

RESEARCH ARTICLE OPEN ACCESS

Antioxidant Capacity and Characterization of Polyphenolic Compounds in Processed Falsa (*Grewia asiatica* L.) Products Using LC-ESI-QTOF-MS/MS Analysis

X. Qin¹ | A. Din² | M. R. Khan² | M. A. Shabbir² | A. Rafi² | A. Siddiqua¹ | O. T. Agar¹ | A. Beyatli¹ | A. Imran¹ | F. Ahmadi¹ | H. A. R. Suleria¹

¹School of Agriculture, Food and Ecosystem Sciences, Faculty of Science, The University of Melbourne, Melbourne, Victoria, Australia | ²National Institute of Food Science and Technology, University of Agriculture, Faisalabad, Pakistan

Correspondence: H. A. R. Suleria (hafiz.suleria@unimelb.edu.au)

Received: 15 September 2025 | **Revised:** 30 March 2026 | **Accepted:** 16 May 2026

Academic Editor: Essam Hebishy

Keywords: antioxidant potential | fruit processing | functional food | phytochemistry | polyphenols

ABSTRACT

Fruits provide abundant vitamins, minerals, dietary fiber, and natural antioxidants. Falsa (*Grewia asiatica*), a lesser-known berry fruit, is gaining attention owing to its diverse bioactive compounds and potential health-promoting properties. This study aimed to evaluate the polyphenol content and antioxidant activity of processed products made from natural and organic Falsa. Natural Falsa fruit leather had the highest total polyphenolic content (6.04 ± 0.31 mg gallic acid equivalents [GAE]/g), followed by organic Falsa fruit leather (3.67 ± 0.13 mg GAE/g), with natural Falsa generally having a greater polyphenolic content. Antioxidant activity, assessed using 2,2'-diphenyl-1-picrylhydrazyl (DPPH), ferrous ion chelating activity (FICA), ferric reducing antioxidant power (FRAP), 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), and total antioxidant capacity assays, was strongest in natural Falsa fruit leather. Liquid chromatography–electrospray ionization–quadrupole time-of-flight tandem mass spectrometry (LC-ESI-QTOF-MS/MS) analysis enabled the tentative identification of 88 polyphenolic compounds across the analyzed samples. These compounds were classified into major groups, including phenolic acids (32), flavonoids (36), lignans (6), stilbenes (2), and other polyphenols (12). HPLC analysis demonstrated a greater concentration of epicatechin and *p*-hydroxybenzoic acid in natural Falsa pulp compared to other Falsa products. In conclusion, our findings suggest that both fruit types (natural or organic) and the processing method have a significant impact on the polyphenolic composition and antioxidant activity of Falsa-based products.

1 | Introduction

Fruits form an integral part of a balanced diet, supplying vital nutrients such as vitamins, minerals, and dietary fiber, along with a rich source of natural antioxidants. The antioxidants are especially important for reducing oxidative stress and promoting health [1]. Berries are especially valued for their flavor, color, and high polyphenolic content, which contributes to both their nutritional and their putative health-promoting properties. Their polyphenolic profile, including phenolic acids, flavonoids, and anthocyanins, is influenced by genetic factors (species and

cultivar) and by environmental and technological variables such as climate, harvest maturity, and postharvest handling and processing conditions [2].

Falsa (*Grewia asiatica* L.) is a shrub or small tree that typically grows up to 8 m tall. Its bark is grayish-white to brown, and the young shoots are covered with stellate hairs. The fruit is a small, dark purple drupe, 5–12 mm in diameter, with a hairy surface and a fibrous, acidic mesocarp. The fruits have a characteristic sweet-sour flavor and are consumed fresh or processed into beverages, jams, and other food products [3]. In addition to its culinary uses,

This is an open access article under the terms of the [Creative Commons Attribution](https://creativecommons.org/licenses/by/4.0/) License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited.

Copyright © 2026 X. Qin et al. *Journal of Food Processing and Preservation* published by John Wiley & Sons Ltd.

G. asiatica is valued in traditional medicine, where its pulp, leaves, and bark are incorporated into various therapeutic formulations and food preparations [3–5]. Despite its promising nutritional and phytochemical profile, comparatively few studies have investigated the polyphenolic composition and antioxidant capacity of Falsa in processed products relative to the growing body of work on fresh fruit and extracts. For example, Koley et al. [6] conducted a comprehensive polyphenolic profiling of fresh Falsa fruits and identified 50 compounds. However, their analysis focused only on the fresh fruit matrices, without examining how processing conditions affect the polyphenolic composition. More recently, Saeed et al. [7] characterized the compositional changes during fruit ripening across six developmental stages (from the initial fruit setting stage to fully ripened fruit) and reported increases in total phenolics, anthocyanins, and antioxidant capacity with progression in Falsa fruit development, yet their work did not address formulation-based or processing-induced changes. Although these reports have provided a detailed characterization of polyphenolic compounds in fresh and developing Falsa fruits, there is limited information regarding how processing conditions alter the polyphenolic profile and antioxidant capacity of Falsa-derived products. This information is important as the perishability of the fruit necessitates processing to extend shelf life, facilitating distribution and supporting commercial utilization. Processing, however, may result in changes in phenolic compounds, thereby altering both their qualitative profile and functional antioxidant properties. Therefore, the primary objective of this investigation was to quantify the total polyphenolic content and to evaluate the antioxidant activity (determined via established chemical assays) in a range of processed Falsa products derived from both naturally and organically cultivated sources. The specific product matrices selected for analysis were fruit squash, nectar, drink, jam, and leather, as these products embody the primary commercial and domestically prepared forms of Falsa. Their inclusion reflects both the cultural significance and economic relevance of these products, owing to their widespread presence in local markets and their frequent preparation in household settings. In addition, the study incorporated samples produced under both conventional (natural) and organic cultivation systems, enabling a direct comparison of production practices that may differentially influence the phytochemical composition. This aspect is important to investigate, as variations in nutrient management strategies and agrochemical inputs might affect polyphenolic content, antioxidant capacity, and the overall phytochemical profile. Liquid chromatography–electrospray ionization–quadrupole time-of-flight tandem mass spectrometry (LC-ESI-QTOF-MS/MS) was employed to tentatively identify the polyphenolic compounds, and HPLC quantification was used to accurately quantify the selected phenolic compounds. This approach aimed to enable direct comparison between natural and organic Falsa products and elucidate the influence of processing on polyphenolic classes and antioxidant capacity.

2 | Materials and Methods

2.1 | Chemicals and Reagents

All standards and reagents were of analytical grade. Unless specified otherwise, they were obtained from Sigma-Aldrich (Castle Hill, New South Wales, Australia), including Folin–Ciocalteu

phenol reagent, anhydrous sodium carbonate, sodium phosphate, gallic acid, sodium acetate, quercetin, aluminum chloride hexahydrate, vanillin, catechin hydrate, potassium persulfate, hydrochloric acid, iron(III) chloride (FeCl_3), *L*-ascorbic acid, 2,2'-diphenyl-1-picrylhydrazyl (DPPH), 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), and 2,4,6-tripyr-idyl-*s*-triazine (TPTZ). HPLC standards (epicatechin, gallic acid, *p*-hydroxybenzoic acid, *p*-coumaric acid, and *trans*-ferulic acid) were also sourced from Sigma-Aldrich. HPLC-grade and liquid chromatography–mass spectrometry (LC-MS)–grade solvents (acetonitrile, glacial acetic acid, ethanol, methanol, and formic acid) were supplied by Thermo Fisher Scientific Inc. (Scoresby, Victoria, Australia). Sulfuric acid (98%) was purchased from RCI Labscan (Rongmuang, Thailand).

2.2 | Sample Preparation

A total of 12 processed Falsa products were analyzed, comprising two categories: organic and natural Falsa, with six processing methods applied to each. Fresh Falsa fruits were sourced from Ayub Agriculture Research Institute (Faisalabad, Pakistan). Organic Falsa was sourced from orchards managed without synthetic inputs, where growers applied only farmyard manure and relied exclusively on canal irrigation, supplied approximately 6–7 times/year. No synthetic pesticides or chemical fertilizers were used in these orchards; however, the fruit was not formally certified under any organic certification scheme. In contrast, natural Falsa was obtained from conventional orchards in which farmers routinely applied farmyard manure in combination with diammonium phosphate and urea fertilizers, with canal-water irrigation conducted 6–7 times annually. The Falsa fruits were initially manually sorted to exclude underripe or damaged specimens, then thoroughly washed with distilled water and air-dried. The edible pulp was manually separated from the seeds using a sanitized stainless-steel knife and immediately stored at -18°C until subsequent processing. For dehydration, the fruit pulp was frozen at -40°C and then freeze-dried for 72 h using a Benchtop freeze dryer (Model SDF-12, Bionics Scientific Technologies, Van Nuys, California, United States). The dry pulps were ground into a fine powder using a commercial grinder and stored in airtight, aluminum foil-sealed bags prior to transportation to the University of Melbourne for further investigations. In addition to powdered pulp, several other processed products, including fruit squash, fruit nectar, fruit drink, fruit jam, and fruit leather, were also prepared from the Falsa fruits, following the detailed procedures described in the subsequent sections.

2.2.1 | Falsa Fruit Pulp Extraction

Fresh Falsa fruits (40 kg) were weighed and mixed with 10 L of reverse osmosis (RO) water. The mixture was passed through a fine pulper to obtain fruit pulp, which was then divided into two portions. The first portion was preserved by adding potassium metabisulfite (0.1%), sodium benzoate (0.1%), and citric acid (0.5%), filled into sterilized cans, and then stored at ambient temperature. The second portion, consisting of untreated, chemical-free pulp, was stored at -18°C for subsequent analysis and fruit leather preparation.

2.2.2 | Falsa Fruit Squash Preparation

The squash was prepared using 50% fruit pulp and 50% RO water. Sugar was added to adjust the sweetness to 45–48°Brix. For sugar-free formulations, stevia was used at a rate of 3 g to replace 1 kg of sugar as a natural sweetener. Citric acid and food-grade color were then added and mixed thoroughly, followed by the addition of potassium metabisulfite immediately before filling. The product was bottled, labeled, and stored at ambient temperature for subsequent analysis.

2.2.3 | Falsa Fruit Nectar Preparation

The nectar was formulated using 20% fruit pulp and 80% RO water. Sugar was added to adjust the sweetness to 15°Brix. Citric acid (0.2%) and carboxymethyl cellulose (0.15%) were weighed and incorporated into the mixture to obtain a uniformly blended pulp. The blend was transferred to a heating pan and heated to 85°C. Once the desired temperature was achieved, the heat source was turned off, and food-grade color (0.05%), sodium citrate (0.1%), and sodium benzoate (0.1%) were incorporated while maintaining constant stirring. The mixture was then filtered through muslin cloth and hot-filled at 65°C into presterilized PET bottles. Finally, the bottles were labeled and stored at ambient temperature for subsequent analysis.

2.2.4 | Falsa Fruit Drink Preparation

The drink was formulated using 10% fruit pulp and 90% RO water. Sugar was added to adjust the sweetness to 13°Brix. Citric acid (0.3%) and carboxymethyl cellulose (0.15%) were accurately weighed and incorporated into the mixture to obtain a uniformly blended pulp. The blend was then transferred to a heating pan and heated to 85°C. Upon reaching the target temperature, heating was discontinued, and food-grade color (0.05%), sodium citrate (0.1%), and sodium benzoate (0.1%) were added with continuous stirring. The mixture was filtered through muslin cloth and hot-filled at 65°C into presterilized PET bottles. Finally, the bottles were labeled and stored at ambient temperature for subsequent product analysis.

2.2.5 | Falsa Fruit Jam Preparation

Pectin was first dissolved in warm water to ensure complete hydration. Fine Falsa pulp (1 kg) was then combined with sorbitol (1 kg) and cooked together with the pectin solution until the mixture reached the desired gel-like consistency. After removal from heat, stevia (3 g/kg) was incorporated as a natural sugar replacer. Citric acid (0.4%), food-grade color (0.05%), and sodium benzoate (0.1%) were added immediately before filling. The prepared jam was allowed to cool slightly, transferred into presterilized glass jars, sealed, labeled, and stored at ambient temperature until further analysis.

2.2.6 | Falsa Fruit Leather Preparation

Falsa fruit pulp, preserved at –18°C without added food chemicals (as described in the pulp extraction method), was used for

leather preparation. Pectin (0.5%) and stevia (0.025%) were incorporated, and the mixture was blended until a smooth consistency was achieved. The blend was then poured into lightly oiled stainless-steel molds. The trays were placed in a dehydrator at 55°C until the moisture content was reduced to approximately 15%. The dried product was cut into rectangular pieces, rolled, packaged, and stored at ambient temperature for subsequent analyses.

2.3 | Extract Preparation

Solid samples (5 g) were placed in test tubes and mixed with 15 mL of pure methanol, then subjected to shaking incubation at 120 rpm and 10°C for 1 h. In the case of liquid or semiliquid samples, 5 mL of the sample was blended with 2.5 mL of pure methanol in a test tube. All sample mixtures were then centrifuged at 8000 rpm for 15 min at 4°C. The resulting supernatants were transferred to clean test tubes and stored at –20°C until analysis.

2.4 | Polyphenolic Compounds

2.4.1 | Total Phenolic Content (TPC)

TPC was assessed using the procedure of Samsonowicz et al. [8] with minor modifications. Aliquots of Falsa sample extracts were dispensed into the wells of a 96-well microplate, followed by the addition of 25 µL of Folin–Ciocalteu reagent. Following a 5-min incubation at 25°C, 200 µL of distilled water and 25 µL of a 10% sodium carbonate solution were introduced into each well. The plate was subsequently incubated at room temperature for 1 h. Absorbance was measured at 725 nm using a microplate reader (Thermo Scientific, Waltham, Massachusetts, United States). Gallic acid standards ranging from 0 to 200 µg/mL (prepared in ethanol) were used to construct the calibration curve. The outcomes were reported as mg of gallic acid equivalents (mg GAE)/g sample.

2.4.2 | Total Flavonoid Content (TFC)

TFC was determined using a colorimetric method [9] with slight modifications. Falsa sample extracts were added to wells of a 96-well microplate, followed by the addition of 2% aluminum chloride solution and 50 g/L sodium acetate. The plate was incubated at 25°C for 2.5 h. After incubation, absorbance was measured at 440 nm. A standard calibration curve was constructed using quercetin at concentrations ranging from 0 to 50 µg/mL. Results were reported as mg of quercetin equivalents (mg QE)/g sample.

2.4.3 | Total Condensed Tannin (TCT)

TCT content was determined according to the procedure of Peng et al. [10] with slight modifications. Briefly, 25 µL of Falsa extract was mixed with 150 µL of 4% (w/v) vanillin solution, after which 25 µL of 32% sulfuric acid was added. The reaction mixture was incubated at room temperature for 15 min, and absorbance was measured at 500 nm. A catechin calibration curve (0–1000 µg/mL in methanol) was prepared, and results were expressed as mg of catechin equivalents (mg CE)/g sample.

2.4.4 | DPPH

The DPPH radical scavenging capacity was determined following the procedure described by Vella et al. [11]. A 0.1 M DPPH solution was prepared in analytical-grade methanol. In each well of a 96-well microplate, 260 μ L of the DPPH solution was combined with 40 μ L of the sample extract. The plate was then kept in the dark at 25°C for 30 min to facilitate the reaction. After incubation, absorbance was recorded at 517 nm. A calibration curve was generated with Trolox standards (0–50 μ g/mL), and results were expressed either as mg of Trolox equivalents (mg TE)/g sample or as percentage radical scavenging activity.

2.4.5 | Ferric Reducing Antioxidant Power (FRAP)

The FRAP assay was conducted according to the procedure of Sogi et al. (2013) with minor adjustments. Fresh FRAP reagent was prepared by combining 300 mM acetate buffer (pH 3.6), 10 mM TPTZ solution (in 40 mM HCl), and 20 mM ferric chloride at a volume ratio of 10:1:1. In a 96-well microplate, 25 μ L of Falsa extract was added to 280 μ L of the FRAP reagent. The mixture was incubated at 37°C for 10 min to facilitate the reduction reaction, after which absorbance was recorded at 593 nm. A calibration curve was generated using Trolox standards (0–50 μ g/mL). Results were expressed as mg TE/g sample.

2.4.6 | ABTS

The ABTS assay was conducted following the method of Vella et al. [11], with modifications. The ABTS⁺ radical cation was generated by mixing 1.25 mL of 7 mM ABTS solution with 22 μ L of 140 mM potassium persulfate, followed by incubation in the dark at room temperature for 16 h. For the assay, 290 μ L of the ABTS⁺ solution was added to 10 μ L of the sample extract in a 96-well microplate. The mixture was allowed to react in the dark at 25°C for 6 min. Absorbance was then recorded at 734 nm. A calibration curve was generated with ascorbic acid standards (0–150 μ g/mL), and the results were expressed as mg AAE/g sample.

2.4.7 | Hydroxyl Radical Scavenging Activity ($\cdot$ OH-RSA)

$\cdot$ OH-RSA was determined following the method of Smirnoff and Cumbe [12]. In a 96-well microplate, 50 μ L of Falsa extract was combined sequentially with 50 μ L of 6 mM FeSO₄·7H₂O and 50 μ L of 6 mM H₂O₂. The mixture was incubated at 25°C for 10 min, after which 50 μ L of 6 mM 3-hydroxybenzoic acid was added. Following a further 10-min incubation at 25°C, absorbance was measured at 510 nm. A calibration curve prepared with ascorbic acid standards (0–300 μ g/mL) was used, and results were expressed as mg AAE/g sample.

2.4.8 | Reducing Power Assay (RPA)

For RPA, 10 μ L of Falsa sample extract was added to a 96-well microplate, followed by 25 μ L of phosphate buffer (prepared from Na₂HPO₄·7H₂O, NaH₂PO₄·H₂O, and distilled water) and 25 μ L of potassium ferricyanide. The mixture was incubated at 25°C

for 20 min. After incubation, 25 μ L of trichloroacetic acid, 85 μ L of distilled water, and 8.5 μ L of FeCl₃ were added. The plate was then incubated for an additional 15 min at 25°C. Absorbance was measured at 750 nm. Trolox standards (0–500 μ g/mL) were used to generate the calibration curve, and the outcomes were reported as mg of Trolox equivalents (mg TE)/g sample.

2.4.9 | Ferrous Ion Chelating Activity (FICA)

The FICA assay was conducted following a modified version of the method by Dinis et al. [13]. In a 96-well microplate, 15 μ L of Falsa extract was combined sequentially with 85 μ L of distilled water, 50 μ L of Buffer A (2 mM FeCl₂ in water), and 50 μ L of Buffer B (5 mM ferrozine in water). The mixture was incubated at 25°C for 10 min, after which absorbance was recorded at 562 nm. A calibration curve was prepared using ethylenediaminetetraacetic acid (EDTA) standards (0–50 μ g/mL). Results were expressed as mg of EDTA equivalents (mg EE)/g sample.

2.4.10 | Total Antioxidant Capacity (TAC)

The TAC assay was undertaken according to the method of Du et al. [14]. In a 96-well microplate, 40 μ L of Falsa extract was mixed with 260 μ L of preprepared antioxidant reagent containing phosphomolybdenum dye. The reaction mixture was incubated at 90°C for 90 min, then cooled to room temperature for 10 min. Absorbance was recorded at 695 nm. A calibration curve was generated using Trolox standards (0–200 μ g/mL). Results were expressed as mg TE/g sample.

2.5 | Characterization of Polyphenolic Compounds Using LC-ESI-QTOF-MS/MS

The identification and characterization of polyphenolic compounds in Falsa products were carried out using LC-ESI-QTOF-MS/MS analysis, as described before [15]. Analyses were performed on an Agilent 1200 Series HPLC system coupled to an Agilent 6520 Accurate-Mass Q-TOF LC/MS (Agilent Technologies, Santa Clara, California, United States). Chromatographic separation was achieved on a Synergi Hydro-RP 80A reversed-phase column (250 $\times$ 4.6 mm, 4 μ m; Phenomenex, Torrance, California, United States) maintained at 25°C. Samples were filtered through 0.45- μ m PTFE syringe filters (Kinesis Australia, Redland, Queensland, Australia) prior to injection (5 μ L). The mobile phases consisted of (A) water:acetic acid (98:2, v/v) and (B) acetonitrile:water:acetic acid (50:49.5:0.5, v/v/v), both degassed ultrasonically (Powersonic 505, Hwashin Technology, South Korea) for 15 min at 25°C. A gradient was run at 0.8 mL/min as follows: 10%–25% B (0–25 min), 25%–35% B (25–35 min), 35%–40% B (35–45 min), 40%–55% B (45–75 min), 55%–80% B (75–79 min), 80%–90% B (79–82 min), 90%–100% B (82–84 min), and 100%–10% B (84–87 min), followed by an isocratic hold at 10% B (87–90 min) for reequilibration. Mass spectra were recorded in both positive and negative electrospray ionization (ESI) modes over an *m/z* range of 50–1300. The nebulizing gas (N₂) was set at 45 psi with a drying-gas flow of 5 L/min at 300°C, while the sheath-gas flow was 11 L/min at 250°C. The capillary voltage was 3.5 kV, the

nozzle potential 500V, and the fragmentor voltage 135V. MS/MS spectra were acquired automatically using stepped collision energies of 10, 15, and 30eV to enhance structural confirmation. The instrument was calibrated internally with reference ions to maintain mass accuracy. Data acquisition and analysis were performed using Agilent MassHunter Qualitative Software B.03.01 (Agilent Technologies, Santa Clara, California, United States).

2.6 | Quantification of Selected Polyphenolics Using HPLC

The quantification of selected individual phenolic constituents in Falsa samples was carried out on an Agilent 1200 Series HPLC system (Agilent Technologies, Santa Clara, California, United States) fitted with a photodiode array (PDA) detector. The chromatographic setup, including the column type and mobile phase composition, matched that used for LC-ESI-QTOF-MS/MS analysis, except that the injection volume was increased to 20μL. Identification and quantification were performed by comparing retention times and spectral characteristics with those of authentic external standards, using calibration curves for each compound. Concentrations were reported as μg/g sample. Data processing was conducted with Agilent MassHunter Qualitative Software B.03.01 (Agilent Technologies, Santa Clara, California, United States).

2.7 | Statistical Analysis

All experiments were conducted in triplicate, and results are reported as mean ± standard deviation (n = 3). Statistical differences among samples were assessed using one-way analysis of variance (ANOVA), followed by Tukey’s post hoc test

at a significance level of $p \leq 0.05$. ANOVA was performed using Minitab Version 19.0 (Minitab LLC, State College, Pennsylvania, United States). Pearson’s correlation analysis between polyphenol content and antioxidant activity was conducted using XLSTAT 2019 (Addinsoft Inc., New York, New York, United States).

3 | Results and Discussion

3.1 | Polyphenolic Content (TPC, TFC, and TCT)

The polyphenolic profile of 12 processed Falsa products, derived from both natural and organic sources, was evaluated using TPC, TFC, and TCT assays. A significant variation in polyphenolic content was observed across different product types and processing methods (Table 1) and will be discussed in the following sections.

3.1.1 | TPC

Among all samples, natural Falsa fruit leather exhibited the highest TPC (6.04 ± 0.31 mg GAE /g), nearly double that of organic Falsa fruit leather (3.67 ± 0.13 mg GAE /g). Generally, most organic Falsa products showed a slightly higher TPC than their natural counterparts, except for fruit leather. This pattern partially agrees with Faller and Fialho [16], who reported an elevation in polyphenol concentration in organic fruits due to reduced agrochemical exposure and enhanced stress-induced metabolite production. The greater TPC concentration in fruit leather is likely attributable to the dehydration processing, which reduces moisture content and concentrates phenolic compounds. In contrast, dilution steps involved in nectar and beverage production may

TABLE 1 | Polyphenolic content in different products of organic and natural Falsa fruit.

Samples	Products	TPC (mg GAE/g)	TFC (mg QE/g)	TCT (mg CE/g)
Organic	Pulp	0.94 ± 0.05^C	—	0.02 ± 0.00^B
	Squash	0.25 ± 0.02^{EF}	—	—
	Drink	0.24 ± 0.01^{EF}	—	—
	Nectar	0.89 ± 0.05^C	—	—
	Jam	0.56 ± 0.05^D	—	—
	Fruit leather	3.67 ± 0.13^B	0.03 ± 0.00^B	—
Natural	Pulp	0.46 ± 0.03^{EF}	0.01 ± 0.00^C	0.07 ± 0.01^A
	Squash	0.19 ± 0.01^{EF}	—	—
	Drink	0.31 ± 0.02^{DF}	—	—
	Nectar	0.34 ± 0.02^{DF}	—	—
	Jam	0.06 ± 0.01^F	—	—
	Fruit leather	6.04 ± 0.31^A	0.09 ± 0.01^A	0.07 ± 0.02^A

Note: Data are presented as mean values with their corresponding standard deviation (n = 3). Different superscript letters (A–F) within the same row denote statistically significant differences (p < 0.05), as determined by one-way ANOVA followed by Tukey’s post hoc test. Abbreviations: CE, catechin equivalents; GAE, gallic acid equivalents; QE, quercetin equivalents; TCT, total condensed tannin; TFC, total flavonoid content; TPC, total phenolic content.

contribute to the lower phenolic values observed in those categories. This effect has been similarly documented by Shende and Datta [17], who demonstrated that drying conditions significantly influence phenolic retention in fruit by-products such as mango peels. High temperatures may enhance the release of bound phenolics through cellular breakdown, resulting in higher extractable phenolic content. Conversely, Carcea et al. [18] reported that thermal treatments involving water absorption can dilute bioactive compounds during cooking, potentially explaining the lower TPC in liquid-based products.

Compared with previously reported values for fresh Falsa [7], the processed products had a generally lower TPC, likely because of thermal degradation, oxidation, and polymerization of phenolics during processing and storage. Previous investigations [19, 20] have further emphasized the impact of temperature, storage duration, and matrix interactions on the loss of phenolics. Overall, these data suggest that the processing method is a critical determinant of phenolic retention, with drying-based techniques (such as fruit leather preparation) offering a greater potential for preservation or concentration of bioactive compounds in Falsa-derived products.

It is important to acknowledge that the use of pure methanol as a solvent during the extraction process represents a methodological limitation. Absolute methanol alone may not provide the optimal polarity or chemical environment offered by acidified or aqueous solvent systems, which makes it less efficient for the complete recovery of the polyphenolic pool. For example, Sultana et al. [21], in a study comparing absolute methanol with aqueous methanol (methanol:water, 80:20, v/v) for the extraction of medicinal plants, reported that the aqueous organic solvent was more effective than absolute organic solvents, as demonstrated by yielding higher extract recoveries, greater phenolic contents, and stronger antioxidant activity of the plant material. This poor efficiency is particularly pronounced for highly polar compounds, such as glycosylated flavonoids and anthocyanins, whose complete recovery and stability may necessitate the presence of water and a low pH [22]. Comparative extraction studies have demonstrated that the choice of solvent system, as well as the incorporation of physical assistance methods (e.g., ultrasonic- or microwave-assisted extraction) or alternative approaches such as accelerated solvent extraction and supercritical fluid extraction, can substantially enhance the recovery of the TPC pool [23–26]. This aspect necessitates further investigation. Another limitation is that only pulp-based samples were evaluated in this experiment, even though the peel, seeds, and leaves may contain greater TPC levels. For example, Lasota et al. [27] reported a substantially greater TPC in the peel compared to the pulp of various fruits. Future research should therefore broaden the sampling matrix to include extracts from the peel, seeds, and potentially the whole fruit to provide a more comprehensive polyphenolic profile of Falsa and its by-products.

3.1.2 | TFC

Flavonoids, a prominent subgroup of polyphenols, are recognized for their antioxidant activity, anti-inflammatory effects, and potential roles in cancer prevention. Therefore, determining TFC is essential for assessing the pharmacological potential

of Falsa-based functional products. In this study, however, TFC levels were undetectable in most of the 12 processed samples, with the exception of fruit leather, where natural Falsa fruit leather recorded the highest TFC (0.09 mg QE/g), followed by organic Falsa fruit leather (0.03 mg QE/g).

The generally low TFC is likely due to thermal degradation during processing. Razzak et al. [28] reported substantial loss of flavonoids (4.27%–82.3%) in heat-treated vegetables. Further, prolonged storage duration may have contributed to flavonoid degradation, as previously noted by Nadeem et al. [29], who observed a decline in both TFC and TPC over time. Furthermore, it is important to acknowledge the limitations inherent to the aluminum chloride colorimetric assay, which likely have contributed to an underestimation of flavonoids. As demonstrated by Pękal and Pyrzynska [30], the response of aluminum-based assays for TFC quantification depends on flavonoid structure, and glycosylation may influence complexation behavior and measured absorbance. Shraim et al. [31] corroborated these limitations and reported large inaccuracies and subclass-dependent biases in quantification. Finally, as discussed for TPC results, the use of absolute methanol as an extraction solvent may have resulted in incomplete recovery of highly polar glycosides that could have been better extracted in acidified or aqueous organic mixtures.

3.1.3 | TCT

Similarly, TCT concentration was generally low, with only a few samples yielding measurable values. Among these, TCT concentration was the greatest in natural Falsa pulp and natural fruit leather (0.07 mg CE/g), while organic Falsa pulp had a lower value (0.02 mg CE/g). Generally, the relatively low tannin content could likely be explained by the use of ripe Falsa fruits in this study, as tannins are known to decrease as ripening progresses in fruits [32, 33]. As ripening proceeds, tannins shift from soluble, readily extractable oligomers to more polymerized, less extractable complexes, resulting in an apparent reduction in TCT. This polymerization process is also known as a reason for the decreasing perceived astringency, as it reduces the capacity to form protein–tannin complexes [32]. Further, the variability observed in TCT measurements may reflect methodological factors, including solvent choice, reagent concentration, and incubation conditions, which are known to influence the quantification of condensed tannins in plant matrices (e.g., vanillin and butanol–HCl assays).

The limited recovery of phenolics and tannins during extraction may result from the formation of nonextractable or bound polymeric forms during processing, which are not likely captured by conventional solvent extraction or colorimetric assays. A substantial portion of phenolic acids and related compounds may occur bound to cell-wall matrices and require alkaline, acid, or enzymatic hydrolysis for maximal release [34]. Thermal processing may further alter the phenolic composition through isomerization and epimerization reactions, potentially changing their extractability. To improve quantification, future analyses should combine optimized solvent systems with advanced spectroscopic methods. For example, nuclear magnetic resonance spectroscopy can potentially provide a complementary, nondestructive means to

profile polyphenolic compounds, including polymeric tannins at the mixture level (solution and solid states) [35].

To accurately assess the health benefits, the results from chemical quantification of polyphenols must be reconciled with their bioavailability and metabolic fate in vivo, as the actual biological effect is dependent on how these compounds are absorbed, metabolized, and distributed to target tissues [36]. Flavonoid glycosides, which are common in fruits, must often be hydrolyzed by gut microbiota before absorption. Similarly, tannins, particularly high-molecular-weight proanthocyanidins, are often poorly absorbed in the small intestine but undergo extensive metabolism by the colonic microflora, yielding low-molecular-weight phenolic acids ([37, 38]). Therefore, the functional relevance of the TFC/TCT values must be viewed in light of these complex metabolic pathways, which produce the circulating, biologically active forms.

3.2 | Antioxidant Activities of Processed Falsa Products

Antioxidant activity is a key indicator of food quality, nutritional value, and shelf stability, as it reflects a product’s capacity to scavenge reactive species and mitigate oxidative degradation of lipids, pigments, and vitamins [39, 40]. To assess the antioxidant potential of the processed Falsa products, multiple chemical assays were employed to capture different underlying antioxidant mechanisms (Table 2). These included free radical scavenging

assays (DPPH, ABTS, FICA, ·OH-RSA) and redox-based assays (FRAP, RPA, and TAC), providing comprehensive coverage of both radical quenching and electron-donating activities.

The DPPH assay, known for its simplicity and reproducibility, revealed a higher antioxidant activity in fruit leather, with values of $9.09 \pm 0.52 \text{ mg TE/g}$ for the natural sample and $7.07 \pm 0.15 \text{ mg TE/g}$ for the organic variant, while all other products remained below 1 mg TE/g . This elevated activity likely reflects phenolic concentration during dehydration. However, the deep purple pigments in Falsa may interfere with DPPH absorbance and lead to an underestimation [41]. This overlap highlights a general limitation of colorimetric radical assays, which cannot fully distinguish radical quenching from background pigment absorbance. Therefore, results from DPPH should be interpreted with caution alongside complementary assays.

To address this limitation, the ABTS assay was used, as it accommodates both hydrophilic and lipophilic antioxidants and is less prone to interference from sample pigmentation [41]. Results mirrored the DPPH assay, with fruit leather again showing the highest radical scavenging activity, while all other samples remained below 1 mg TE/g . The agreement between the ABTS and DPPH results may suggest that Falsa phenolics include a mix of hydrophobic and hydrophilic compounds. The generally lower ABTS values compared to DPPH likely suggest a predominance of hydrophobic antioxidant molecules in the Falsa matrix.

TABLE 2 | Antioxidant activities in different products of organic and natural Falsa fruit.

Falsa products		Antioxidant assays						
		DPPH (mg TE/g)	FRAP (mg TE/g)	ABTS (mg AAE/g)	RPA (mg TE/g)	·OH-RSA (mg AAE/g)	FICA (mg EDTA/g)	TAC (mg TE/g)
Organic	Pulp	0.39 ± 0.00^D	0.41 ± 0.01^A	0.39 ± 0.02^C	0.46 ± 0.02^{DE}	0.15 ± 0.00^{AB}	0.16 ± 0.01^{CD}	—
	Squash	0.49 ± 0.02^{CD}	0.75 ± 0.07^A	0.48 ± 0.00^C	0.40 ± 0.02^{EF}	0.16 ± 0.00^A	0.20 ± 0.01^C	—
	Drink	0.12 ± 0.00^D	0.08 ± 0.00^A	0.11 ± 0.01^D	0.39 ± 0.00^{EF}	0.15 ± 0.00^{AB}	0.09 ± 0.00^D	—
	Nectar	0.21 ± 0.00^D	0.39 ± 0.02^A	0.19 ± 0.00^D	0.09 ± 0.01^H	0.16 ± 0.00^{AB}	0.13 ± 0.01^{CD}	—
	Jam	0.30 ± 0.02^D	0.23 ± 0.02^A	0.13 ± 0.01^D	0.30 ± 0.00^G	0.15 ± 0.00^{AB}	0.15 ± 0.01^{CD}	—
	Fruit leather	7.07 ± 0.15^B	1.63 ± 0.03^A	5.58 ± 0.11^A	0.38 ± 0.02^F	0.10 ± 0.01^C	1.38 ± 0.10^A	1.22 ± 0.11^B
Natural	Pulp	0.91 ± 0.03^C	0.82 ± 0.03^A	0.75 ± 0.04^B	0.56 ± 0.03^C	0.16 ± 0.00^A	0.22 ± 0.00^C	—
	Squash	0.48 ± 0.01^{CD}	0.88 ± 0.00^A	0.37 ± 0.03^C	1.06 ± 0.06^A	0.15 ± 0.00^{AB}	0.13 ± 0.01^{CD}	—
	Drink	0.17 ± 0.01^D	0.11 ± 0.01^A	0.14 ± 0.02^D	0.03 ± 0.00^H	0.15 ± 0.00^{AB}	0.22 ± 0.00^C	—
	Nectar	0.23 ± 0.02^D	0.13 ± 0.01^A	0.16 ± 0.01^D	0.06 ± 0.00^H	0.15 ± 0.00^B	0.15 ± 0.00^{CD}	—
	Jam	0.33 ± 0.01^D	0.40 ± 0.02^A	0.14 ± 0.01^D	0.52 ± 0.02^{CD}	0.15 ± 0.00^{AB}	0.13 ± 0.00^{CD}	—
	Fruit leather	9.09 ± 0.52^A	2.94 ± 0.00^A	5.50 ± 0.03^A	0.83 ± 0.03^B	0.03 ± 0.01^D	0.88 ± 0.30^B	3.19 ± 0.12^A

Note: Values are presented as mean ± standard deviation (n = 3). Different superscript letters (A–F) within the same row indicate statistically significant differences at p < 0.05, as determined by one-way ANOVA followed by Tukey’s post hoc test. Abbreviations: OH-RSA, hydroxyl radical scavenging activity; AAE, ascorbic acid equivalents; ABTS, 2,2’-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid); DPPH, 2,2’-diphenyl-1-picrylhydrazyl; EDTA, ethylenediaminetetraacetic acid; FICA, ferrous ion chelating activity; FRAP, ferric reducing antioxidant power; RPA, reducing power assay; TAC, total antioxidant capacity; TE, Trolox equivalents.

The ·OH-RSA assay showed relatively consistent activity across samples (approximately 0.15 mg AAE/g), with the exception of the natural fruit leather, which exhibited the lowest value (0.03 mg AAE/g). This is an unexpected outcome, as flavonoids are potent hydroxyl radical scavengers [42], and the fruit leather contained higher detectable TFC than the other samples, in which TFC was undetectable. This discrepancy between TFC and ·OH-RSA performance likely indicates that hydroxyl radical quenching in Falsa products may not depend on TFC abundance but rather on the presence of specific structural subclasses or interactions with other matrix components formed during processing.

The FICA assay, which assesses metal chelation by inhibiting the formation of ferrous complexes, showed a markedly higher activity in organic Falsa fruit leather (1.38 mg EDTA/g) compared to other products (ranging from 0.09 to 0.22 mg EDTA/g). Transition metals are known to trigger lipid oxidation and decrease the storage stability and quality of foods, and metal chelation is an important pathway for limiting metal-catalyzed lipid peroxidation [43]. This high activity may highlight the potential functionality of Falsa fruit leather components, whether when consumed, released during processing, or incorporated as natural antioxidant ingredients, to support oxidative stability in lipid-containing systems. However, their contribution to oxidative stability needs to be demonstrated within real or model lipid systems.

The FRAP assay, based on the reduction of ferric to ferrous ions, demonstrated a broader range of reducing power, from 0.08 to 1.63 mg TE/g. Organic Falsa fruit leather displayed the highest FRAP value, while organic Falsa drink had the lowest. Although FeSO_4 is often used as a standard in FRAP assays, Stratil et al. [44] recommended Trolox for standardization across multiple antioxidant assays, suggesting that variations in standard selection may influence comparability. A limitation of the FRAP assay, however, is that this assay requires a highly acidic, nonphysiological pH (around 3.6) for performance, which significantly restricts its relevance for simulating antioxidant activity under in vivo human physiological conditions. The low-pH environment limits the dissociation of phenolic compounds and makes them less reactive to oxidation by the reagent. This low pH also slows the reaction kinetics, preventing complete oxidation of certain compounds such as thiols and hydroxycinnamic acids. In addition, the FRAP assay has exhibited greater sensitivity to hydrophilic than to hydrophobic antioxidants, potentially underestimating TAC in complex matrices [45].

RPA showed activities between 0.03 and 1.06 mg TE/g, with natural Falsa squash displaying the highest value. Finally, the TAC assay, which integrates overall electron transfer potential, yielded measurable responses only for fruit leather samples, consistent with their high phenolic concentration and redox density.

The variability in antioxidant results across assays may highlight the multifaceted nature of antioxidant mechanisms (e.g., electron transfer, hydrogen atom transfer, and metal chelation) [46]. Differences in sample ranking likely arise from both compositional diversity and interactions within the Falsa

matrix. Sugars, organic acids, and minor phenolics can influence antioxidant outcomes through synergistic or antagonistic effects [47]. For example, the combined action of different phenolics can lead to activity greater than the sum of their individual activities (synergism). To pinpoint the phenolic classes responsible for these activities, future studies should apply chromatographic fractionation or purified standards in activity-guided assays to link compound subclasses (e.g., anthocyanins vs. flavones) to specific antioxidant mechanisms. These targeted analyses would clarify whether the strong FICA results arise from particular flavonoid glycosides or whether the radical scavenging (DPPH/ABTS) is mainly driven by quercetin derivatives.

Overall, Falsa fruit leather consistently demonstrated the highest antioxidant potential across all assay types, particularly in the natural variant. This superior performance is likely due to phenolic concentration through dehydration, which minimizes dilution and preserves antioxidant integrity. Integrating data from multiple assays offers a more accurate reflection of TAC. Despite their speed and simplicity, chemical antioxidant assays use reactions that differ significantly from those in the body, and thus, the observed in vitro activity could not be a reliable indicator of actual in vivo efficacy [36]. Polyphenols are known to be effective antioxidants but are susceptible to instability in the gastrointestinal environment and undergo extensive microbial biotransformation, producing metabolites with altered or reduced antioxidant potential. In addition, limited intestinal absorption further restricts the fraction that becomes biologically active [36]. To determine the physiological relevance, future research should incorporate cell-based oxidative stress models or in vivo animal studies assessing biomarkers of oxidative protection. The specific phenolic compounds identified through LC-MS/MS profiling will be discussed in the following section.

3.3 | Correlation Analysis of Phenolic Compounds With Antioxidant Assays

The relationship between polyphenol content and antioxidant activity in the 12 processed Falsa products was evaluated using Pearson's correlation analysis (Table 3). A strong positive correlation was observed between TPC and TFC ($r = 0.958$, $p < 0.01$), suggesting that flavonoids are the predominant class of polyphenolic compounds in these samples. This finding agrees with previous reports indicating that Falsa fruits are rich in flavonoids [6]. TPC also showed a significant positive correlation with TAC ($r = 0.979$, $p < 0.01$), as well as with both the DPPH ($r = 0.977$, $p < 0.01$) and ABTS ($r = 0.945$, $p < 0.01$) radical scavenging assays. These strong associations indicate that phenolic compounds, particularly flavonoids, play a central role in the free radical scavenging activity and overall antioxidant potential of processed Falsa products.

In addition, TFC demonstrated positive correlations with multiple antioxidant assays, including DPPH, FRAP, ABTS, FICA, and TAC, further supporting the hypothesis that flavonoids are key contributors to antioxidant function. Conversely, TCT was not significantly correlated with any of the antioxidant assays. This may be attributed to the generally low tannin levels

TABLE 3 | Pearson's correlation coefficients (*r*) between antioxidant assays and polyphenolic content in Falsa fruit products.

Variables	TPC	TFC	TCT	DPPH	FRAP	ABTS	·OH-RSA	RPA	FICA
TFC	0.958*								
TCT	−0.033	0.192							
DPPH	0.977*	0.931*	−0.153						
FRAP	0.927*	0.954*	0.093	0.937*					
ABTS	0.945*	0.869*	−0.273	0.988*	0.899*				
OH-RSA	−0.970*	−0.968*	−0.034	−0.951*	−0.916*	−0.897*			
RPA	0.312	0.410	0.258	0.353	0.574	0.314	−0.388		
FICA	0.835*	0.713*	−0.493	0.911*	0.762*	0.961*	−0.760*	0.166	
TAC	0.979*	0.984*	0.096	0.954*	0.943*	0.899*	−0.990*	0.388	0.752*

*Significant correlation with $p \leq 0.01$.

detected in the samples and the possibility that tannins in Falsa do not contribute significantly to free radical stabilization or electron donation in these assay systems.

Strong interassay correlations were also observed. In particular, DPPH exhibited significant positive correlations with FRAP, ABTS, FICA, and TAC, with the strongest correlation observed between DPPH and ABTS ($r = 0.988$, $p < 0.01$). These results support the complementary nature of these assays in capturing different aspects of antioxidant capacity. TAC also correlated positively with all other antioxidant assays (except RPA and OH-RSA), indicating its utility as a robust indicator of antioxidant potential.

An unexpectedly strong negative correlation ($r = -0.97$) existed between TPC/TFC and ·OH-RSA, which could likely be explained by the unique behavior of Fenton-type hydroxyl radical assays, in which the analytical signal reflects not only direct radical quenching but also metal-mediated radical generation. Polyphenolic compounds are able to chelate or reduce $\text{Fe}^{3+}/\text{Fe}^{2+}$, thereby altering the kinetics of ·OH formation and decay in the reaction system [48]. As a result, ·OH-RSA may not correlate linearly with TPC but instead depends on phenolic structure, redox characteristics, and metal-chelating ability, often displaying inverse or nonlinear trends relative to single-electron transfer-based assays (e.g., DPPH, ABTS, and FRAP). In support of this observation, Rangani et al. [49] reported that hydroxyl radical scavenging capacity was negatively correlated with TPC, while its association with TFC in unripe areca nut extracts was weak. However, in ripe areca nuts, both correlation coefficients were negative, with coefficients of -0.47 for TPC and -0.67 for TFC, respectively. Similarly, a recent investigation in our group on citrus peels [15] demonstrated a weak negative correlation between ·OH-RSA and both TPC (-0.09) and TFC (-0.26).

Despite these consistent and mechanistically interpretable relationships, the complexity of phenolic interactions necessitates caution when drawing conclusions from in vitro antioxidant assays alone. The antioxidant performance of polyphenols is influenced by synergistic and antagonistic interactions, structural diversity, and matrix effects. Therefore, no single method can

comprehensively describe the antioxidant profile of a complex food matrix such as Falsa. Further LC-MS/MS-based studies are warranted to elucidate the qualitative and quantitative contributions of individual phenolics, providing deeper mechanistic insight into their antioxidant behavior and processing stability.

3.4 | LC-ESI-QTOF-MS/MS Characterization of Falsa Fruit Products

LC-MS is a powerful analytical platform that enables the separation, detection, and structural identification of complex mixtures with high sensitivity and specificity [50]. In this study, LC-ESI-QTOF-MS/MS was employed to characterize the phenolic profiles of 12 processed Falsa products. The analysis was performed in both positive and negative ionization modes ($[\text{M} - \text{H}]^-/[\text{M} + \text{H}]^+$), using Agilent MassHunter Qualitative Software and the Personal Compound Database and Library. Identification of phenolic compounds was based on mass-to-charge ratio (m/z), MS/MS fragmentation patterns, and matching to library standards, with compounds selected under the criteria of mass accuracy within ± 5 ppm and a library match score above 80.

A total of 88 polyphenolic compounds were tentatively identified across the analyzed samples (Table 4), comprising 32 phenolic acids, 36 flavonoids, 6 lignans, 2 stilbenes, and 12 other polyphenols. This comprehensive profiling highlights the substantial chemical diversity of phenolic constituents present in Falsa-derived products. This diversity may provide a strong molecular basis for the antioxidant activity observed in earlier assays. Moreover, the presence of several flavonoids also agrees with the high TFC values and strong antioxidant correlations observed in previous sections. The hierarchical clustering heatmaps derived from LC-ESI-QTOF-MS/MS data (negative and positive ESI modes) illustrate the distribution of tentatively identified polyphenolic compounds across the 12 Falsa products (Figure 1). In general, compared to the negative ESI mode, the positive ESI mode displayed comparatively more compact clustering patterns, suggesting a more uniform distribution of detected compounds across samples relative to the negative mode. Samples in both ionization modes were grouped based on similarities in

TABLE 4 | Tentative characterization of polyphenolic compounds in Falsa products by LC-ESI-QTOF-MS/MS.

No.	Proposed compounds	Molecular formula	RT (min)	Ionization (ESI ⁺ /ESI ⁻)	Molecular weight	Theoretical (m/z)	Observed (m/z)	Error (ppm)	MS ² product ions	Sample
Phenolic acid										
<i>Hydroxybenzoic acids</i>										
1	3-O-Methylgallic acid	C ₈ H ₈ O ₅	9.004	**[M + H] ⁺	184.0377	185.0450	185.0451	0.5	170, 142	*FNS, FNFL, FOD, FOJ
2	Ellagic acid	C ₁₄ H ₆ O ₈	9.348	[M - H] ⁻	302.0034	300.9961	300.9962	0.3	284, 229, 201	FND
3	Ellagic acid glucoside	C ₂₀ H ₁₆ O ₁₃	9.770	[M - H] ⁻	464.0562	463.0489	463.0491	0.4	301	FON
4	Ellagic acid arabinoside	C ₁₉ H ₁₄ O ₁₂	39.842	**[M - H] ⁻	434.0508	433.0435	433.0000	1.2	300	*FND, FON, FOP
5	Paeoniflorin	C ₂₃ H ₂₈ O ₁₁	41.105	[M - H] ⁻	480.1672	479.1599	479.1600	0.2	449, 357, 327	*FNS, FON
6	Gallic acid	C ₇ H ₆ O ₅	55.508	**[M + H] ⁺	170.0216	171.0289	171.0289	0.1	125	*FOS, FOP, FOJ, FNJ
7	2,3-Dihydroxybenzoic acid	C ₇ H ₆ O ₄	56.563	**[M - H] ⁻	154.0254	153.0181	153.0181	0.1	109	*FOS, FOJ, FNJ
<i>Hydroxycinnamic acids</i>										
8	Ferulic acid 4-O-glucuronide	C ₁₆ H ₁₈ O ₁₀	8.656	**[M - H] ⁻	370.0881	369.0808	369.0801	-1.9	193	*FOP, FOJ, FND
9	Hydroxycaffeic acid	C ₉ H ₈ O ₅	9.413	[M - H] ⁻	196.0354	195.0281	195.0281	0.1	151	*FND, FOS, FOP
10	Caffeic acid 4-sulfate	C ₉ H ₈ O ₇ S	9.446	[M - H] ⁻	259.9979	258.9906	258.9905	-0.4	179, 135	FND
11	1-Sinapoyl-2-feruloylgentiobiose	C ₃₃ H ₄₀ O ₁₈	9.577	[M - H] ⁻	724.2189	723.2116	723.2119	0.4	529, 499	*FND, FNJ
12	Sinapic acid	C ₁₁ H ₁₂ O ₅	9.613	[M - H] ⁻	224.0692	223.0619	223.0620	0.4	205, 163	*FOD, FOS, FNFL, FNN, FON, FNS
13	Caffeic acid	C ₉ H ₈ O ₄	10.913	[M - H] ⁻	180.0422	179.0349	179.0349	0.1	143, 133	*FND, FOP, FOJ, FON

(Continues)

TABLE 4 | (CONTINUED)

No.	Proposed compounds	Molecular formula	RT (min)	Ionization (ESI ⁺ /ESI ⁻)	Molecular weight	Theoretical (<i>m/z</i>)	Observed (<i>m/z</i>)	Error (ppm)	MS ² product ions	Sample
14	1-Sinapoyl-2,2'-diferuloylgentiobiose	C ₄₃ H ₄₈ O ₂₁	10.806	**[M - H] ⁻	900.2672	899.2599	899.2619	2.2	613, 201	*FND
15	Rosmarinic acid	C ₁₈ H ₁₆ O ₈	11.661	**[M - H] ⁻	360.0843	359.077	359.0770	0.1	179	*FNN, FOP, FNS, FND, FON
16	Caffeoyl tartaric acid	C ₁₃ H ₁₂ O ₉	11.722	[M - H] ⁻	312.0485	311.0412	311.0415	1.0	161	FND
17	<i>p</i> -Coumaroyl tartaric acid	C ₁₃ H ₁₂ O ₈	11.789	[M - H] ⁻	296.0521	295.0448	295.0455	2.4	115	*FND, FNN
18	3-Caffeoylquinic acid	C ₁₆ H ₁₈ O ₉	18.898	**[M - H] ⁻	354.0939	353.0866	353.0866	0.1	253, 190, 144	*FOP, FOD, FNN, FOJ, FNS, FOFL
19	3- <i>p</i> -Coumaroylquinic acid	C ₁₆ H ₁₈ O ₈	19.989	[M - H] ⁻	338.0970	337.0897	337.0895	-0.6	265, 173, 162	*FOP, FOJ
20	Caffeic acid 3- <i>O</i> -glucuronide	C ₁₅ H ₁₆ O ₁₀	20.845	**[M - H] ⁻	356.0772	355.0699	355.0704	1.4	179	*FND, FNN
21	5-Feruloylquinic acid	C ₁₇ H ₂₀ O ₉	36.720	**[M - H] ⁻	368.1132	367.1059	367.1000	4.9	298, 288, 192, 191	*FOJ, FND, FOP
22	Cinnamic acid	C ₉ H ₈ O ₂	44.070	[M - H] ⁻	148.0513	147.0440	147.0438	-1.4	103	FOS
23	2-Hydroxy-2-phenylacetic acid	C ₈ H ₈ O ₃	46.063	[M - H] ⁻	152.0483	151.0410	151.0411	0.7	136, 92	FOS
24	Ferulic acid 4- <i>O</i> -glucoside	C ₁₆ H ₂₀ O ₉	57.271	**[M - H] ⁻	356.1101	355.1028	355.1027	-0.3	193, 178, 149, 134	*FOP, FOD, FOS, FOJ, FNS
25	<i>p</i> -Coumaric acid 4- <i>O</i> -glucoside	C ₁₅ H ₁₈ O ₈	58.711	[M - H] ⁻	326.0978	325.0905	325.0903	-0.6	163	FOJ
26	Verbascoside	C ₂₉ H ₃₆ O ₁₅	62.113	[M - H] ⁻	624.2054	623.1981	623.1985	0.6	477, 461, 315, 135	FOP
27	Caffeoyl glucose	C ₁₅ H ₁₈ O ₉	66.984	[M - H] ⁻	342.0969	341.0896	341.0895	-0.3	179, 161	*FOJ, FNS, FOS, FND, FOP

(Continues)

TABLE 4 | (CONTINUED)

No.	Proposed compounds	Molecular formula	RT (min)	Ionization (ESI ⁺ /ESI ⁻)	Molecular weight	Theoretical (<i>m/z</i>)	Observed (<i>m/z</i>)	Error (ppm)	MS ² product ions	Sample
28	<i>m</i> -Coumaric acid	C ₉ H ₈ O ₃	67.023	**[M – H] ⁻	164.0489	163.0416	163.0416	0.1	119	*FOJ, FND, FNN, FOS
29	Ferulic acid	C ₁₀ H ₁₀ O ₄	67.503	**[M – H] ⁻	194.0565	193.0492	193.0493	0.5	178, 149, 134	*FOJ, FOS, FOP, FON, FNS
<i>Hydroxyphenylacetic acids</i>										
30	3,4-Dihydroxyphenylacetic acid	C ₈ H ₈ O ₄	11.290	**[M – H] ⁻	168.0420	167.0347	167.0347	0.1	149, 123	*FOS, FOJ, FNS
<i>Hydroxyphenylpropanoic acids</i>										
31	3-Hydroxy-3-(3-hydroxyphenyl)propionic acid	C ₉ H ₁₀ O ₄	10.719	**[M – H] ⁻	182.0568	181.0495	181.0495	0.1	163, 135, 119	**FND, FOS, FNN, FON
32	Dihydroferulic acid 4-sulfate	C ₁₀ H ₁₂ O ₇ S	12.983	[M – H] ⁻	276.0317	275.0244	275.0244	0.1	195, 151, 177	*FNFL, FOFL, FON, FNN, FNS
<i>Flavonoids</i>										
<i>Flavanols</i>										
33	Theaflavin 3,3'-O-digallate	C ₄₃ H ₃₂ O ₂₀	9.842	[M – H] ⁻	868.1518	867.1445	867.1451	0.7	715, 563, 545	FND
34	4''-O-Methylepigallocatechin 3-O-gallate	C ₂₃ H ₂₀ O ₁₁	10.902	[M – H] ⁻	472.1004	471.0931	471.0937	1.3	169, 319	FON
35	Theaflavin	C ₂₉ H ₂₄ O ₁₂	33.496	[M – H] ⁻	564.1275	563.1202	563.1206	0.7	545	*FOS, FON, FOP
36	4'-O-Methyl-(-)-epigallocatechin 7-O-glucuronide	C ₂₂ H ₂₄ O ₁₃	55.979	**[M – H] ⁻	496.1176	495.1103	495.1103	0.1	451, 313	*FOP, FOS, FNN
37	Prodelphinidin dimer B3	C ₃₀ H ₂₆ O ₁₄	59.284	**[M + H] ⁺	610.132	611.1393	611.1396	0.5	469, 311, 291	*FOJ, FOP
38	(+)-Catechin 3-O-gallate	C ₂₂ H ₁₈ O ₁₀	66.452	[M – H] ⁻	442.0910	441.0837	441.0838	0.2	289, 169, 125	*FNI, FNS, FOJ

(Continues)

TABLE 4 | (CONTINUED)

No.	Proposed compounds	Molecular formula	RT (min)	Ionization (ESI ⁺ /ESI ⁻)	Molecular weight	Theoretical (<i>m/z</i>)	Observed (<i>m/z</i>)	Error (ppm)	MS ² product ions	Sample
39	Procyanidin dimer B1	C ₃₀ H ₂₆ O ₁₂	68.660	**[M - H] ⁻	578.1452	577.1379	577.1381	0.3	451	*FND, FNN, FOS, FNS, FOJ
<i>Flavones</i>										
40	Cirsilineol	C ₁₈ H ₁₆ O ₇	11.772	**[M + H] ⁺	344.0883	345.0956	345.0958	0.6	330, 312, 297, 284	*FNS, FNN, FOP
41	Apigenin 7- <i>O</i> -apiosylglucoside	C ₂₆ H ₂₈ O ₁₄	65.009	[M + H] ⁺	564.1462	565.1535	565.1534	-0.2	296	*FOJ, FNN
42	Chrysoeriol 7- <i>O</i> -glucoside	C ₂₂ H ₂₂ O ₁₁	66.049	**[M + H] ⁺	462.1175	463.1248	463.1248	0.1	445, 427, 409, 381	**FOJ, FOP, FOFL, FNN, FON
43	6-Hydroxyluteolin 7-rhamnoside	C ₂₁ H ₂₀ O ₁₁	67.273	**[M - H] ⁻	448.1039	447.0966	447.0971	1.1	301	*FOJ, FNN, FON, FOS, FNI
<i>Flavanones</i>										
44	Naringenin	C ₁₅ H ₁₂ O ₅	11.960	**[M - H] ⁻	272.0679	271.0606	271.0604	-0.7	107, 119, 151, 177	*FOS, FND
45	3',4',7-Trihydroxyisoflavanone	C ₃₃ H ₄₂ O ₁₉	28.207	**[M - H] ⁻	742.2271	741.2198	741.2201	0.4	433, 271	*FNI, FON, FNS, FOJ
46	Narirutin	C ₂₇ H ₃₂ O ₁₄	50.387	[M - H] ⁻	580.1788	579.1715	579.1709	-1.0	271	*FOP, FOS, FOJ
47	Hesperidin	C ₂₈ H ₃₄ O ₁₅	62.953	**[M + H] ⁺	610.1888	611.1961	611.1958	-0.5	593, 465, 449, 303	*FOJ, FNI, FOS, FNN
48	Isoxanthohumol	C ₂₁ H ₂₂ O ₅	65.279	[M - H] ⁻	354.1445	353.1372	353.1372	0.1	338, 309	*FOS, FNS, FON
<i>Flavonols</i>										
49	Quercetin 3- <i>O</i> -arabinoside	C ₂₀ H ₁₈ O ₁₁	35.837	**[M - H] ⁻	434.0853	433.0780	433.0780	0.1	301	*FOD, FOP, FNN, FOJ, FNS, FON

(Continues)

TABLE 4 | (CONTINUED)

No.	Proposed compounds	Molecular formula	RT (min)	Ionization (ESI ⁺ /ESI ⁻)	Molecular weight	Theoretical (<i>m/z</i>)	Observed (<i>m/z</i>)	Error (ppm)	MS ² product ions	Sample
50	Kaempferol 3- <i>O</i> -glucosyl-rhamnosyl-galactoside	C ₃₃ H ₄₀ O ₂₀	38.166	**[M - H] ⁻	756.2105	755.2032	755.2033	0.1	285	*FNS, FON, FNI, FOP, FNN
51	Isorhamnetin	C ₁₆ H ₁₂ O ₇	46.950	**[M - H] ⁻	316.0588	315.0515	315.0515	0.1	300, 271	*FND, FOJ, FOS
52	Kaempferol 3- <i>O</i> -(2''-rhamnosyl-galactoside) 7- <i>O</i> -rhamnoside	C ₃₃ H ₄₀ O ₁₉	56.585	**[M - H] ⁻	740.2201	739.2128	739.2126	-0.3	593, 447, 285	*FOP, FNI, FOJ, FOS
53	Quercetin 3- <i>O</i> -(6''-malonyl-glucoside) 7- <i>O</i> -glucoside	C ₃₀ H ₃₂ O ₂₀	58.132	[M + H] ⁺	712.1493	713.1566	713.1572	0.8	303, 551	FOS, FOFL
<i>Dihydrochalcones</i>										
54	3-Hydroxyphloretin 2'- <i>O</i> -glucoside	C ₂₁ H ₂₄ O ₁₁	64.526	[M - H] ⁻	452.1323	451.1250	451.1250	0.1	289, 273	FOS
55	Phloridzin	C ₂₁ H ₂₄ O ₁₀	65.127	**[M - H] ⁻	436.1367	435.1294	435.1307	3.0	273	*FNS, FON, FOS
<i>Dihydroflavonols</i>										
56	Dihydromyricetin 3- <i>O</i> -rhamnoside	C ₂₁ H ₂₂ O ₁₂	40.970	[M - H] ⁻	466.1148	465.1075	465.1077	0.4	301	*FOS, FOP, FNS
57	Dihydroquercetin	C ₁₅ H ₁₂ O ₇	41.884	**[M - H] ⁻	304.0608	303.0535	303.0540	1.6	285, 275, 151	*FNS, FOJ, FNN, FOS
58	Dihydroquercetin 3- <i>O</i> -rhamnoside	C ₂₁ H ₂₂ O ₁₁	65.630	**[M - H] ⁻	450.1142	449.1069	449.1068	-0.2	303	*FNS, FOJ, FOS
<i>Anthocyanins</i>										
59	Cyanidin 3- <i>O</i> -(6''-succinyl-glucoside)	C ₂₅ H ₂₅ O ₁₄	45.393	[M - H] ⁻	549.1213	548.1140	548.1132	-1.5	459, 399, 369	FOS
60	Cyanidin 3,5- <i>O</i> -diglucoside	C ₂₇ H ₃₁ O ₁₆	59.995	**[M + H] ⁺	611.1612	612.1685	612.1685	0.1	449, 287	*FOP, FNS
61	Cyanidin 3- <i>O</i> -(6''- <i>p</i> -coumaroyl-glucoside)	C ₃₀ H ₂₇ O ₁₃	61.495	**[M + H] ⁺	595.1457	596.1530	596.1547	2.9	287	*FNN, FON

(Continues)

TABLE 4 | (CONTINUED)

No.	Proposed compounds	Molecular formula	RT (min)	Ionization (ESI ⁺ /ESI ⁻)	Molecular weight	Theoretical (<i>m/z</i>)	Observed (<i>m/z</i>)	Error (ppm)	MS ² product ions	Sample
<i>Isoflavonoids</i>										
62	Equol	C ₁₅ H ₁₄ O ₃	29.421	[M + H] ⁺	242.0948	243.1021	243.1021	0.1	255, 211, 197	*FOP, FOS, FNN
63	Sativanone	C ₁₇ H ₁₆ O ₅	41.322	[M – H] ⁻	300.0977	299.0904	299.0913	3.0	284, 269, 225	FOS
64	3'-Hydroxydaidzein	C ₁₅ H ₁₀ O ₅	47.947	**[M + H] ⁺	270.0533	271.0606	271.0608	0.7	253, 241, 225	*FOS, FON, FNS, FOJ
65	Violanone	C ₁₇ H ₁₆ O ₆	56.611	**[M – H] ⁻	316.0965	315.0892	315.0894	0.6	300, 285, 135	*FOS, FNS, FNI, FOJ, FND
66	2'-Hydroxyformnonetin	C ₁₆ H ₁₂ O ₅	63.860	[M + H] ⁺	284.0675	285.0748	285.0745	-1.1	270, 229	FOS
67	5,6,7,3',4'-Pentahydroxyisoflavone	C ₁₅ H ₁₀ O ₇	64.683	**[M + H] ⁺	302.0432	303.0505	303.0501	-1.3	285, 257	*FON, FOJ, FNJ
68	6''-O-Malonyldaidzin	C ₂₄ H ₂₂ O ₁₂	69.449	[M + H] ⁺	502.1110	503.1183	503.1186	2.2	255	*FOP, FOS
Other polyphenols										
<i>Other polyphenols</i>										
69	Lithospermic acid	C ₂₇ H ₂₂ O ₁₂	37.984	**[M – H] ⁻	538.1090	537.1017	537.0994	-4.3	493, 339, 295	*FNS, FOS
<i>Hydroxycoumarins</i>										
70	Urolithin A	C ₁₃ H ₈ O ₄	9.420	[M – H] ⁻	228.0428	227.0355	227.0354	-0.4	198, 182	FNN
71	Esculetin	C ₉ H ₆ O ₄	32.161	**[M – H] ⁻	178.0260	177.0187	177.0187	0.1	149, 133, 89	*FOJ, FOS, FND, FNN
72	Scopoletin	C ₁₀ H ₈ O ₄	49.505	**[M – H] ⁻	192.0421	191.0348	191.0348	0.1	176	*FOP, FND, FNJ, FNN, FNS
<i>Furanocoumarins</i>										
73	Isopimpinellin	C ₁₃ H ₁₀ O ₅	58.832	**[M + H] ⁺	246.0524	247.0597	247.0599	0.8	232, 217, 205, 203	*FOJ, FNN, FOS, FOP, FON

(Continues)

TABLE 4 | (CONTINUED)

No.	Proposed compounds	Molecular formula	RT (min)	Ionization (ESI ⁺ /ESI ⁻)	Molecular weight	Theoretical (<i>m/z</i>)	Observed (<i>m/z</i>)	Error (ppm)	MS ² product ions	Sample
<i>Hydroxybenzaldehydes</i>										
74	4-Hydroxybenzaldehyde	C ₇ H ₆ O ₂	64.190	**[M - H] ⁻	122.0359	121.0286	121.0286	0.1	77	*FNJ, FNS, FOS, FNP, FOJ, FNN
<i>Hydroxybenzoketones</i>										
75	2-Hydroxy-4-methoxyacetophenone 5-sulfate	C ₉ H ₁₀ O ₇ S	20.664	[M - H] ⁻	262.0170	261.0097	261.0098	0.4	181, 97	*FON, FNS
<i>Curcuminoids</i>										
76	Curcumin	C ₂₁ H ₂₀ O ₆	9.816	[M - H] ⁻	368.1266	367.1193	367.1193	0.1	217	*FOD, FOP, FOJ
77	Demethoxycurcumin	C ₂₀ H ₁₈ O ₅	12.817	**[M - H] ⁻	338.1162	337.1089	337.1091	0.6	217	*FNJ, FOJ
78	Bisdemethoxycurcumin	C ₁₉ H ₁₆ O ₄	64.716	[M + H] ⁺	308.1051	309.1124	309.1124	0.1	291, 263	*FOD, FOJ, FOS
<i>Tyrosols</i>										
79	3,4-DHPEA-AC	C ₁₀ H ₁₂ O ₄	36.503	[M - H] ⁻	196.0727	195.0654	195.0654	0.1	135	FOP
80	Demethyloleuropein	C ₂₄ H ₃₀ O ₁₃	49.852	[M - H] ⁻	526.1694	525.1621	525.1648	5.0	495	FOP
<i>Lignans</i>										
81	Arctigenin	C ₂₁ H ₂₄ O ₆	10.240	[M - H] ⁻	372.1549	371.1476	371.1478	0.5	356, 312, 295	FOJ
82	7-Oxomatairesinol	C ₂₀ H ₂₀ O ₇	19.247	**[M + H] ⁺	372.1192	373.1265	373.1264	-0.3	358, 343, 328, 325	*FNS, FOP
83	Pinoresinol	C ₂₀ H ₂₂ O ₆	19.515	[M - H] ⁻	358.1411	357.1338	357.1338	0.1	342, 327, 313, 221	*FNJ, FOJ, FNN
84	7-Hydroxymatairesinol	C ₂₀ H ₂₂ O ₇	51.293	[M - H] ⁻	374.1360	373.1287	373.1281	-1.6	343, 313, 298, 285	FOP
85	Schisandrin B	C ₂₃ H ₂₈ O ₆	57.684	[M + H] ⁺	400.1876	401.1949	401.1957	2.0	386	FOJ
86	Schisanhenol	C ₂₃ H ₃₀ O ₆	61.667	[M + H] ⁺	402.2024	403.2097	403.2102	1.2	331, 354, 385	FON

(Continues)

TABLE 4 | (CONTINUED)

No.	Proposed compounds	Molecular formula	RT (min)	Ionization (ESI ⁺ /ESI ⁻)	Molecular weight	Theoretical (<i>m/z</i>)	Observed (<i>m/z</i>)	Error (ppm)	MS ² product ions	Sample
<i>Stilbenes</i>										
87	Resveratrol 5- <i>O</i> -glucoside	C ₂₀ H ₂₂ O ₈	58.790	[M - H] ⁻	390.1345	389.1272	389.1273	0.3	227	*FOJ, FOP
88	4-Hydroxy-3,5,4'-trimethoxystilbene	C ₁₇ H ₁₈ O ₄	62.535	[M + H] ⁺	286.1211	287.1284	287.1291	2.4	271, 241, 225	*FOJ, FOP, FOD, FON

Abbreviations: FND, Falsa natural drink; FNFL, Falsa natural fruit leather; FNN, Falsa natural nectar; FNP, Falsa natural pulp; FNS, Falsa natural squash; FOD, Falsa organic drink; FOFL, Falsa organic fruit leather; FOJ, Falsa organic jam; FON, Falsa organic nectar; FOP, Falsa organic pulp; FOS, Falsa organic squash.

*The compound was detected in multiple Falsa samples; the data presented in this table correspond to the sample marked with an asterisk.

**Compounds were identified in both negative [M - H]⁻ and positive [M + H]⁺ ionization modes; however, only data from a single mode are presented.

their polyphenolic profiles, with no clear separation according to fruit cultivation type (organic vs. natural). The clustering patterns indicate variability in the relative distribution of compounds among the processed products. These differences may be associated with variations in processing.

3.4.1 | Phenolic Acids

Phenolic acids are key bioactive constituents in berries and contribute significantly to their antioxidant potential [51]. In this study, 32 phenolic acids were tentatively identified in the processed Falsa products, including 7 hydroxybenzoic acids, 21 hydroxycinnamic acids, 1 hydroxyphenylacetic acid, and 2 hydroxyphenylpropanoic acids (Table 4). Within the hydroxybenzoic acid subclass, Compound 5 was tentatively identified as paeoniflorin ([M - H]⁻ at *m/z* 479.16). Other hydroxybenzoic acids included 2,3-dihydroxybenzoic acid (*m/z* 153.0181), gallic acid (*m/z* 171.0289), and 3-*O*-methylgallic acid (*m/z* 185.0451), which produced fragment ions at *m/z* 109, 125, and 142, respectively, indicative of CO₂ loss.

Hydroxycinnamic acids were the most abundant phenolic acid subclass detected, consistent with their common occurrence in fruits and cereals [52, 53]. A total of 21 hydroxycinnamic acid derivatives were tentatively characterized. In particular, ferulic acid (Compound 29) was detected at *m/z* 193.0493, along with its conjugated forms ferulic acid 4-*O*-glucuronide (Compound 8, *m/z* 369.0808) and ferulic acid 4-*O*-glucoside (Compound 24, *m/z* 355.1027). These were identified based on characteristic losses of glucuronide (176 Da) and glycoside moieties, producing fragments at *m/z* 193, 178, 149, and 134.

Quinic acid derivatives were also present, including 3-*p*-coumaroylquinic acid (Compound 19, *m/z* 337.0895) and 3-caffeoylquinic acid (Compound 18, *m/z* 353.0866). Sinapic acid (Compound 12, *m/z* 223.0620) was confirmed through water loss to *m/z* 205, consistent with Grzegorzczak-Karolak et al. [54]. Further notable compounds included caffeoyl glucose (Compound 27, *m/z* 341.0895), identified by glucose loss (162 Da) and fragment ions at *m/z* 179 and 161 [55]. *m*-Coumaric acid (Compound 28, *m/z* 163.0416) showed decarboxylation to *m/z* 119 [56]. Caffeic acid (Compound 13, *m/z* 179.0349) exhibited base peaks at *m/z* 143 (loss of 2H₂O) and *m/z* 133 (loss of HCOOH), supporting its identification [57]. Cinnamic acid (Compound 22) was identified in the negative ionization mode at *m/z* 147.0438 only in the Falsa organic squash sample.

The presence of glycosylated and quinic acid conjugates might indicate the activity of transferases involved in phenolic modification. Thermal processing can markedly influence these compounds; heating promotes glycoside hydrolysis, increasing the levels of free, more bioavailable aglycones such as ferulic acid, whereas excessive heat can induce degradation, contributing to the overall decline in TPC, as discussed in Section 3.1.

Among the identified hydroxycinnamic acids, caffeic acid is of particular interest due to its reported anticancer activity, especially against hepatocellular carcinoma [58]. This bioactivity is attributed to the compound's structure, particularly its catechol hydroxyl groups and conjugated double bonds, which enable efficient radical scavenging and metal chelation.

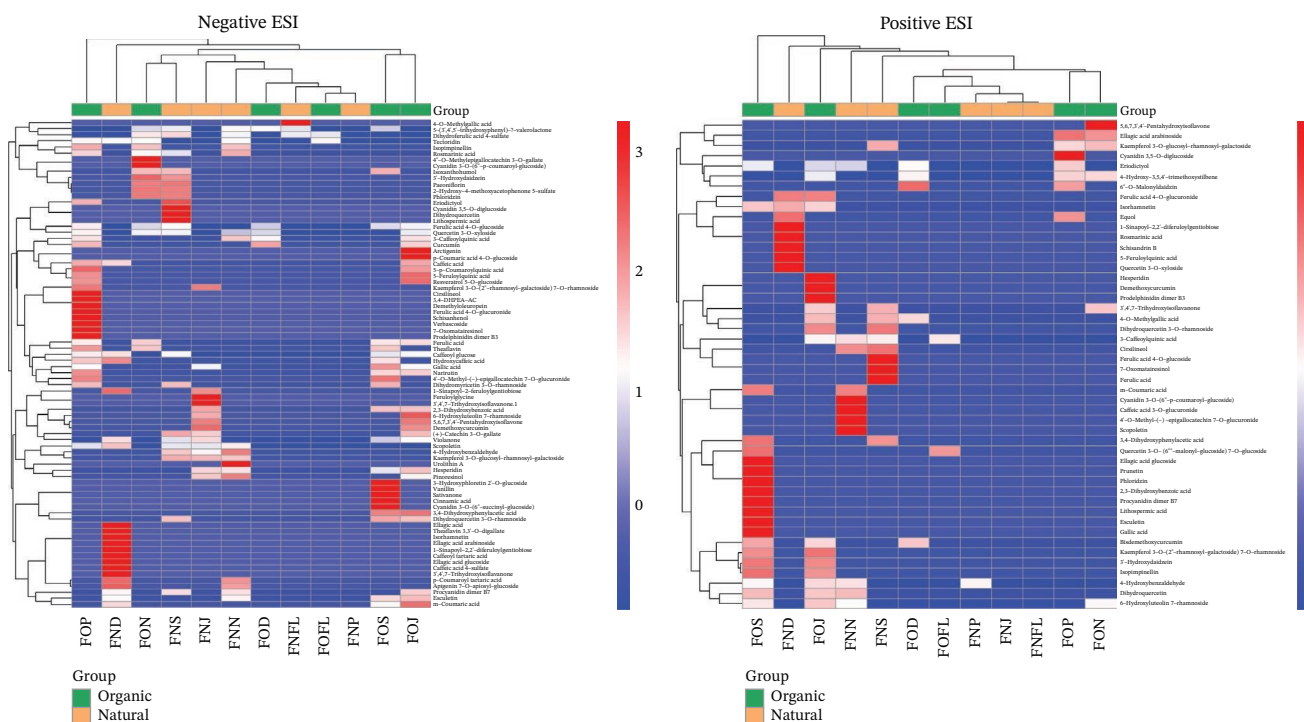


FIGURE 1 | Heatmaps representing the relative abundance of identified polyphenolic compounds across the processed Falsa products obtained by LC-ESI-QTOF-MS/MS analysis in negative and positive ionization modes. Color intensity (blue to red) indicates low to high relative abundance of compounds. Hierarchical clustering illustrates similarities and differences among samples, highlighting distinct grouping patterns between organic and natural products and variations associated with processing methods. Abbreviations: FND, Falsa natural drink; FNFL, Falsa natural fruit leather; FNJ, Falsa natural jam; FNN, Falsa natural nectar; FNP, Falsa natural pulp; FNS, Falsa natural squash; FOD, Falsa organic drink; FOFL, Falsa organic fruit leather; FOJ, Falsa organic jam; FON, Falsa organic nectar; FOP, Falsa organic pulp; FOS, Falsa organic squash.

3.4.2 | Other Polyphenols

In addition to phenolic acids, flavonoids, stilbenes, and lignans, a total of 12 additional polyphenolic compounds were identified across the 12 processed Falsa products. These included lithospermic acid (1), hydroxycoumarins (3), furanocoumarins (1), hydroxybenzaldehydes (1), hydroxybenzoketones (1), curcuminoids (3), and tyrosols (2). Among the hydroxycoumarins, scopoletin (Compound 72) and esculetin (Compound 71) were detected, with precursor ions at m/z 191.0348 and 177.0187, respectively. In the MS^2 spectrum, scopoletin produced a characteristic fragment at m/z 176, resulting from the loss of a methyl group (CH_3 , 15 Da). Esculetin displayed a product ion at m/z 133, corresponding to the loss of both water (18 Da) and carbon monoxide (28 Da), consistent with previously reported fragmentation patterns.

These minor polyphenolic classes, although present in lower abundance, contribute to the overall chemical diversity of Falsa and may play synergistic roles in antioxidant activity. Their presence highlights the complex phytochemical composition of Falsa-based processed products and supports their characterization as a functional food source.

3.4.3 | Flavonoids

A total of 36 flavonoids were identified in the processed Falsa products, encompassing various subclasses: flavanols (7), flavones (4), flavanones (5), flavonols (5), dihydrochalcones (2),

dihydroflavonols (3), anthocyanins (3), and isoflavonoids (7) (Table 4). These compounds are known for their antioxidant, anti-inflammatory, and health-promoting properties and contribute substantially to the bioactivity of fruit-based functional foods.

3.4.3.1 | Flavanols. Among the flavanols, procyanidin dimer B1 (Compound 39) was detected in multiple samples (Falsa natural drink, Falsa organic jam, Falsa natural squash, Falsa natural nectar, and Falsa organic squash). It was tentatively identified based on the precursor ion $[M-H]^-$ at m/z 577.138. Prodelphinidin dimer B3 (Compound 37) was identified in both ionization modes with a molecular ion at m/z 611.1396. Fragment ions at m/z 469, 311, and 291 were attributed to heterocyclic fission and subsequent loss of phloroglucinol and hydroxyl groups, respectively [59].

3.4.3.2 | Flavones and Flavanones. Four flavones (Compounds 40–43) and five flavanones (Compounds 44–48) were also detected. In positive ionization mode, Compound 40 (m/z 345.0958) was identified as cirsilineol, Compound 42 (m/z 463.1248) as chrysoeriol 7-*O*-glucoside, and Compound 41 (m/z 565.1534) as apigenin 7-*O*-apiosylglucoside. Flavanones included narirutin (Compound 46, m/z 579.1709) and naringenin (Compound 44, m/z 271.0604) detected in negative ionization mode.

Flavanols are an important subclass of flavonoids, characterized by a ketone group and a 3-hydroxyl group on the central pyran (C) ring. Structurally, they consist of two aromatic rings (A and

B) connected by a heterocyclic C ring and are known to contribute to proanthocyanidin biosynthesis. Flavonols such as quercetin, kaempferol, and myricetin are commonly found in fruits and vegetables and exhibit strong antioxidant properties, including free radical neutralization and protection against oxidative stress-related diseases [60].

In this study, five flavonols were identified: isorhamnetin (Compound 51, m/z 315.0515), quercetin 3-*O*-arabinoside (Compound 49, m/z 433.0780), and kaempferol 3-*O*-glucosyl-rhamnosyl-galactoside (Compound 50, m/z 755.2032). Fragmentation of Compound 51 produced a base peak at m/z 300, corresponding to the loss of a methyl radical. Product ions at m/z 271 and 285 from Compounds 49 and 50 indicated characteristic fragmentation patterns, confirming their identities [61].

3.4.3.3 | Dihydrochalcones. Dihydrochalcones are a subclass of flavonoid derivatives with a distinctive structure consisting of two aromatic rings connected by a three-carbon aliphatic chain. They exhibit diverse bioactivities, including antioxidant, anti-inflammatory, antimicrobial, and antidiabetic effects, often enhanced by glycosylation [62, 63]. In this study, 3-hydroxyphloretin 2'-*O*-glucoside (Compound 54) was identified only in Falsa organic squash, with a precursor ion of m/z 451.125 in negative ionization mode.

3.4.3.4 | Dihydroflavonols. Dihydroflavonols are formed via the oxidative hydroxylation of flavanones, introducing a hydroxyl group at the C-3 position. These intermediates are critical in flavonoid biosynthesis and also display antioxidant and cardioprotective properties [64]. Dihydromyricetin 3-*O*-rhamnoside was detected in negative mode at m/z 465.1077. Fragmentation yielded a product ion at m/z 301, corresponding to the loss of a rhamnose moiety (164 Da), consistent with reported data [65].

3.4.3.5 | Isoflavonoids. Isoflavonoids are structurally similar to flavonoids but differ in the positioning of the B ring on the central C ring. They are largely plant-derived and are recognized for their phytoestrogenic activity and therapeutic potential in cancer, inflammation, osteoporosis, and metabolic disorders [66, 67]. A total of seven isoflavonoids were identified across the processed Falsa samples investigated in this study.

Equol (Compound 62, m/z 243.1021 $[M+H]^+$) was detected in Falsa organic pulp, Falsa organic squash, and Falsa natural nectar, while 2'-hydroxyformononetin (Compound 66) was found in Falsa organic squash, with m/z 285.0745 and product ions at m/z 270 and 229. Sativanone (Compound 63) was also observed in Falsa organic squash, identified in negative mode at m/z 299.0913, showing characteristic fragment ions at m/z 284 and 269 due to the loss of a methyl group [68]. 6''-*O*-Malonyldaidzin (Compound 68, m/z 503.1186 $[M+H]^+$) was identified in Falsa organic pulp and Falsa organic squash, with a key MS/MS fragment at m/z 255, confirming its identity.

3.4.4 | Anthocyanins

Anthocyanins are water-soluble flavonoid pigments responsible for the red, purple, and blue hues found in many fruits, vegetables, and flowers, particularly in berries and dark-colored plant

materials. Structurally, anthocyanins consist of a benzopyran backbone with varying hydroxyl and methoxy substitutions, which influence their color expression and chemical stability [69]. Common anthocyanins include cyanidin, petunidin, malvidin, and pelargonidin, which are often glycosylated, enhancing their solubility and bioavailability.

In this study, three anthocyanins were tentatively identified in processed Falsa products. Compound 60 was detected in Falsa organic pulp and Falsa natural squash, with a precursor ion at m/z 612.1685 in positive ionization mode. MS/MS analysis produced characteristic fragment ions at m/z 449 (indicative of $[M - \text{glucose} - \text{H}_2\text{O}]^+$) and m/z 287 (resulting from further loss of glucose), supporting the identification of cyanidin 3,5-*O*-diglucoside. Compound 61 was found in Falsa natural nectar and Falsa organic nectar and was tentatively identified as cyanidin 3-*O*-(6''-*p*-coumaroyl-glucoside), based on its precursor ion at m/z 596.1547 in positive ionization mode. The coumaroyl conjugation on the glucose moiety suggests enhanced antioxidant activity and increased stability in acidic food matrices, making these compounds particularly relevant to the color and functional properties of Falsa-based products.

3.4.5 | Other Polyphenols

In addition to the major flavonoid subclasses and phenolic acids, other polyphenolic compounds were identified across the 12 processed Falsa products. These included hydroxycoumarins (3), furanocoumarins (1), hydroxybenzaldehydes (1), hydroxybenzoketones (1), curcuminoids (3), and tyrosols (2) (Table 4). Among the hydroxycoumarins, scopoletin and esculetin were detected in both positive and negative ionization modes, with precursor ions at m/z 191.0348 and 177.0187, respectively. The MS² spectrum of scopoletin showed a major fragment at m/z 176, consistent with the loss of a methyl group (15 Da), confirming its structure [70]. Esculetin generated a product ion at m/z 133, corresponding to successive losses of H₂O (18 Da) and CO (28 Da) from the precursor ion, supporting its identification [71].

3.4.6 | Lignans and Stilbenes

Lignans are a class of plant-derived polyphenolic compounds and represent a major group of phytoestrogens. Structurally, lignans are typically composed of two phenylpropanoid units (C6–C3) linked through carbon–carbon or carbon–oxygen bonds, forming dimers or more complex oligomers [72]. These secondary metabolites are widely distributed in the plant kingdom, particularly in seeds, whole grains, vegetables, and fruits. Flaxseed is considered one of the richest dietary sources of lignans. Numerous *in vitro* and *in vivo* studies have demonstrated the anticancer, antioxidant, cardioprotective, and antiestrogenic effects of lignans, supporting their relevance in health-promoting foods [73].

Stilbenes are another important group of plant polyphenols, found predominantly in roots, rhizomes, leaves, and bark. They are synthesized as defense compounds in response to biotic and abiotic stresses. Stilbenes typically feature a C6–C2–C6 backbone with various hydroxyl, methoxy, or sugar substituents, giving rise to compounds such as resveratrol, piceatannol, and

pterostilbene [74]. These compounds exhibit a wide range of bioactivities, including antioxidant, anticancer, neuroprotective, and cardioprotective effects [75]. The distribution and concentration of stilbenes in plants are known to vary depending on tissue type, developmental stage, and seasonal conditions [76].

In this study, Compound 87 was tentatively identified in Falsa organic jam and Falsa organic pulp as resveratrol 5-*O*-glucoside, with a precursor ion at m/z 389.1273. The MS² spectrum exhibited a major fragment ion at m/z 227, corresponding to the loss of a glucoside moiety (162 Da), confirming the compound's structural identity [77].

The Falsa samples analyzed in this study were found to contain several phenolic compounds known for their antioxidant properties, including various derivatives of caffeic acid, ferulic acid, and procyanidin. Caffeic acid derivatives are recognized for their ability to scavenge free radicals and inhibit oxidative stress by donating hydrogen atoms and chelating metal ions, thereby contributing to cellular protection against oxidative damage [78]. Similarly, ferulic acid derivatives possess potent antioxidant activity through their capacity to stabilize free radicals and modulate antioxidant enzyme activities [79]. Procyanidins, which are oligomeric flavan-3-ols, have been extensively studied for their high radical scavenging capacity and are also linked to cardioprotective and neuroprotective effects due to their ability to reduce lipid peroxidation and inflammation [80]. These compounds collectively contribute to the enhanced antioxidant potential observed in the fruit pulps, underlying their possible health benefits in mitigating oxidative stress-related diseases. To validate these untargeted findings into validated functional benefits, future studies must focus on two critical areas: first, employing targeted

quantification to establish the precise absolute concentrations of key compounds for correlation with specific in vitro or in vivo biological effects; and second, utilizing advanced metabolomics approaches in cell-based assays or postdigestion models to monitor the biotransformation and bioavailability of these compounds, thereby establishing a definitive link between the identified chemical structures and their validated biological activity within a physiological context.

3.5 | Heatmap Visualization of HPLC Data

Hierarchical clustering analysis of HPLC-PDA data was performed to evaluate the phenolic profiles of 12 processed Falsa products, and the results are presented as a heatmap (Figure 2). This approach enables comparative visualization of the relative distribution of selected phenolic acids and flavonoids, facilitating the identification of similarities and differences among product types and processing methods. The clustered heatmap revealed distinct grouping patterns based on the phenolic composition. One cluster comprised Falsa organic nectar, Falsa organic squash, Falsa organic drink, and Falsa organic jam, characterized by consistently low epicatechin levels and generally lower to moderate concentrations of phenolic acids, including gallic, *p*-hydroxybenzoic, *p*-coumaric, and *trans*-ferulic acids. A second cluster included products such as Falsa natural squash, Falsa organic pulp, and Falsa organic fruit leather, which exhibited comparable phenolic profiles with some variability, particularly in gallic acid content. In contrast, Falsa natural pulp displayed a distinct phenolic signature, clearly separated in the clustering analysis, with markedly elevated epicatechin and increased *p*-hydroxybenzoic acid relative to the other samples. Overall, phenolic

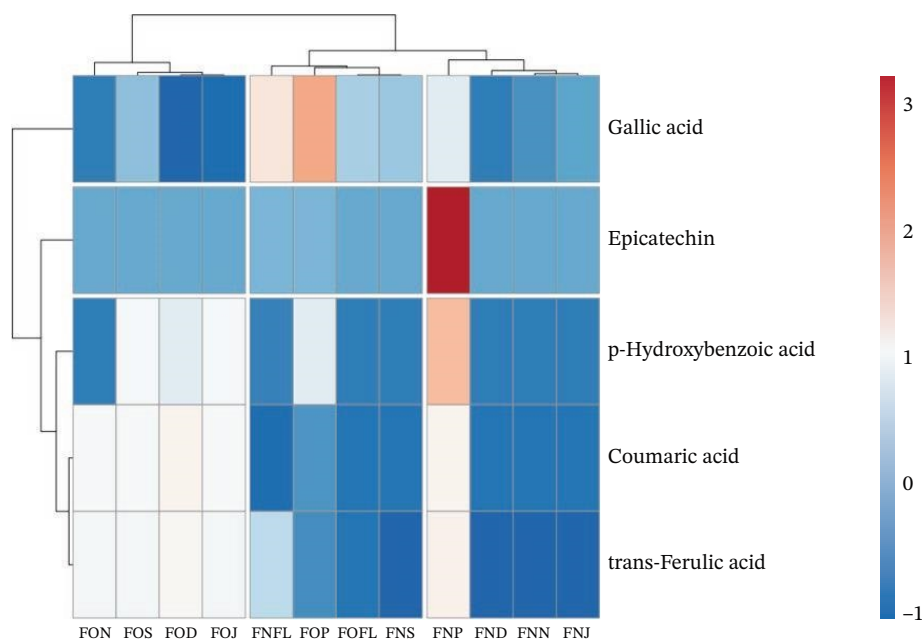


FIGURE 2 | Heatmap showing the distribution and relative concentrations of selected phenolic compounds across 12 processed Falsa products. Color gradients represent compound abundance, with red indicating higher concentrations and blue indicating lower concentrations. Hierarchical clustering was applied to group both samples and compounds based on similarity in phenolic profiles. Abbreviations: FND, Falsa natural drink; FNFL, Falsa natural fruit leather; FNJ, Falsa natural jam; FNN, Falsa natural nectar; FNP, Falsa natural pulp; FNS, Falsa natural squash; FOD, Falsa organic drink; FOFL, Falsa organic fruit leather; FOJ, Falsa organic jam; FON, Falsa organic nectar; FOP, Falsa organic pulp; FOS, Falsa organic squash.

concentrations across processed products appeared variable and, in several cases, reduced, likely reflecting the impact of processing conditions such as thermal treatment and storage. This observation is consistent with previous reports indicating that processing can influence phenolic composition and associated antioxidant potential.

4 | Conclusions

This study highlights the potential of processed Falsa products as a source of bioactive phenolic compounds, with natural Falsa fruit leather being the most phenolic-rich and antioxidant-active formulation. The identification of 88 distinct polyphenolic compounds supports Falsa's chemical diversity and its relevance as a functional food ingredient. The results demonstrate that processing methods significantly influence the retention of bioactive compounds, highlighting the importance of optimized drying and minimal thermal exposure in preserving phenolic integrity and antioxidant capacity. From a practical perspective, these findings provide guidance for developing polyphenolic-enriched, health-oriented Falsa-based products. Food processors and product developers may exploit the fruit's natural antioxidant properties through minimal processing techniques, controlled dehydration, and protective packaging strategies that minimize oxidative degradation. Further, incorporating Falsa extracts into active packaging, microencapsulation systems, or natural preservative formulations could enhance both nutritional and storage stability in functional food applications. Future research should focus on understanding the bioavailability and in vivo efficacy of Falsa-derived phenolics, as well as evaluating sensory acceptance, product stability during storage, and scalability of processing methods. These investigations will be essential for validating health claims, optimizing processing parameters, and advancing the commercial application of Falsa-based functional foods.

However, several limitations should be acknowledged. The use of absolute methanol as the extraction solvent may have limited the recovery of certain phenolics compared with mixed or acidified solvent systems. Furthermore, this study focused only on the pulp fraction, excluding peel or seed components that might be a richer source of polyphenols. Additionally, the antioxidant activity was assessed through chemical assays, which may not fully reflect physiological efficacy. Future research should therefore optimize extraction solvents for enhanced recovery of polyphenols, use in vivo assays that are more accurately reflective of biological activity given their mechanistic similarity to the human body, and evaluate consumer acceptability of phenolic-rich formulations such as fruit leather. Further work should also examine storage stability, packaging interactions, and scalability of production processes to support the development of commercially viable Falsa-based functional products.

Author Contributions

X. Qin: conceptualization, formal analysis, investigation, methodology, data curation, software, writing – original draft. **A. Din:** methodology, data curation, writing – original draft. **M. R. Khan:** methodology, writing – original draft. **M. A. Shabbir:** methodology, writing – review and editing. **A. Rafi:** methodology, writing – review and editing.

A. Siddiqua: methodology, formal analysis, writing – review and editing. **O. T. Agar:** methodology, software, writing – review and editing. **A. Beyatli:** methodology, visualization, writing – review and editing. **A. Imran:** methodology, software, writing – review and editing. **F. Ahmadi:** data curation, writing – review and editing. **H. A. R. Suleria:** conceptualization, investigation, methodology, project administration, resources, validation, supervision, writing – review and editing.

Acknowledgments

During the preparation of this work, the authors used OpenAI's tool ChatGPT-4 to enhance the readability, clarity, and fluency of the text. After using this tool, the authors reviewed and edited the content as needed and took full responsibility for the content of the publication.

Funding

No funding was received for this manuscript. Open access publishing facilitated by The University of Melbourne, as part of the Wiley - The University of Melbourne agreement via the Council of Australasian University Librarians.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

References

1. I. Mozos, C. Flangea, D. C. Vlad, et al., "Effects of Anthocyanins on Vascular Health," *Biomolecules* 11, no. 6 (2021): 811, <https://doi.org/10.3390/biom11060811>.
2. B. Vahapoglu, E. Erskine, B. Gultekin Subasi, and E. Capanoglu, "Recent Studies on Berry Bioactives and Their Health-Promoting Roles," *Molecules* 27, no. 1 (2022): 108, <https://doi.org/10.3390/molecules27010108>.
3. M. Zia-Ul-Haq, M. S. Stanković, K. Rizwan, and V. De Feo, "Grewia asiatica L., A Food Plant With Multiple Uses," *Molecules* 18, no. 3 (2013): 2663–2682, <https://doi.org/10.3390/molecules18032663>.
4. F. Mirheidari, A. Khadivi, and Y. Moradi, "Selection of Promising Accessions of Phalsa (*Grewia asiatica* L.) Based on Fruit-Related Traits," *Food Science & Nutrition* 10, no. 11 (2022): 3680–3688, <https://doi.org/10.1002/fsn3.2967>.
5. R. Shukla, D. C. Sharma, M. H. Baig, et al., "Antioxidant, Antimicrobial Activity and Medicinal Properties of *Grewia asiatica* L.," *Medicinal Chemistry* 12, no. 3 (2016): 211–216, <https://doi.org/10.2174/1573406411666151030110530>.
6. T. K. Koley, Z. Khan, D. Oulkar, B. Singh, B. P. Bhatt, and K. Banerjee, "Profiling of Polyphenols in Phalsa (*Grewia asiatica* L.) Fruits Based on Liquid Chromatography High Resolution Mass Spectrometry," *Journal of Food Science and Technology* 57, no. 2 (2020): 606–616, <https://doi.org/10.1007/s13197-019-04092-y>.
7. A. Saeed, S. Kauser, A. Hussain, et al., "Tracking the Variations in Trace Elements, Some Nutrients, Phenolics, and Anthocyanins in *Grewia asiatica* L. (Phalsa) at Different Fruit Development Stages," *Biological Trace Element Research* 202, no. 4 (2024): 1784–1801, <https://doi.org/10.1007/s12011-023-03763-4>.
8. M. Samsonowicz, E. Regulska, D. Karpowicz, and B. Leśniewska, "Antioxidant Properties of Coffee Substitutes Rich in Polyphenols and Minerals," *Food Chemistry* 278 (2019): 101–109, <https://doi.org/10.1016/j.foodchem.2018.11.057>.

9. B. Christ, and K. H. Müller, "Zur Serienmäßigen Bestimmung Des Gehaltes An Flavonol-Derivaten In Drogen," *Archiv Der Pharmazie* 293, no. 12 (1960): 1033–1042.
10. D. Peng, H. F. Zahid, S. Ajlouni, F. R. Dunshea, and H. A. Suleria, "LC-ESI-QTOF/MS Profiling of Australian Mango Peel By-Product Polyphenols and Their Potential Antioxidant Activities," *Processes* 7, no. 10 (2019): 764, <https://doi.org/10.3390/pr7100764>.
11. F. M. Vella, D. Cautela, and B. Laratta, "Characterization of Polyphenolic Compounds in Cantaloupe Melon By-Products," *Foods* 8, no. 6 (2019): 196, <https://doi.org/10.3390/foods8060196>.
12. N. Smirnoff, and Q. J. Cumbes, "Hydroxyl Radical Scavenging Activity of Compatible Solutes," *Phytochemistry* 28, no. 4 (1989): 1057–1060, [https://doi.org/10.1016/0031-9422\(89\)80182-7](https://doi.org/10.1016/0031-9422(89)80182-7).
13. T. C. Dinis, V. M. Madeira, and L. M. Almeida, "Action of Phenolic Derivatives (Acetaminophen, Salicylate, and 5-Aminosalicylate) as Inhibitors of Membrane Lipid Peroxidation and as Peroxyl Radical Scavengers," *Archives of Biochemistry and Biophysics* 315, no. 1 (1994): 161–169, <https://doi.org/10.1006/abbi.1994.1485>.
14. J. Du, B. Zhong, V. Subbiah, C. J. Barrow, F. R. Dunshea, and H. A. Suleria, "LC-ESI-QTOF-MS/MS Profiling and Antioxidant Activity of Phenolics From Custard Apple Fruit and By-Products," *Separations* 8, no. 5 (2021): 62, <https://doi.org/10.3390/separations8050062>.
15. M. B. Tanujaya, F. Ahmadi, A. Beyatli, A. Imran, and H. A. R. Suleria, "Antioxidant Capacity and Polyphenol Compounds Characterization Through LC-ESI-QTOF-MS/MS in Citrus Peels From Different Varieties," *Journal of Food Biochemistry* 2025, no. 1 (2025): 6515172, <https://doi.org/10.1155/jfbc/6515172>.
16. A. L. K. Faller, and E. Fialho, "Polyphenol Content and Antioxidant Capacity in Organic and Conventional Plant Foods," *Journal of Food Composition and Analysis* 23, no. 6 (2010): 561–568, <https://doi.org/10.1016/j.jfca.2010.01.003>.
17. D. Shende, and A. K. Datta, "Optimization Study for Refractance Window Drying Process of Langra Variety Mango," *Journal of Food Science and Technology* 57, no. 2 (2020): 683–692, <https://doi.org/10.1007/s13197-019-04101-0>.
18. M. Carcea, V. Narducci, V. Turfani, and V. Giannini, "Polyphenols in Raw and Cooked Cereals/Pseudocereals/Legume Pasta and Couscous," *Foods* 6, no. 9 (2017): 80, <https://doi.org/10.3390/foods6090080>.
19. M. T. Rashid, F. Sarpong, M. M. Hashim, M. A. Jatoi, B. Safdar, and M. S. Baloch, "Quality Changes in Diet Phalsa Squash Formulation During Storage: A Kinetic and Statistical Interpretation of Key Parameters Degradation Mechanism," *International Journal of Fruit Science* 21, no. 1 (2021): 804–818, <https://doi.org/10.1080/15538362.2021.1936346>.
20. Y. Zhou, Y. Cao, J. Li, et al., "Screening and Characterization of Phenolic Compounds by LC-ESI-QTOF-MS/MS and Their Antioxidant Potentials in Papaya Fruit and Their By-Products Activities," *Food Bioscience* 52 (2023): 102480, <https://doi.org/10.1016/j.fbio.2023.102480>.
21. B. Sultana, F. Anwar, and M. Ashraf, "Effect of Extraction Solvent/Technique on the Antioxidant Activity of Selected Medicinal Plant Extracts," *Molecules* 14, no. 6 (2009): 2167–2180, <https://doi.org/10.3390/molecules14062167>.
22. J. Dai, and R. J. Mumper, "Plant Phenolics: Extraction, Analysis and Their Antioxidant and Anticancer Properties," *Molecules* 15, no. 10 (2010): 7313–7352, <https://www.mdpi.com/1420-3049/15/10/7313>, <https://doi.org/10.3390/molecules15107313>.
23. A. Arceusz, M. Wesolowski, and P. Konieczynski, "Methods for Extraction and Determination of Phenolic Acids in Medicinal Plants: A Review," *Natural Product Communications* 8, no. 12 (2013): 1934578X1300801238.
24. A. Oufighou, F. Brahmi, S. Achat, et al., "Optimization of Microwave-Assisted Extraction of Phenolic Compounds From *Opuntia ficus-indica* Cladodes," *Processes* 13, no. 3 (2025): 724, <https://doi.org/10.3390/pr13030724>.
25. M. M. A. N. Ranjha, S. Amjad, S. Ashraf, et al., "Extraction of Polyphenols From Apple and Pomegranate Peels Employing Different Extraction Techniques for the Development of Functional Date Bars," Supplement 3, *International Journal of Fruit Science* 20 (2020): S1201–S1221, <https://doi.org/10.1080/15538362.2020.1782804>.
26. B. J. Xu, and S. K. Chang, "A Comparative Study on Phenolic Profiles and antioxidant Activities of Legumes as Affected by Extraction Solvents," *Journal of Food Science* 72, no. 2 (2007): S159–S166, <https://doi.org/10.1111/j.1750-3841.2006.00260.x>.
27. M. Lasota, P. Lechwar, W. Kukula-Koch, M. Czop, K. Czech, and K. Gawel-Bęben, "Pulp or Peel? Comparative Analysis of the Phytochemical Content and Selected Cosmetic-Related Properties of *Annona cherimola* L., *Diospyros kaki* Thumb., *Cydonia oblonga* Mill. and *Fortunella margarita* Swingle Pulp and Peel Extracts," *Molecules* 29, no. 5 (2024): 1133, <https://www.mdpi.com/1420-3049/29/5/1133>, <https://doi.org/10.3390/molecules29051133>.
28. A. Razzak, T. Mahjabin, M. R. M. Khan, M. Hossain, U. Sadia, and W. Zzaman, "Effect of Cooking Methods on the Nutritional Quality of Selected Vegetables at Sylhet City," *Heliyon* 9, no. 11 (2023): e21709, <https://doi.org/10.1016/j.heliyon.2023.e21709>.
29. M. Nadeem, A. Ghaffar, M. M. Hashim, et al., "Sonication and Microwave Processing of Phalsa Drink: A Synergistic Approach," *International Journal of Fruit Science* 21, no. 1 (2021): 993–1007, <https://doi.org/10.1080/15538362.2021.1965942>.
30. A. Pękal, and K. Pyrzynska, "Evaluation of Aluminium Complexation Reaction for Flavonoid Content Assay," *Food Analytical Methods* 7, no. 9 (2014): 1776–1782, <https://doi.org/10.1007/s12161-014-9814-x>.
31. A. M. Shraim, T. A. Ahmed, M. M. Rahman, and Y. M. Hijji, "Determination of Total Flavonoid Content by Aluminum Chloride Assay: A Critical Evaluation," *LWT* 150 (2021): 111932, <https://doi.org/10.1016/j.lwt.2021.111932>.
32. J. L. Goldstein, and T. Swain, "Changes in Tannins in Ripening Fruits," *Phytochemistry* 2, no. 4 (1963): 371–383, [https://doi.org/10.1016/S0031-9422\(00\)84860-8](https://doi.org/10.1016/S0031-9422(00)84860-8).
33. T. Ozawa, T. H. Lilley, and E. Haslam, "Polyphenol Interactions: Astringency and the Loss of Astringency in Ripening Fruit," *Phytochemistry* 26, no. 11 (1987): 2937–2942, [https://doi.org/10.1016/S0031-9422\(00\)84566-5](https://doi.org/10.1016/S0031-9422(00)84566-5).
34. J. Perez-Jimenez, V. Neveu, F. Vos, and A. Scalbert, "Systematic Analysis of the Content of 502 Polyphenols in 452 Foods and Beverages: An Application of the Phenol-Explorer Database," *Journal of Agricultural and Food Chemistry* 58, no. 8 (2010): 4959–4969, <https://doi.org/10.1021/jf100128b>.
35. Q. Ye, H. Wang, and J. Xu, "Application of NMR Spectroscopy for the Characterization of Dietary Polyphenols," in *Applications of NMR Spectroscopy: Volume 3* (Bentham Science Publishers, 2015), 37–77, <https://doi.org/10.2174/9781681080628115030004>.
36. Y. Lang, N. Gao, Z. Zang, et al., "Classification and Antioxidant Assays of Polyphenols: A Review," *Journal of Future Foods* 4, no. 3 (2024): 193–204, <https://doi.org/10.1016/j.jfutfo.2023.07.002>.
37. C. Manach, A. Scalbert, C. Morand, C. Rémésy, and L. Jiménez, "Polyphenols: Food Sources and Bioavailability," *American Journal of Clinical Nutrition* 79, no. 5 (2004): 727–747, <https://doi.org/10.1093/ajcn/79.5.727>.
38. A. González-Sarriás, R. García-Villalba, F. A. Tomás-Barberán, and J. C. Espín, "Metabolism of Proanthocyanidins by Human Gut Microflora: Role of the Colon on the Bioavailability of Polyphenols,"

- Journal of Agricultural and Food Chemistry* 56, no. 10 (2008): 3848–3856, <https://doi.org/10.1021/jf073405b>.
39. B. Poljsak, V. Kovač, and I. Milisav, “Antioxidants, Food Processing and Health,” *Antioxidants* 10, no. 3 (2021): 433, <https://doi.org/10.3390/antiox10030433>.
 40. F. Shahidi, and Y. Zhong, “Measurement of Antioxidant Activity,” *Journal of Functional Foods* 18 (2015): 757–781, <https://doi.org/10.1016/j.jff.2015.01.047>.
 41. J. Rumpf, R. Burger, and M. Schulze, “Statistical Evaluation of DPPH, ABTS, FRAP, and Folin-Ciocalteu Assays to Assess the Antioxidant Capacity of Lignins,” *International Journal of Biological Macromolecules* 233 (2023): 123470, <https://doi.org/10.1016/j.ijbiomac.2023.123470>.
 42. J. Treml, and K. Šmejkal, “Flavonoids as Potent Scavengers of Hydroxyl Radicals,” *Comprehensive Reviews in Food Science and Food Safety* 15, no. 4 (2016): 720–738, <https://doi.org/10.1111/1541-4337.12204>.
 43. C. Mussio, P. Garcia-Perez, E. Moret, S. Catena, and L. Lucini, “The Role, Mechanisms and Evaluation of Natural Chelating Agents in Food Stability: A Review,” *Food Chemistry* 496 (2025): Pt 1, 146682, <https://doi.org/10.1016/j.foodchem.2025.146682>.
 44. P. Stratil, B. Klejdus, and V. Kubáň, “Determination of Total Content of Phenolic Compounds and Their Antioxidant Activity in Vegetables Evaluation of Spectrophotometric Methods,” *Journal of Agricultural and Food Chemistry* 54, no. 3 (2006): 607–616, <https://doi.org/10.1021/jf052334j>.
 45. K. M. Naji, F. H. Thamer, A. A. Numan, E. M. Dauqan, Y. M. Alshaibi, and M. R. D’souza, “Ferric-Bipyridine Assay: A Novel Spectrophotometric Method for Measurement of Antioxidant Capacity,” *Heliyon* 6, no. 1 (2020): e03162, <https://doi.org/10.1016/j.heliyon.2020.e03162>.
 46. R. L. Prior, X. Wu, and K. Schaich, “Standardized Methods for the Determination of Antioxidant Capacity and Phenolics in Foods and Dietary Supplements,” *Journal of Agricultural and Food Chemistry* 53, no. 10 (2005): 4290–4302, <https://doi.org/10.1021/jf0502698>.
 47. D. Huang, B. Ou, and R. L. Prior, “The Chemistry Behind Antioxidant Capacity Assays,” *Journal of Agricultural and Food Chemistry* 53, no. 6 (2005): 1841–1856, <https://doi.org/10.1021/jf030723c>.
 48. Z. Cheng, J. Ren, Y. Li, W. Chang, and Z. Chen, “Study on the Multiple Mechanisms Underlying the Reaction Between Hydroxyl Radical and Phenolic Compounds by Qualitative Structure and Activity Relationship,” *Bioorganic & Medicinal Chemistry* 10, no. 12 (2002): 4067–4073, [https://doi.org/10.1016/S0968-0896\(02\)00267-5](https://doi.org/10.1016/S0968-0896(02)00267-5).
 49. S. C. Rangani, R. A. U. J. Marapana, G. S. A. Senanayake, P. R. D. Perera, M. M. Pathmalal, and H. K. Amarasinghe, “Correlation Analysis of Phenolic Compounds, Antioxidant Potential, Oxygen Radical Scavenging Capacity, and Alkaloid Content in Ripe and Unripe *Areca catechu* From Major Cultivation Areas in Sri Lanka,” *Applied Food Research* 3, no. 2 (2023): 100361, <https://doi.org/10.1016/j.afres.2023.100361>.
 50. P. K. Mukherjee, P. K. Mukherjee, “LC–MS: A Rapid Technique for Understanding the Plant Metabolite Analysis,” in *Quality Control and Evaluation of Herbal Drugs* (Elsevier, 2019), 459–479, <https://doi.org/10.1016/B978-0-12-813374-3.00011-9>.
 51. P. Padmanabhan, J. Correa-Betanzo, and G. Paliyath, B. Caballero, P. M. Finglas, and F. Toldrá, “Berries and Related Fruits,” in *Encyclopedia of Food and Health* (Academic Press, 2016), 364–371, <https://doi.org/10.1016/B978-0-12-384947-2.00060-X>.
 52. N. P. Seeram, D. Heber, “Berries,” in *Nutritional Oncology*, (2nd ed. (Academic Press, 2006), 615–628, <https://doi.org/10.1016/B978-012088393-6/50093-2>.
 53. M. G. Tosato, D. E. Orallo, M. F. Fangio, V. Diz, L. E. Dicelio, and M. S. Churio, A. M. Grumezescu, “Nanomaterials and Natural Products for UV-Photoprotection,” in *Surface Chemistry of Nanobiomaterials* (William Andrew Publishing, 2016), 359–392, <https://doi.org/10.1016/B978-0-323-42861-3.00012-1>.
 54. I. Grzegorzczak-Karolak, M. Krzemińska, A. K. Kiss, M. A. Olszewska, and A. Owczarek, “Phytochemical Profile and Antioxidant Activity of Aerial and Underground Parts of *Salvia bulleyana* Diels. Plants,” *Metabolites* 10, no. 12 (2020): , <https://doi.org/10.3390/metabo10120497>.
 55. X. Liao, Y. Hong, and Z. Chen, “Identification and Quantification of the Bioactive Components in *Osmanthus fragrans* Roots by HPLC-MS/MS,” *Journal of Pharmaceutical Analysis* 11, no. 3 (2021): 299–307, <https://doi.org/10.1016/j.jpha.2020.06.010>.
 56. A. Ali, H. Wu, E. N. Ponnampalam, J. J. Cottrell, F. R. Dunshea, and H. A. R. Suleria, “Comprehensive Profiling of Most Widely Used Spices for Their Phenolic Compounds Through LC-ESI-QTOF-MS2 and Their Antioxidant Potential,” *Antioxidants* 10, no. 5 (2021): 721, <https://www.mdpi.com/2076-3921/10/5/721>, <https://doi.org/10.3390/antiox10050721>.
 57. E. Schiano, V. Piccolo, E. Novellino, et al., “Thinned Nectarines, an Agro-Food Waste With Antidiabetic Potential: HPLC-HESI-MS/MS Phenolic Characterization and In Vitro Evaluation of Their Beneficial Activities,” *Foods* 11, no. 7 (2022): 1010, <https://doi.org/10.3390/foods11071010>.
 58. K. M. M. Espíndola, R. G. Ferreira, L. E. M. Narvaez, et al., “Chemical and Pharmacological Aspects of Caffeic Acid and Its Activity in Hepatocarcinoma,” *Frontiers in Oncology* 9 (2019): 541, <https://doi.org/10.3389/fonc.2019.00541>.
 59. V. Subbiah, B. Zhong, M. A. Nawaz, C. J. Barrow, F. R. Dunshea, and H. A. R. Suleria, “Screening of Phenolic Compounds in Australian Grown Berries by LC-ESI-QTOF-MS/MS and Determination of Their Antioxidant Potential,” *Antioxidants* 10, no. 1 (2021): 26, <https://www.mdpi.com/2076-3921/10/1/26>, <https://doi.org/10.3390/antiox10010026>.
 60. A. N. Panche, A. D. Diwan, and S. R. Chandra, “Flavonoids: An Overview,” *Journal of Nutritional Science* 5 (2016): e47, <https://doi.org/10.1017/jns.2016.41>.
 61. Y. Zhang, H. Xiong, X. Xu, et al., “Compounds Identification in Semen Cuscutae by Ultra-High-Performance Liquid Chromatography (UPLCs) Coupled to Electrospray Ionization Mass Spectrometry,” *Molecules* 23, no. 5 (2018): 1199, <https://www.mdpi.com/1420-3049/23/5/1199>, <https://doi.org/10.3390/molecules23051199>.
 62. D. Smailagić, N. Banjac, S. Ninković, et al., “New Insights Into the Activity of Apple Dihydrochalcone Phloretin: Disturbance of Auxin Homeostasis as Physiological Basis of Phloretin Phytotoxic Action,” *Frontiers in Plant Science* 13 (2022): 875528, <https://doi.org/10.3389/fpls.2022.875528>.
 63. M. Stomp, D. Broda, and A. Bajek-Bil, “Dihydrochalcones: Methods of Acquisition and Pharmacological Properties—A First Systematic Review,” *Molecules* 24, no. 24 (2019): 4468, <https://doi.org/10.3390/molecules24244468>.
 64. M. C. Dias, D. Pinto, and A. M. S. Silva, “Plant Flavonoids: Chemical Characteristics and Biological Activity,” *Molecules* 26, no. 17 (2021): 5377, <https://doi.org/10.3390/molecules26175377>.
 65. Y. Hong, Z. Wang, C. J. Barrow, F. R. Dunshea, and H. A. R. Suleria, “High-Throughput Screening and Characterization of Phenolic Compounds in Stone Fruits Waste by LC-ESI-QTOF-MS/MS and Their Potential Antioxidant Activities,” *Antioxidants* 10, no. 2 (2021): 234, <https://www.mdpi.com/2076-3921/10/2/234>, <https://doi.org/10.3390/antiox10020234>.
 66. D. M. Kopustinskiene, V. Jakstas, A. Savickas, and J. Bernatoniene, “Flavonoids as Anticancer Agents,” *Nutrients* 12, no. 2 (2020): 457, <https://doi.org/10.3390/nu12020457>.
 67. K. Niaz, and F. Khan, A. Sanches Silva, S. F. Nabavi, M. Saeedi, and S. M. Nabavi, “Analysis of Polyphenolics,” in *Recent Advances in Natural*

Products Analysis (Elsevier, 2020), 39–197, <https://doi.org/10.1016/B978-0-12-816455-6.00003-2>.

68. X. Zhao, S. Zhang, D. Liu, M. Yang, and J. Wei, “Analysis of Flavonoids in *Dalbergia odorifera* by Ultra-Performance Liquid Chromatography With Tandem Mass Spectrometry,” *Molecules* 25, no. 2 (2020): , <https://doi.org/10.3390/molecules25020389>.

69. R. Mattioli, A. Francioso, L. Mosca, and P. Silva, “Anthocyanins: A Comprehensive Review of Their Chemical Properties and Health Effects on Cardiovascular and Neurodegenerative Diseases,” *Molecules* 25, no. 17 (2020): 3809, <https://doi.org/10.3390/molecules25173809>.

70. Q. Tong, Y. Chang, G. Shang, et al., “Integrated Chemical Characterization, Metabolite Profiling, and Pharmacokinetics Analysis of Zhijun Tangshen Decoction by UPLC-Q/TOF-MS,” *Frontiers in Pharmacology* 15 (2024): 1363678, <https://doi.org/10.3389/fphar.2024.1363678>.

71. Z. Chen, Y. Yang, H. Tao, L. Liao, Y. Li, and Z. Zhang, “Direct Analysis in Real-Time Mass Spectrometry for Rapid Identification of Traditional Chinese Medicines With Coumarins as Primary Characteristics,” *Phytochemical Analysis* 28, no. 3 (2017): 137–143, <https://doi.org/10.1002/pca.2650>.

72. G. B. Maru, G. Kumar, S. Ghantasala, and P. Tajpara, R. R. Watson, V. R. Preedy, and S. Zibadi, “Polyphenol-Mediated *In Vivo* Cellular Responses During Carcinogenesis,” in *Polyphenols in Human Health and Disease* (Academic Press, 2014), 1141–1179, <https://doi.org/10.1016/B978-0-12-398456-2.00088-8>.

73. A. Hazafa, M. O. Iqbal, U. Javaid, et al., “Inhibitory Effect of Polyphenols (Phenolic Acids, Lignans, and Stilbenes) on Cancer by Regulating Signal Transduction Pathways: A Review,” *Clinical and Translational Oncology* 24, no. 3 (2022): 432–445, <https://doi.org/10.1007/s12094-021-02709-3>.

74. S. Navarro-Orcajada, I. Conesa, F. J. Vidal-Sánchez, et al., “Stilbenes: Characterization, Bioactivity, Encapsulation and Structural Modifications. A Review of Their Current Limitations and Promising Approaches,” *Critical Reviews in Food Science and Nutrition* 63, no. 25 (2023): 7269–7287, <https://doi.org/10.1080/10408398.2022.2045558>.

75. T. Teka, L. Zhang, X. Ge, Y. Li, L. Han, and X. Yan, “Stilbenes: Source Plants, Chemistry, Biosynthesis, Pharmacology, Application and Problems Related to Their Clinical Application-A Comprehensive Review,” *Phytochemistry* 197 (2022): 113128, <https://doi.org/10.1016/j.phytochem.2022.113128>.

76. J. M. Al-Khayri, R. Mascarenhas, H. M. Harish, et al., “Stilbenes, a Versatile Class of Natural Metabolites for Inflammation-An Overview,” *Molecules* 28, no. 9 (2023): 3786, <https://doi.org/10.3390/molecules28093786>.

77. H. A. R. Suleria, C. J. Barrow, and F. R. Dunshea, “Screening and Characterization of Phenolic Compounds and Their Antioxidant Capacity in Different Fruit Peels,” *Foods* 9, no. 9 (2020): 1206, <https://www.mdpi.com/2304-8158/9/9/1206>, <https://doi.org/10.3390/foods9091206>.

78. A. Tajner-Czopek, M. Gertchen, E. Rytel, A. Kita, A. Z. Kucharska, and A. Sokół-Łętowska, “Study of Antioxidant Activity of Some Medicinal Plants Having High Content of Caffeic Acid Derivatives,” *Antioxidants* 9, no. 5 (2020): 412, <https://doi.org/10.3390/antiox9050412>.

79. M. Srinivasan, A. R. Sudheer, and V. P. Menon, “Ferulic Acid: Therapeutic Potential Through Its Antioxidant Property,” *Journal of Clinical Biochemistry and Nutrition* 40, no. 2 (2007): 92–100, <https://doi.org/10.3164/jcbrn.40.92>.

80. G. R. Beecher, “Proanthocyanidins: Biological Activities Associated With Human Health,” Supplement 1, *Pharmaceutical Biology* 42 (2004): 2–20, <https://doi.org/10.3109/13880200490893474>.