely attributable to differences in raw materials, harvest maturity and processing conditions, underscoring the need for standardized production processes to ensure consistent phenolic profiles and bioactive properties. Although the present findings highlight the functional potential of quince and persimmon vinegars, all biological effects reported in this study are based on in vitro assays and should therefore be interpreted with caution, pending further in vivo and clinical validation. From an application perspective, these results suggest that quince and persimmon vinegars may be considered as value-added fermented fruit products or functional food ingredients for food and beverage formulations.

Within the broader context of functional fruit vinegars, quince and persimmon vinegars demonstrated comparable and, in some assays, relatively stronger in vitro bioactivities when compared with previously reported vinegars such as apple cider, grape and pomegranate vinegars. These differences may be attributed to the distinct phenolic and organic acid profiles of quince and persimmon matrices, as well as fermentation-related compositional changes. However, such comparisons should be interpreted cautiously, as differences in raw materials, processing conditions and analytical approaches across studies limit direct equivalence. Accordingly, the present findings position quince and persimmon vinegars as promising additions to the functional fruit vinegar category, warranting further investigation under controlled conditions.

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Author contributions Güleyfe Beyza Yıldırım: Methodology, Investigation, Extraction, Data curation. Çağlayan Ünsal Gürer: Methodology, Supervision of extraction, Review. İnci Kurt Celep: Investigation, Biological analysis. Mehmet Akdeniz, Ismail Yener and Serkan Yigitkan: Biological analysis, Data curation. Mustafa Abdullah Yılmaz: Organic acid and phenolic content analysis (LC–MS/MS). Suzan Tireki: Statistical analysis, Writing. Abdulselam Ertas: Data analysis, Writing, Review, and Editing. Aslı Barla Demirkoz: Conceptualization, Project

administration, Writing, Review and Editing.

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Data availability Data will be made available on request.

Declarations

Conflict of interest The authors declare no conflicts of interest.

Ethical approval This article does not contain any studies with human or living animal subjects.

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