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Phenolic Compounds in Plant-Based Milk Alternatives from the Greek Market

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Abstract

Plant-based milk alternatives (PBMAs) are plant-based fluid products that are marketed as substitutes for regular milk. The nutrient composition of PBMA products can vary widely, depending on the plant source, processing methods, potential additives, etc., and in recent years, considerable research effort has been devoted to the exploration of the nutritional content of PBMAs, which are increasingly consumed worldwide. In the present study, an established UHPLC–Orbitrap MS method was employed for the extensive characterization of phenolic compounds in PBMAs available in the Greek market. Twenty-eight PBMAs were studied, including a variety of almond-, soy-, coconut-, oat-, walnut-, and rice-based products. In almond-based milk products, low total concentrations and a broad distribution across compound classes were observed, with *trans*-chlorogenic acid and neochlorogenic acid being the most abundant constituents, whereas coconut-based milk samples were generally not rich in phenolic compounds. In soy-based milk samples, the presence of isoflavones including daidzein, genistein, and glycitein was uniquely detected, while oat-based products were the samples richer in phenolic content, in particular for hydroxycinnamic acids, such as *trans*-chlorogenic acid and neochlorogenic acid. In addition, a suspect screening approach, using Exactive Plus Orbitrap, enabled the exploration and semi-quantification of three avenanthramides (A, B, C) in the studied oat-based milk samples and six isoflavonoids, namely daidzein and genistein derivatives, in soy-based milk. Such compounds are known for their antioxidant and anti-inflammatory properties, and their occurrence in PBMAs highlights the potential health-promoting effects of these dairy alternatives.

Keywords: avenanthramides; milk alternatives; Orbitrap MS; phenolics; plant-based milk alternatives

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1. Introduction

The increasing adoption of veganism, combined with environmental concerns about dairy farming and production and ethical concerns among the population regarding the consumption of animal-based products, has driven a plant-based dairy market trend, resulting in a rising availability of dairy alternatives in the global market. According to an updated report, the global plant-based dairy market was forecasted to reach a valuation of USD 14.7 billion in 2024 to USD 36.69 billion by the end of 2034 [1]. Plant-based milk

alternatives (PBMAs) are plant-based fluid products, which are also referred to as plant-based milk substitutes or plant beverages. These water-soluble extracts are produced by maceration and grinding of the plant material in water, followed by homogenization that results in an emulsion resembling dairy milk [2,3]. According to the FDA, "plant-based products that are marketed and sold as alternatives to milk are made from nuts (including hazelnuts, walnuts, cashews, and almonds), seeds (including sesame, flax, and hemp), rice, coconuts, oats, or legumes (including soy)" [4].

In recent years research efforts have been devoted to the characterization of PBMA properties due to the lack of standardized methods regarding these newly emerging products [5]. Some examples include particle and physicochemical properties and sensory attributes [5]. For particle properties (that is, size, morphology, and charge), different methods and techniques such as light scattering [6,7], and optical or electron microscopy have been explored [8,9]. Physicochemical and sensory properties have been assessed using a variety of methods, such as colorimetry [10], differential pulse voltammetry [11], and a plethora of conventional chromatographic methods such as gas chromatography [12,13], and liquid chromatography [9], coupled with different detectors (flame ionization, mass spectrometry, etc.) [14,15].

Although PBMAs cannot be considered milk analogs, but rather different food products with their own nutritional profile and functional properties [16], in many countries, terms such as "almond milk", "oat milk", and so on are widely used. The composition of PBMA products, their predominant ingredients, and their nutrient profiles vary widely, depending on the plant source, processing methods, and added ingredients. Sweeteners and flavorings may be added, while these products may also be enriched by minerals and vitamins. Over recent years, considerable research effort has been devoted to the exploration of the nutritional content of PBMAs and the potential health issues related to PBMA consumption [17–19].

A recent report discusses the study of key ingredients of different brands of almond-, oat-, rice-, coconut-, and soya-based beverages in comparison to cow and goat milk [14]. PBMAs presented lower contents of total protein, lipids, amino acids, and minerals than cow and goat milk. However, the antioxidant activity of PBMAs and potential ingredients such as unsaturated fatty acids, phenolic compounds, and phytosterols make them an alternative choice to dairy milk that may offer specific health benefits to consumers [20,21].

The studies on the antioxidant capacity and phenolic compound contents of PBMAs are limited [20,21]. The antioxidant profile of commercial non-cocoa- and cocoa-flavored PBMAs has been recently studied, showing an increase in the total phenolic content in cocoa-flavored PBMAs [13]. Soy milk is perhaps the most studied PBMA regarding its contents in phenolic compounds, especially isoflavones, which have been found to be significantly impacted by the thermal processes and the preparation methods applied [22–24]. In 2024, Grainger et al. studied the bioactive (poly)phenol content in twenty-seven PBMA products of six types (almond, coconut, oat, pea, rice, and soy) in the US market [15]. They found that the (poly)phenol contents significantly varied and were dependent on plant source, brand, and added flavorings.

Phenolic compounds are a large and heterogeneous class of plant-derived secondary metabolites that include phenolic acids, flavonoids, and stilbenes, among others. They are widely recognized for their antioxidant activity and potential health-promoting effects, including anti-inflammatory, cardioprotective, and anticancer properties [15,25]. In the context of PBMAs, these compounds are primarily inherited from the plant sources used. Phenolic acids such as ferulic, caffeic, and *p*-hydroxybenzoic acid, along with flavonoids like quercetin, kaempferol, apigenin, and luteolin, are among the most commonly expected phenolics in PBMAs. More precisely, soy-based beverages are typically rich in isoflavones

like daidzein, genistein, and glycitein and their derivatives [26], while oat-based drinks are known to contain ferulic acid, *p*-coumaric acid, and a high content of their derivatives, that is, avenanthramides [15].

The highly increasing global consumption of PBMAs, in combination with the lack of detailed studies on the PBMAs' bioactive phenolic compound contents in the literature, prompted us to undertake the present study. Herein, we describe the application of an ultra-high-performance liquid chromatography–mass spectrometry (UHPLC–Orbitrap MS) method in order to explore the presence of a large set of phenolic compounds in commercial PBMAs available in the Greek market. The samples studied include almond, soy, coconut, oat, walnut, and rice PBMAs.

2. Materials and Methods

2.1. Chemicals and Reagents

The solvents used were of LC–MS analytical grade. Water, acetonitrile, isopropanol, and methanol were purchased from Thermo Fisher Scientific (Pittsburgh, PA, USA). Optima™ LC/MS-grade formic acid 99.0%+ was used as an ultra-pure additive in the mobile phase along with LC/MS-grade solvents. Analytical standards of phenolic compounds, all of HPLC-grade purity, were obtained from various suppliers. Standards purchased from Supelco (Bellefonte, PA, USA) were apigenin, chrysin, formononetin, genistein, daidzein, catechin, (−)-epicatechin, (−)-epigallocatechin, homovanillic acid, syringic acid, kaempferol, luteolin, (±)-naringenin, caftaric acid, (+)-*trans*-chlorogenic acid, neochlorogenic acid, procyanidin B2, *trans*-resveratrol, tyrosol, 3-hydroxytyrosol, vanillin, sinapic acid, *p*-coumaric acid, ferulic acid, and *trans*-cinnamic acid. Additional standards obtained from Sigma-Aldrich (St. Louis, MO, USA) included gallic acid, *p*-hydroxybenzoic acid, caffeic acid, astragalin (kaempferol-3-*O*-glucoside), epicatechin gallate, ε-viniferin, protocatechuic acid, *o*-coumaric acid, *trans*-coutaric acid, myricetin, and vanillic acid. Compounds sourced from Extrasynthese (Genay, France) were (−)-epigallocatechin gallate, procyanidin B1, isoquercetin (quercetin-3-*O*-glucoside), isorhamnetin (3-*O*-methylquercetin), miquelianin (quercetin-3-*O*-glucuronide), glycitein, quercetin, hesperidin, and rutin (quercetin-3-*O*-rutinoside). Piceid was acquired from Fluorochem (Hadfield, UK). Isotopically labeled quercetin (quercetin-d_3), used as an internal standard under negative ionization mode, was purchased from Cayman Chemical (Ann Arbor, MI, USA).

2.2. Stock and Working Solutions

The analytical standards were dissolved in methanol at a concentration of 1000 μg/mL (stock solutions) and stored at −20 °C. Daily preparation of fresh working solutions at concentrations of 0.50 and 1.00 μg/mL was achieved through appropriate dilution.

2.3. Instrumentation

An ultra-high-performance liquid chromatography (UHPLC) system (Dionex UltiMate™ 3000 Rapid Separation UHPLC+; Thermo Fisher Scientific, Bremen, Germany), equipped with a binary pump, integrated degasser, autosampler, and column compartment, and controlled via Chromeleon™ 6.8 software, was used for chromatographic analysis. The UHPLC system was coupled to an Exactive Plus™ Orbitrap mass spectrometer (Thermo Fisher Scientific, Bremen, Germany) equipped with a Heated Electrospray Ionization (HESI-II) source. Instrumental parameters were set as previously described by Gerogianni et al. [27,28]. Briefly, the spray voltage was maintained at 3.8 kV, with sheath gas, auxiliary gas, and sweep gas flow rates set to 40, 11, and 2 arbitrary units, respectively. The capillary and auxiliary gas heater temperatures were set at 320 °C and 200 °C, respectively. Chromatographic separation was achieved on a Hypersil Gold™ C18 column (100 × 2.1 mm,

1.9 µm; Thermo Fisher Scientific) fitted with a suitable guard cartridge (10 × 2.1 mm, 4.6 µm). The mobile phases consisted of water and acetonitrile, both containing 0.1% formic acid (*v*/*v*), delivered at a constant flow rate of 0.120 mL/min under an 18-minute gradient elution program. The exact masses $[M–H]^-$, retention times (R_t), and the limits of detection (LOD) and quantification (LOQ) for the phenolic compounds are presented in Tables S1 and S2 (Supplementary Material). The Exactive Plus Orbitrap MS was externally calibrated according to the manufacturer's protocol and additionally before each analytical sequence, ensuring mass accuracy within the specified tolerance (±5 ppm).

The limits of detection (LOD) and quantification (LOQ) were estimated from the calibration curves using the ratio of the standard deviation of the response to the slope (σ/S), determined from low-concentration standards near the origin of the curve. Solvent blanks were systematically injected at the beginning of each sequence, immediately after the highest calibration standard, and after every triplicate of sample injections. This approach ensured continuous monitoring for carry-over and background contamination. No interfering peaks were observed at the retention times of the target analytes.

Data acquisition was performed with the use of Thermo XCalibur 4.0. Exactive Plus Tune software (Version 2.8 SP1, Thermo Fisher Scientific, Bremen, Germany) for the direct control of the mass spectrometer. Data acquisition was carried out in full scan mode, incorporating all-ion fragmentation (AIF) to allow concurrent acquisition of both precursor and fragment ion spectra. Extracted ion chromatograms (EICs) were obtained through Qual XCalibur and FreeStyle (Thermo Fisher Scientific, Bremen, Germany), which produced base peak chromatograms for masses achieving a mass accuracy width of 5 ppm. Quantitative data processing and handling were performed using TraceFinder ™ software (Version 4.1, Thermo Fisher Scientific, San Jose, CA, USA).

2.4. Sample Preparation

Each PBMA sample (1 mL) was extracted with methanol (4 mL) in a screw-cap glass centrifuge tube. The mixture was vortexed for approximately 30 s and then centrifuged at 4000× *g* for 10 min to facilitate protein precipitation. A volume of 500 µL of the resulting clear supernatant was diluted with water (500 µL) in an LC vial, and the mixture was directly subjected to LC-MS analysis. All samples, blanks, and calibration standards were spiked with quercetin-d_3, which served as the internal standard. A similar sample preparation approach has been effectively applied in PBMAs by Grainger et al. [15], and by the authors in previous studies for the analysis of free fatty acids in dairy matrices, such as milk and yogurt [29,30].

2.5. Sampling

Twenty-eight commercially available PBMA products were procured from the local market in Athens, Greece. These products were chosen based on their availability in the Greek market at the time of sampling, reflecting the main PBMAs consumed locally, without additional selection criteria. Specifically, ten nut-based (nine almond-based, one walnut-based), three legume-based (soy-based), five coconut-based, and ten cereal-based (nine oat-based, one rice-based) milk alternative samples were analyzed. Among the oat-based samples, one additionally contained almond, while another one contained hazelnut, though in lower quantities than oat, and as a result, these two samples were included in the oat-based samples. The key ingredients of the commercial PBMA samples, as declared by the producers, are collectively summarized in Table 1.

Table 1. Key ingredients of commercial PBMA samples used in this study.

Code	Brand	Ingredients Declared by the Producers	Nutritional Information Declared by the Producers per 100 mL
		Almond milk	
A	Almond brand 1	Water, almond (3%), calcium, sea salt, emulsifier (sunflower lecithin), stabilizer (gellan gum), vitamins (B2, B12, D, E).	Fat: 1.7 g. Saturated fatty acids: 0.1 g. Carbohydrates: 3.0 g. Sugars: 2.0 g. Fibers: 0.4 g. Proteins: 0.7 g. Salt: 0.10 g.
B	Almond brand 2	Water, almond (3%), dietary fiber, stabilizers (gellan gum, locust bean gum), emulsifier (sunflower lecithin), sea salt, flavorings, calcium phosphate, sodium bicarbonate, vitamins (B2, B12, D2, E).	Fat: 1.7 g. Saturated fatty acids: 0.1 g. Carbohydrates: 0.5 g. Sugars: 0.5 g. Fibers: 1.8 g. Proteins: 0.7 g. Salt: 0.10 g.
C	Almond brand 3	Water, almond (2.3%), calcium (calcium carbonate), sea salt, stabilizers (guar gum, gellan gum), emulsifier (lecithins), natural flavorings, vitamins (B12, D2, E).	Fat: 1.2 g. Saturated fatty acids: 0.1 g. Carbohydrates: 2.6 g. Sugars: 2.3 g. Fibers: 0.3 g. Proteins: 0.5 g. Salt: 0.15 g.
D	Almond brand 4	Water, almond (2.5%), sugar, fructose, acidity regulator (potassium phosphates), calcium carbonate, natural flavors, stabilizers (gellan gum, guar gum), sea salt.	Fat: 1.2 g. Saturated fatty acids: 0.1 g. Carbohydrates: 2.6 g. Sugars: 2.5 g. Fibers: 0.3 g. Proteins: 0.5 g. Salt: 0.08 g.
E	Almond brand 5	Water, almond (2.3%), pea protein, calcium salts of orthophosphoric acid, sea salt, acidity regulator (potassium phosphate), stabilizer (gellan gum), emulsifier (sunflower lecithin), natural flavorings, vitamins (B2, B12, D).	Fat: 1.6 g. Saturated fatty acids: 0.1 g. Carbohydrates: 0.0 g. Sugars: 0.0 g. Fibers: 0.2 g. Proteins: 1.7 g. Salt: 0.16 g.
F	Almond brand 6	Water, almond (2.3%), neutral calcium phosphate, sea salt, stabilizers (locust bean gum, gellan gum), emulsifier (sunflower lecithins), vitamins (B2, B12, E, D2).	Fat: 1.2 g. Saturated fatty acids: 0.1 g. Carbohydrates: 0.0 g. Sugars: 0.0 g. Fibers: 0.3 g. Proteins: 0.5 g. Salt: - g.
G	Almond brand 7	Water, almond (3%), calcium, sea salt, emulsifier: sunflower lecithin, stabilizer: gellan gum, vitamins (B2, B12, D, E).	Fat: 1.7 g. Saturated fatty acids: 0.1 g. Carbohydrates: 4.4 g. Sugars: 3.6 g. Fibers: 0.4 g. Proteins: 0.7 g. Salt: 0.10 g.
H	Almond brand 8	Water, almond * (6%), tapioca starch *, natural almond flavor *. * Organic ingredient.	Fat: 1.9 g. Saturated fatty acids: 0.3 g. Carbohydrates: 3.2 g. Sugars: <0.3 g. Fibers: - g. Proteins: 1.0 g. Salt: 0.14 g.
I	Almond brand 9	Water, almonds* (2.8%), rice starch *, natural almond flavor, sea salt, stabilizers (guar gum *, xanthan gum). * Organic ingredient.	Fat: 1.7 g. Saturated fatty acids: 0.2 g. Carbohydrates: - g. Sugars: <0.5 g. Fibers: - g. Proteins: 1.6 g. Salt: 0.05 g
		Soy milk	
J	Soy brand 1	Soy base [water, peeled soy beans (8.7%)], acidity regulators (potassium phosphates), calcium carbonate, flavors, sea salt, stabilizer (gellan gum), vitamins (B2, B12, D2).	Fat: 1.8 g. Saturated fatty acids: 0.3 g. Carbohydrates: 0.0 g. Sugars: 0.0 g. Fibers: 0.6 g. Proteins: 3.3 g. Salt: - g.
K	Soy brand 2	Soy base, water, peeled soy beans (8%), sugar, acidity regulators (potassium phosphates), calcium carbonate, sea salt, stabilizer (gellan gum), vitamins (B2, B12, D2).	Fat: 1.8 g. Saturated fatty acids: 0.3 g. Carbohydrates: 2.5 g. Sugars: 2.5 g. Fibers: 0.5 g. Proteins: 3.0 g. Salt: 0.09 g.
L	Soy brand 3	Tonyu preparation * (water, dehulled non-GMO soy beans * 8%). * Organic farming.	Fat: 2.6 g. Saturated fatty acids: 0.6 g. Carbohydrates: 0.7 g. Sugars: <0.5 g. Fibers: 0.5 g. Proteins: 3.9 g. Salt: 0.03 g.

Table 1. *Cont.*

Code	Brand	Ingredients Declared by the Producers	Nutritional Information Declared by the Producers per 100 mL
		Coconut milk	
M	Coconut brand 1	Water, coconut (7%), rice (3%), calcium, sea salt, natural flavor, stabilizer (gellan gum), vitamins (B2, B12, D2, E).	Fat: 1.5 g. Saturated fatty acids: 1.0 g. Carbohydrates: 5.0 g. Sugars: 2.7 g. Fibers: 0.2 g. Proteins: 0.2 g. Salt: 0.10 g.
N	Coconut brand 2	Water, coconut milk (5.3%) (coconut cream, water), rice (3.3%), neutral calcium phosphate, stabilizers (guar gum, gellan gum, xanthan gum), sea salt, flavors, vitamins (B12, D2).	Fat: 1.2 g. Saturated fatty acids: 1.1 g. Carbohydrates: 0.0 g. Sugars: 0.0 g. Fibers: 0.0 g. Proteins: 0.1 g. Salt: 0.3 g.
O	Coconut brand 3	Water, coconut milk (7%) (coconut cream, water), coconut water (2.6%), neutral calcium phosphate, natural coconut flavor, stabilizers (guar gum, xanthan gum, gellan gum), sea salt, vitamins (B12, D2).	Fat: 0.8 g. Saturated fatty acids: 0.8 g. Carbohydrates: 2.7 g. Sugars: 1.9 g. Fibers: 0.1 g. Proteins: 0.1 g. Salt: 0.12 g.
P	Coconut brand 4	Water, coconut milk * (8.5%), tapioca starch *, natural coconut flavor *, sea salt *, stabilizer (gellan gum). * Product of organic farming.	Fat: 2.7 g. Saturated fatty acids: 2.5 g. Carbohydrates: 2.0 g. Sugars: <0.3 g. Fibers: - g. Proteins: 0.2 g. Salt: 0.1 g.
Q	Coconut brand 5	Water, coconut * (59.6%), guar gum *. * Product of organic farming.	Fat: 1.6 g. Saturated fatty acids: 1.4 g. Carbohydrates: 2.4 g. Sugars: 2.1 g. Fibers: <0.5 g. Proteins: <0.5 g. Salt: 0.09 g.
		Oat milk	
R	Oat brand 1	Oat base [water, oat (EU/non-EU) (8.7%)], soluble corn fiber, sunflower oil, calcium carbonate, sea salt, stabilizer (gellan gum), vitamins (B2, B12, D2).	Fat: 1.5 g. Saturated fatty acids: 0.2 g. Carbohydrates: 5.8 g. Sugars: 0.0 g. Fibers: 1.2 g. Proteins: 0.2 g. Salt: 0.10 g.
S	Oat brand 2	Water, oats (12%), dietary fiber, sunflower oil, calcium phosphate, sea salt, sodium bicarbonate, stabilizer (gellan gum), emulsifier (sunflower lecithin), vitamins (B2, B12, D2, E).	Fat: 1.5 g. Saturated fatty acids: 0.3 g. Carbohydrates: 8.0 g. Sugars: 4.9 g. Fibers: 1.7 g. Proteins: 1.2 g. Salt: 0.11 g.
T	Oat brand 3	Water, oats (10%), pea protein, sunflower oil, calcium salts of orthophosphoric acid, sea salt, acidity regulator (potassium phosphate), stabilizer (gellan gum), natural flavors, vitamins (B2, B12, D).	Fat: 1.5 g. Saturated fatty acids: 0.2 g. Carbohydrates: 6.2 g. Sugars: 3.5 g. Fibers: 0.5 g. Proteins: 1.7 g. Salt: 0.18 g.
U	Oat brand 4	Water, oats * (16%), sunflower oil *. * Product of organic farming.	Fat: 1.2 g. Saturated fatty acids: 0.2 g. Carbohydrates: 9.6 g. Sugars: 5.7 g. Fibers: - g. Proteins: 1.1 g. Salt: 0.12 g.
V	Oat brand 5	Oat base, water, oat (8.7%), soluble corn fiber, sunflower oil, calcium carbonate, sea salt, stabilizer (gellan gum), vitamins (B2, B12, D2).	Fat: 1.5 g. Saturated fatty acids: 0.2 g. Carbohydrates: 6.6 g. Sugars: 3.3 g. Fibers: 1.4 g. Proteins: 0.8 g. Salt: 0.08 g.
W	Oat brand 6	Water, oats (8%), sunflower seeds (1%), pumpkin seeds (0.5%), sesame (0.5%), calcium, sea salt, emulsifier (sunflower lecithin), stabilizer (gellan gum), vitamins (B12, D, E).	Fat: 0.8 g. Saturated fatty acids: 0.1 g. Carbohydrates: 2.7 g. Sugars: 1.6 g. Fibers: 0.2 g. Proteins: 0.3 g. Salt: 0.10 g.
X	Oat brand 7	Water, oats * (11%), cold-pressed sunflower oil *, sea salt. * Organic farming.	Fat: 0.9 g. Saturated fatty acids: 0.2 g. Carbohydrates: 4.5 g. Sugars: <0.5 g. Fibers: - g. Proteins: 0.6 g. Salt: 0.09 g.

Table 1. *Cont.*

Code	Brand	Ingredients Declared by the Producers	Nutritional Information Declared by the Producers per 100 mL
		Oat and hazelnut or almond milk	
Y	Oat and hazelnut	Water, oats (8%), hazelnuts (2.5%), dietary fiber, calcium phosphate, sea salt, potassium phosphate, stabilizer (gellan gum), emulsifier (sunflower lecithin), flavorings, vitamins (B2, B12, D2, E).	Fat: 2.2 g. Saturated fatty acids: 0.2 g. Carbohydrates: 5.6 g. Sugars: 3.5 g. Fibers: 1.7 g. Proteins: 1.2 g. Salt: 0.11 g.
Z	Oat and almond	Oat base (water, oat (3.2%)), almond (1.7%), vegetable fiber from chicory roots, neutral calcium phosphate, stabilizers (locust bean gum, gellan gum), sea salt, natural flavors, vitamins (B2, B12, E, D2).	Fat: 1.0 g. Saturated fatty acids: 1.0 g. Carbohydrates: 2.4 g. Sugars: 2.4 g. Fibers: 1.5 g. Proteins: 0.3 g. Salt: 0.11 g.
		Walnut milk	
AA	Walnut	Water, cane sugar, walnut paste (2%), calcium phosphate, stabilizers (locust bean gum, gellan gum), salt, emulsifier (mono- and diglycerides of fatty acids), flavoring, vitamins (B2, B12, D2).	Fat: 1.3 g. Saturated fatty acids: 0.2 g. Carbohydrates: 2.9 g. Sugars: 2.8 g. Fibers: 0.1 g. Proteins: 0.3 g. Salt: 0.10 g.
		Rice milk	
AB	Rice	Water, rice * (13.7%), cold-pressed sunflower oil *, sea salt. * Organic farming.	Fat: 0.7 g. Saturated fatty acids: 0.1 g. Carbohydrates: 8.8 g. Sugars: <0.5 g. Fibers: - g. Proteins: <0.5 g. Salt: 0.04 g.

2.6. Statistical Analysis

All experiments were carried out in triplicate, and the results are expressed as average ± standard deviation. Microsoft Excel was used for data handling. Principal component analysis (PCA) was conducted with XLSTAT 2018 version (Addinsoft, New York, NY, USA).

3. Results

3.1. PBMAs' Phenolic Content

The phenolic composition of the PBMA samples was characterized using a previously established UHPLC–Orbitrap MS method [27]. More than 50 phenolic compounds were used for this targeted analysis, and 28 were identified and quantified (Table 2). These included representatives from various structural classes, such as phenolic aldehydes, and hydroxybenzoic and hydroxycinnamic acid derivatives, as well as flavonoids, including flavan-3-ols, flavonols, flavones, and isoflavones. Representative EICs of selected phenolic compounds are presented in Figure 1.

All PBMA types contained detectable phenolic compounds, and the quantitative results are expressed as μg/mL and relative percentage of total phenolics identified (Table 2). Almond-based milk products showed lower total concentrations but a broader distribution across compound classes. Notably, *trans*-chlorogenic acid (0.78 μg/mL, 31.40%) and neochlorogenic acid (0.71 μg/mL, 28.70%) were predominant, followed by quercetin (0.40 μg/mL, 16.06%) and ferulic acid (0.18 μg/mL, 7.11%). Additionally, vanillin, vanillic acid, caffeic acid, and *p*-hydroxybenzoic acid were found at low contents (0.01–0.14 μg/mL); this was also the case for epicatechin gallate and epigallocatechin gallate, luteolin, apigenin, kaempferol, isorhamnetin, and chrysin (0.01–0.04 μg/mL). Almonds are known to contain a diverse range of flavonoids, and the detection of kaempferol, isorhamnetin, and flavan-3-ols, though at low levels, supports their botanical origin [31,32].

Soy-based milk samples were uniquely characterized by the presence of isoflavones, including daidzein (0.52 μg/mL, 1.34%), genistein (0.41 μg/mL, 1.05%), and glycitein (0.05 μg/mL, 0.11%), which were not detected in the other matrices. These compounds are characteristic of the starting plant material, that is, soy beans. It is worth noting that vanillin (36.85 μg/mL) accounted for over 94% of total phenolics identified in soy-based drinks, markedly distinguishing them from the other formulations. However, the dominance of vanillin likely stems from added flavoring agents [33]. Importantly, *p*-coumaric acid, quercetin, ferulic acid, vanillic acid, rutin, and syringic acid were found at quantities ranging from 0.06 to 0.28 μg/mL, while the remaining phenolic compounds were detected at quantities of 0.01 μg/mL or at trace amounts.

Coconut-based milk samples were generally not rich in phenolic compounds, though they contained appreciable quantities of quercetin (0.24 μg/mL, 14.12%), vanillin (1.23 μg/mL, 72.35%), and ferulic acid (0.19 μg/mL, 10.94%). These compounds have been previously reported in cocos and their by-products [34]. *p*-Hydroxybenzoic acid, vanillic acid, and rutin were found at low quantities (0.01–0.03 μg/mL), and the remaining phenolic compounds were only detected in traces.

Oat-based milk samples exhibited the highest overall phenolic content, particularly for hydroxycinnamic acids, with *trans*-chlorogenic acid (2.68 μg/mL, 29.11%) and neochlorogenic acid (2.08 μg/mL, 22.63%) dominating the profile. Ferulic acid (1.22 μg/mL, 13.29%), vanillin (1.21 μg/mL, 13.20%), syringic acid (0.56 μg/mL, 6.05%), and sinapic acid (0.38 μg/mL, 4.18%) were also abundant in oat-based milk, followed by vanillic acid, piceid, and quercetin (0.26–0.30 μg/mL). Finally, caffeic acid, *p*-coumaric acid, homovanillic acid, protocatechuic acid, and *p*-hydroxybenzoic acid were quantified at low quantities (0.01 to 0.08 μg/mL), while the remaining phenolic compounds were only detected in trace amounts.

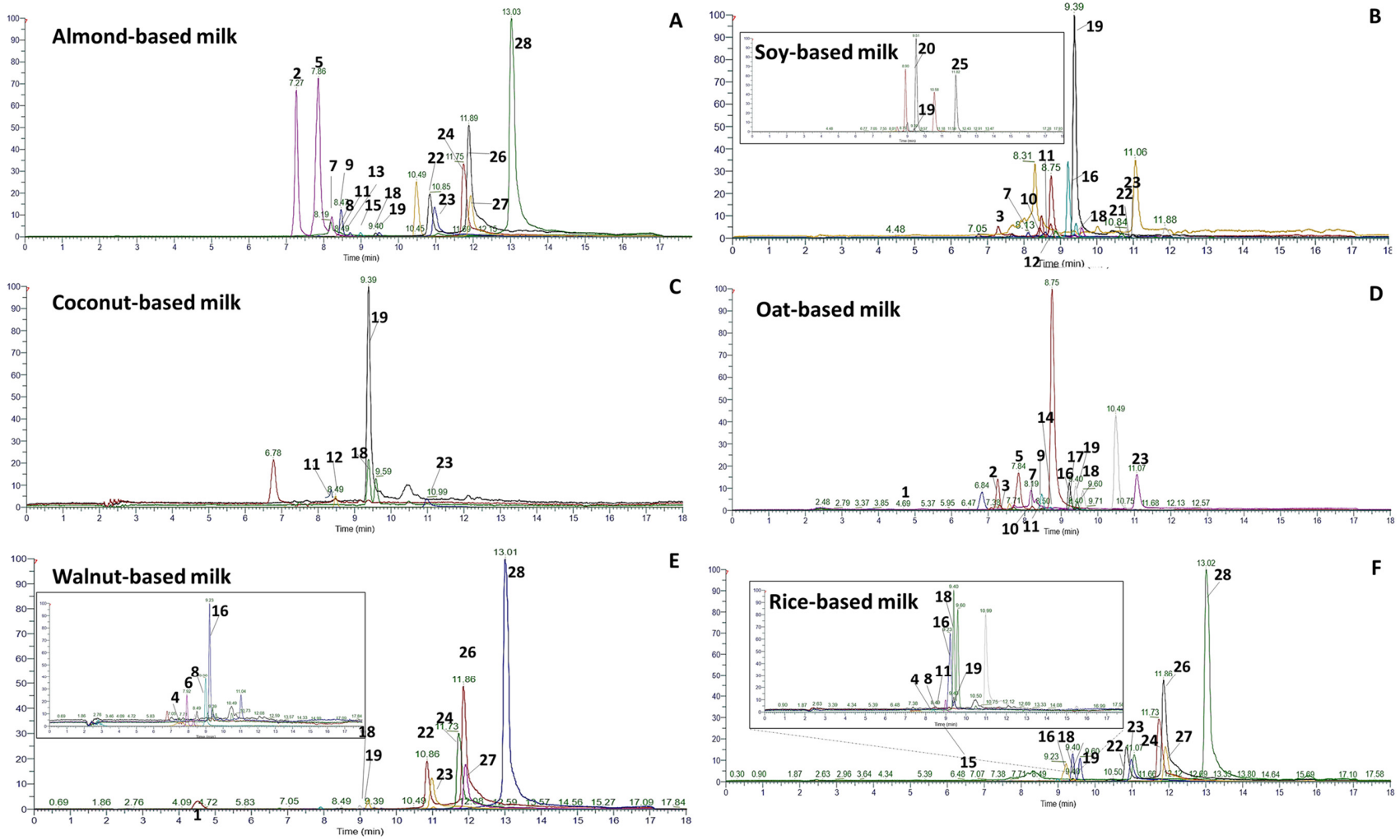


Figure 1. Extracted ion chromatograms (EICs) of phenolic compounds in a representative sample of almond-based milk (**A**), soy-based milk (**B**), coconut-based milk (**C**), oat-based milk (**D**), walnut-based milk (**E**), and rice-based milk (**F**). Peaks: gallic acid (1), chlorogenic acid (2), protocatechuic acid (3), caftaric acid (4), neochlorogenic acid (5), catechin (6), p-hydroxybenzoic acid (7), epigallocatechin gallate (8), caffeic acid (9), syringic acid (10), vanillic acid (11), rutin (12), homovanillic acid (13), piceid (14), epicatechin gallate (15), p-coumaric acid (16), sinapic acid (17), ferulic acid (18), vanillin (19), daidzein (20), glycitein (21), luteolin (22), quercetin (23), apigenin (24), genistein (25), kaempferol (26), isorhamnetin (27), and chrysin (28).

Table 2. Contents of phenolic compounds in plant-based milk alternatives (μg/mL).

Sample	Almond (*n* = 9)		Soy (*n* = 3)		Coconut (*n* = 5)		Oat (*n* = 9)		Walnut (*n* = 1)		Rice (*n* = 1)	
	Mean ± SD (μg/mL)	%	Mean ± SD (μg/mL)	%	Mean ± SD (μg/mL)	%	Mean ± SD (μg/mL)	%	(μg/mL)	%	(μg/mL)	%
Phenolic aldehyde												
Vanillin	0.14 ± 0.12	5.62	36.85 ± 52.9	94.72	1.23 ± 1.98	72.35	1.21 ± 2.02	13.20	0.08 ± 0.00	3.48	0.11 ± 0.00	9.17
Benzoic acid derivatives												
Gallic acid	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	0.16 ± 0.04	6.96	<LOQ	<LOQ
Protocatechuic acid	<LOQ	<LOQ	0.01 ± 0.01	0.03	<LOQ	<LOQ	0.02 ± 0.03	0.22	<LOQ	<LOQ	<LOQ	<LOQ
Syringic acid	<LOQ	<LOQ	0.10 ± 0.15	0.26	<LOQ	<LOQ	0.56 ± 1.13	6.05	<LOQ	<LOQ	<LOQ	<LOQ
Vanillic acid	0.08 ± 0.11	3.37	0.22 ± 0.14	0.56	0.03 ± 0.02	2.00	0.30 ± 0.54	3.22	0.01 ± 0.00	0.43	0.02 ± 0.00	1.67
Homovanillic acid	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	0.06 ± 0.18	0.66	<LOQ	<LOQ	<LOQ	<LOQ
p-Hydroxybenzoic acid	0.01 ± 0.00	0.40	0.01 ± 0.00	0.03	0.01 ± 0.00	0.59	0.01 ± 0.00	0.11	0.01 ± 0.00	0.43	0.01 ± 0.00	0.83
Cinnamic acid derivatives												
Caffeic acid	0.02 ± 0.04	0.94	<LOQ	<LOQ	<LOQ	<LOQ	0.08 ± 0.11	0.82	<LOQ	<LOQ	<LOQ	<LOQ
Ferulic acid	0.18 ± 0.01	7.11	0.22 ± 0.02	0.57	0.19 ± 0.03	10.94	1.22 ± 1.53	13.29	0.18 ± 0.00	7.83	0.36 ± 0.08	30.00
p-Coumaric acid	<LOQ	<LOQ	0.06 ± 0.02	0.15	<LOQ	<LOQ	0.08 ± 0.11	0.86	0.01 ± 0.00	0.43	0.01 ± 0.00	0.83
Neochlorogenic acid	0.71 ± 1.26	28.70	<LOQ	<LOQ	<LOQ	<LOQ	2.08 ± 4.72	22.63	<LOQ	<LOQ	<LOQ	<LOQ
trans-Chlorogenic acid	0.78 ± 1.38	31.40	<LOQ	<LOQ	<LOQ	<LOQ	2.68 ± 6.10	29.11	<LOQ	<LOQ	<LOQ	<LOQ
Sinapic acid	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	0.38 ± 0.89	4.18	<LOQ	<LOQ	<LOQ	<LOQ
Caftaric acid	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	0.02 ± 0.01	0.87	0.01 ± 0.00	0.83
Flavan-3-ols												
Catechin	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	0.01 ± 0.00	0.43	<LOQ	<LOQ
Epicatechin gallate	0.01 ± 0.02	0.49	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	0.04 ± 0.02	1.74	0.01 ± 0.00	0.83
Epigallocatechin gallate	0.03 ± 0.03	1.21	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	0.09 ± 0.04	3.91	0.04 ± 0.01	3.33
Flavones												
Luteolin	0.02 ± 0.04	0.90	0.01 ± 0.01	0.02	<LOQ	<LOQ	<LOQ	<LOQ	0.12 ± 0.06	5.22	0.03 ± 0.01	2.50
Apigenin	0.01 ± 0.01	0.13	0.01 ± 0.01	0.02	<LOQ	<LOQ	<LOQ	<LOQ	0.02 ± 0.01	0.87	<LOQ	<LOQ
Chrysin	0.02 ± 0.03	0.85	0.01 ± 0.01	0.01	<LOQ	<LOQ	<LOQ	<LOQ	0.12 ± 0.05	5.22	0.04 ± 0.01	3.33

Table 2. *Cont.*

Sample	Almond (*n* = 9)		Soy (*n* = 3)		Coconut (*n* = 5)		Oat (*n* = 9)		Walnut (*n* = 1)		Rice (*n* = 1)	
	Mean ± SD (μg/mL)	%	Mean ± SD (μg/mL)	%	Mean ± SD (μg/mL)	%	Mean ± SD (μg/mL)	%	(μg/mL)	%	(μg/mL)	%
Flavonols												
Quercetin	0.40 ± 0.25	16.06	0.28 ± 0.04	0.71	0.24 ± 0.00	14.12	0.26 ± 0.05	2.84	1.05 ± 0.38	45.65	0.46 ± 0.06	38.33
Isorhamnetin	0.04 ± 0.07	1.53	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	0.22 ± 0.09	9.57	0.06 ± 0.02	5.00
Kaempferol	0.03 ± 0.05	1.26	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	0.16 ± 0.06	6.96	0.05 ± 0.01	4.17
Flavonol glucoside												
Rutin	<LOQ	<LOQ	0.12 ± 0.10	0.32	0.01 ± 0.02	0.71	0.01 ± 0.00	0.01	<LOQ	<LOQ	<LOQ	<LOQ
Isoflavones												
Genistein	<LOQ	<LOQ	0.41 ± 0.29	1.05	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Glycitein	<LOQ	<LOQ	0.05 ± 0.03	0.11	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Daidzein	<LOQ	<LOQ	0.52 ± 0.38	1.34	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Stilbenoids												
Piceid	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	0.29 ± 0.64	3.18	<LOQ	<LOQ	<LOQ	<LOQ

<LOQ: below the limit of quantification. All experiments were performed in triplicate. Phenolic compounds not listed in the table were not detected (<LOD) in the analyzed PBMA samples.

The walnut-based sample exhibited the highest levels of quercetin (1.05 μg/mL, 45.65%); notable amounts of flavonols such as isorhamnetin (0.22 μg/mL, 9.57%) and kaempferol (0.16 μg/mL, 6.96%) were also detected, suggesting a polyphenol profile dominated by flavonols. Flavones such as luteolin, chrysin, and apigenin (0.02–0.12 μg/mL) were also present, followed by the flavan-3-ols epigallocatechin gallate, epicatechin gallate, and catechin (0.01–0.09 μg/mL). Ferulic acid, gallic acid, vanillin, and caftaric acid were found at concentrations ranging from 0.02 to 0.18 μg/mL, while the remaining compounds were detected as traces. As a starting material for the production of PBMAs, walnut is reportedly rich in flavonols, catechin, and a variety of benzoic and cinnamic acids and their derivatives [32,35].

The rice-based milk alternative exhibited generally low phenolic concentrations, with quercetin (0.46 μg/mL, 38.33%), ferulic acid (0.36 μg/mL, 30.00%), and vanillin (0.11 μg/mL, 9.17%) comprising the majority of the quantified phenolic content, followed by low quantities of isorhamnetin, kaempferol, chrysin, epigallocatechin gallate, luteolin, and vanillic acid (0.02–0.06 μg/mL).

It is worth noting that the concentrations of phenolic compounds were found to vary greatly between different manufacturers/brands, which is evident by the high standard deviations presented among PBMAs of the same type, especially in the cases of almond-, soy-, and oat-based samples. This variability can be attributed to the differences in formulation between the commercial products. Additionally, the incorporation of flavoring agents, such as vanilla, may have also influenced the overall phenolic contents of these products, which was also observed by Grainger et al. [15]. Vanilla extract is frequently used in PBMA production and can result in high quantities of vanillin, as was already mentioned in the case of soy-based milk samples.

Overall, comparison across PBMA categories revealed clear differences in phenolic composition. Soy- and almond-based samples contained the highest total concentrations of phenolic compounds, with isoflavonoids and flavonols, respectively, as the major contributing classes. Oat- and coconut-based products showed intermediate levels, whereas rice- and walnut-based samples consistently exhibited the lowest contents. These results reflect both the intrinsic phenolic composition of the raw materials and the effects of formulation and processing, which are not standardized among products. The observed variability therefore highlights the heterogeneity of PBMAs with respect to phenolic content.

3.2. Principal Component Analysis (PCA)

Multivariate statistical analysis was employed to study the correlation of phenolic contents in relation to the plant source of PBMA samples. Data were analyzed via PCA to establish any "clustering" with respect to four PBMA groups (almond-, soy-, coconut-, and oat-based milk samples). A PCA model was constructed using 18 variables (chlorogenic acid, protocatechuic acid, neochlorogenic acid, epigallocatechin gallate, caffeic acid, syringic acid, vanillic acid, rutin, epicatechin gallate, *p*-coumaric acid, ferulic acid, vanillin, luteolin, quercetin, genistein, kaempferol, isorhamnetin, chrysin), corresponding to the phenolic compounds that were examined. The scree plot is presented in Figure S2 (Supplementary Material), showing Eigen values of 7.17 and 4.46 for PC1 and PC2, respectively. As depicted in Figure 2, the first two components of the model (PC1 and PC2) explain 64.58% of the variance, and the score plot of PC1 (39.82%) versus PC2 (24.76%) indicates moderate discrimination between the groups of PBMA samples. Three clusters can be observed in Figure 2; the almond-based milk samples are broadly located in the right part of the plot, while the oat-, soy-, and coconut-based milk samples are located in the left part of the plot. The coconut-based milk samples are closely clustered and can be distinguished from the remaining samples, while oat-based and soy-based milk samples

are indistinguishable. The walnut-based (AA) and rice-based (AB) samples are located in the same part of the plot as almond-based samples.

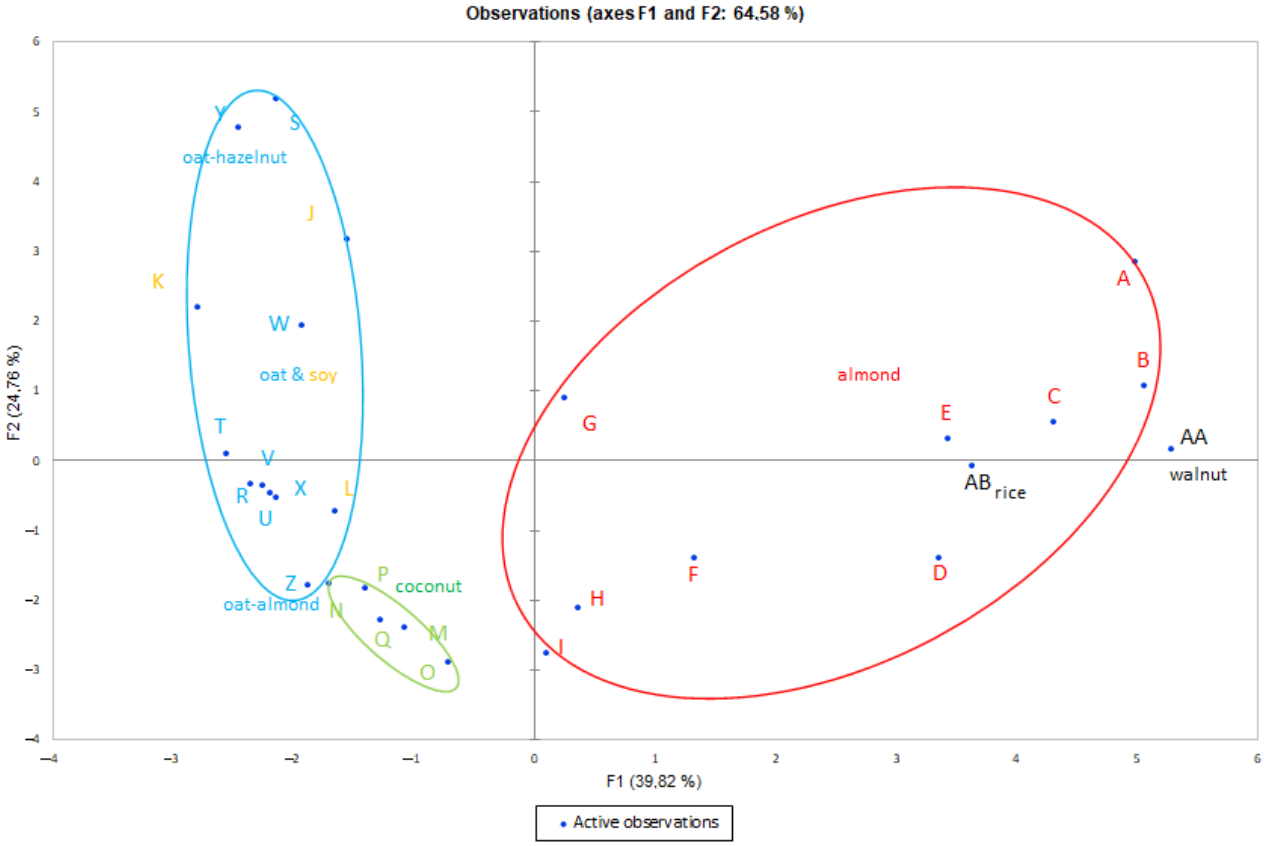


Figure 2. Principal component analysis (PCA) plot of phenolic compounds from PBMA samples using 18 variables. Red circle indicates the cluster of almond-based milk samples, blue circle the cluster of oat-based milk samples, and green circle the cluster of coconut-based milk samples.

3.3. Suspect Screening of Phenolic Compounds

In addition to the targeted profiling of phenolic compounds, a suspect screening approach was employed to explore the presence of matrix-specific polyphenols in oat- and soy-based milk alternatives. In oat-based samples, particular attention was given to avenanthramides, a class of phenolic alkaloids unique to oats and known for their potent antioxidant and anti-inflammatory properties [36,37]. Harnessing high-resolution Orbitrap MS, three major avenanthramides (A, B, and C) were tentatively identified based on their exact masses and MS/MS fragmentation patterns (Figure 3). Additional chromatographic data, extracted ion chromatograms, and mass spectra of these compounds are provided in Figure S3 (Supplementary Material). All compounds met key identification criteria, including mass error lower than 5 ppm and the presence of diagnostic fragment ions, previously reported in the literature [38,39]. Semi-quantification was performed by comparing the peak areas to those of structurally related standards (*p*-coumaric acid for avenanthramide A, ferulic acid for B, and caffeic acid for C) [40,41]. Among these, avenanthramide B was present at the highest relative abundance, followed by A and C, supporting prior observations on their natural distribution in oat products (Table 3). However, high standard deviations in all avenanthramide contents indicate high variability among the commercial oat-based milk samples.

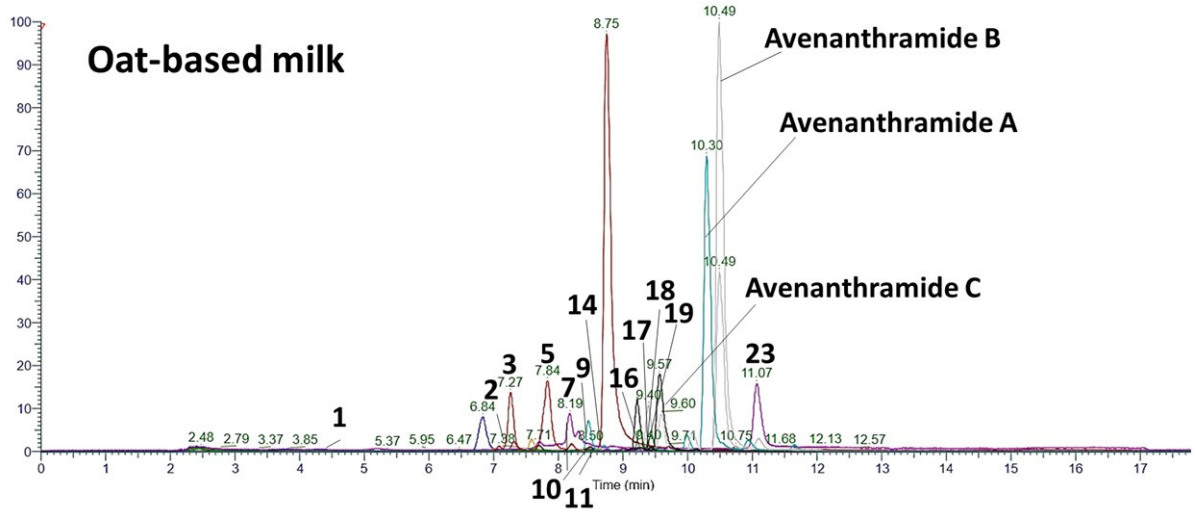


Figure 3. EICs of phenolic compounds in a representative sample of oat-based milk. Peaks: gallic acid (1), chlorogenic acid (2), protocatechuic acid (3), neochlorogenic acid (5), p-hydroxybenzoic acid (7), caffeic acid (9), syringic acid (10), vanillic acid (11), piceid (14), p-coumaric acid (16), sinapic acid (17), ferulic acid (18), vanillin (19), quercetin (23), and avenanthramides A, B, and C identified using suspect screening.

Table 3. Avenanthramides' suspect analysis data using an Exactive Plus Orbitrap mass spectrometer in negative ionization mode and their contents in oat-based milk alternatives (μg/mL).

Compound Name	Molecular Formula	Theoretical Mass $[M\text{-}H]^-$	Measured Mass $[M\text{-}H]^-$	Mass Error (ppm)	MS Rank	R_t (min)	Ion Fragments			Content μg/mL ± SD
Avenanthramide A	$C_{16}H_{13}NO_5$	298.0721	298.0723	−0.67	97.9	10.30	254.08	163.06	161.04	25.22 ± 36.88
Avenanthramide B	$C_{17}H_{15}NO_6$	328.0827	328.0829	−0.61	97.1	10.50	284.09	193.05	191.06	38.42 ± 57.97
Avenanthramide C	$C_{16}H_{13}O_6$	314.0670	314.0675	−1.59	96.6	9.58	270.08	179.03	177.04	0.93 ± 1.28

R_t, retention time; MS Rank, the rank order based on the MS data, defined as a measure of the correlation between the theoretical and measured isotopic patterns, reflecting the quality of the isotopic match of the peak.

Similarly, suspect screening of soy-based milk alternatives revealed the presence of glycosylated and acylated isoflavonoid derivatives, including daidzin, genistin, and glycitin, as well as their acetyl- and malonyl- conjugates (Figure 4). Such derivatives have been reported in soy milk and soy beans [15,22,23,42–44]. These compounds were tentatively identified through accurate mass measurements and characteristic mass spectra according to the literature [45]. Chromatographic data, EICs, and mass spectra of these compounds are provided in Figure S4 (Supplementary Material). Semi-quantification was performed by comparing the peak areas to those of structurally related standards (genistein for the genistein derivatives, namely genistin, acetylgenistin, and malonylgenistin, and daidzein for the daidzein derivatives, which are daidzin, acetyldaidzin, and malonyldaidzin) (Table 4). Notably, genistin emerged as the predominant isoflavone derivative, quantified at 0.12 ± 0.03 μg/mL, in line with previous findings describing it as the major conjugated isoflavone in soy [24,45]. Glycitein derivatives were observed at trace levels, and their low signal intensity limited further spectral characterization. Nonetheless, their tentative identification was based on accurate mass measurements and diagnostic fragment ions, in line with suspect screening criteria.

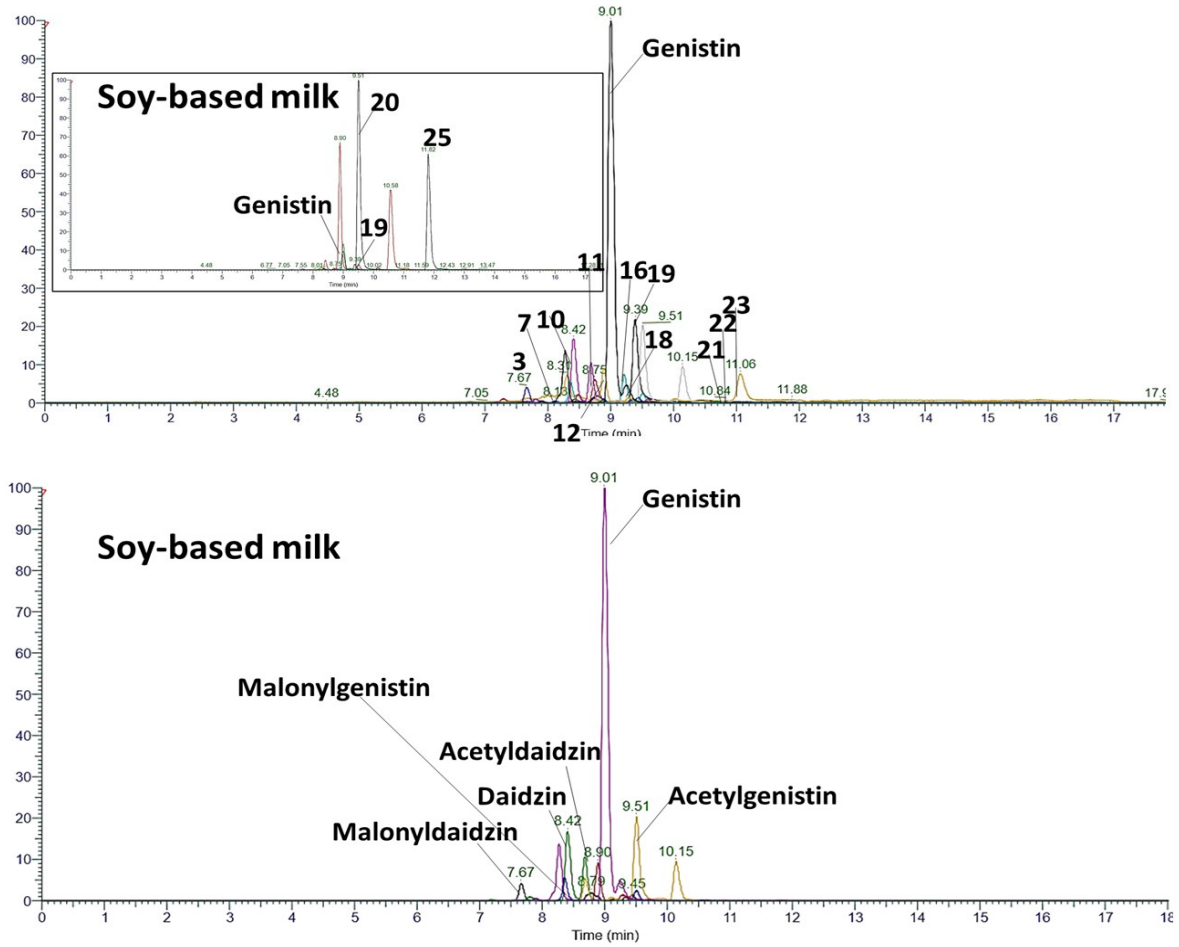


Figure 4. EICs of phenolic compounds in a representative sample of soy-based milk. Peaks: protocatechuic acid (3), p-hydroxybenzoic acid (7), syringic acid (10), vanillic acid (11), rutin (12), p-coumaric acid (16), ferulic acid (18), vanillin (19), daidzein (20), glycitein (21), luteolin (22), quercetin (23), genistein (25), and daidzin, acetyldaidzin, malonyldaidzin, genistin, acetylgenistin, and malonylgenistin identified using suspect screening analysis.

Table 4. Isoflavonoids' suspect analysis data using an Exactive Plus Orbitrap mass spectrometer in negative ionization mode and their contents in soy-based milk alternatives (μg/mL).

Compound Name	Molecular Formula	Theoretical Mass $[M-H]^-$	Measured Mass $[M-H]^-$	Mass Error (ppm)	MS Rank	R_t (min)	Ion Fragments		Content μg/mL ± SD
Isoflavonoids									
Malonyldaidzin	$C_{24}H_{22}O_{12}$	501.1038	501.1024	2.79	94.5	7.67	415.10	253.05	0.01 ± 0.00
Acetyldaidzin	$C_{23}H_{22}O_{10}$	457.1140	457.1147	−1.53	94.8	8.88	415.10	253.05	0.01 ± 0.00
Daidzin	$C_{21}H_{20}O_9$	415.1035	415.1039	−0.96	93.7	8.41	253.05		0.02 ± 0.00
Malonylgenistin	$C_{24}H_{22}O_{13}$	517.0988	517.0977	2.13	95.3	8.36	431.10	269.04	0.01 ± 0.00
Acetylgenistin	$C_{23}H_{22}O_{11}$	473.1089	473.1092	−0.63	97.8	9.51	431.10	269.04	0.01 ± 0.00
Genistin	$C_{21}H_{20}O_{10}$	431.0984	431.0987	−0.70	98.8	9.01	269.04		0.12 ± 0.03

R_t, retention time; MS Rank, the rank order based on the MS data, defined as a measure of the correlation between the theoretical and measured isotopic patterns, reflecting the quality of the isotopic match of the peak.

4. Discussion

As previously stated, nuts, seeds, grains, and legumes are rich in antioxidant phenolic compounds with promising health benefits; a plethora of studies report the occurrence and characterization of different phenolic compounds found in such plant sources or their by-products [31,32,34,36,44,46]. Recently, the development of plant-based alternatives, such

as PBMAs, has also gained traction [20,21,33,47]. However, a limited amount of studies provide detailed information regarding the profiling of individual phenolic compounds in PBMAs [15,22,24], while in several studies, only total phenolic content is estimated, often as gallic acid equivalents [20,21,47,48]. In any case, when studying plant-based products, it is important to note that their specific nutritional and/or bioactive compound profiles may vary based on the raw materials and potential additives used in their production and on the processing methods applied to the starting plant material [33]. During the manufacture of PBMAs, processes such as soaking, blanching, filtration, and enzymatic treatment are likely to contribute to the overall reduction in phenolic compound content. These steps may lead to partial degradation or removal of soluble phenolics, resulting in significantly lower levels compared to the raw ingredients [20,21,49].

In their study, Grainger et al. reported that almond-based milk alternatives obtained in the US market contained high amounts of chlorogenic acid isomers (0.00–2.25 mg/100 mL), ferulic acid (0.0–0.09 mg/100 mL), and vanillin (0.00–13.4 mg/100 mL), which were assumed to be derived from added flavors during processing, as well as other unknown phenolic compounds [15]. Compared to the samples studied herein, chlorogenic acid isomers, ferulic acid, and vanillin were also detected, albeit in lower quantities, constituting the major phenolic compounds in almond-based milk samples, while 11 phenolic compounds were additionally identified and quantified. The same analytes were reported in coconut milk, namely chlorogenic acid isomers (0.72–0.97 mg/100 mL), vanillin (8.99–11.57 mg/100 mL), and unknown compounds [15], though in our samples, chlorogenic acid isomers were only found as traces and the main phenolic compounds identified were vanillin (1.23 μg/mL, 72.35%), quercetin (0.24 μg/mL, 14.12%), and ferulic acid (0.19 μg/mL, 10.94%). In the case of rice milk, coumaric acid isomers and ferulic acid were identified in low quantities (0.2 mg/100 mL) [15], with these compounds also present in our sample, though ferulic acid (0.36 μg/mL) and quercetin (0.46 μg/mL) were the most prominent constituents. In oat-based milk samples, ferulic acid (0.14–0.81 mg/100 mL) and vanillin (0.00–8.18 mg/100 mL) were reported among common phenolic compounds, while avenanthramides A, B, and C were also quantified and ranged from 0.1 to 0.44 mg/100 mL, 0.05 to 0.27 mg/100 mL, and 0.1 to 0.32 mg/100 mL, respectively [15]. In our study, avenanthramides were semi-quantified and estimated at higher quantities than those reported by Grainger et al. [15], exhibiting differences in abundance, as avenanthramide B dominated in our samples, similar to other reports [50,51]. Finally, in literature reports studying soy-based milk samples, the most abundant phenolic compounds were flavonoids such as catechin (2.69 mg/100 mL), rutin (1.06 mg/100 mL), and quercetin (1.43 mg/100 mL), followed by cinnamic derivatives such as ferulic acid (0.20 mg/100 mL), *p*-coumaric acid (0.23 mg/100 mL), and sinapic acid (0.35 mg/100 mL) [24]. Isoflavones have also been reportedly detected in high abundance in soy milk, namely genistein and its derivatives (0.29–14.27 mg/100 mL), daidzein and its derivatives (0.20–10.48 mg/100 mL), and, to a lesser extent, glycitin (0.48 mg/100 mL) [15]. While the quantities measured in these reports do not align with those found in our study, the identified compounds are in general agreement with those reported herein, following similar trends with regard to the plant source. The lower values obtained in the present work may be attributed to the approximate quantification inherent to suspect screening [40,41], as well as to potential losses of phenolic compounds during processing steps such as soaking, filtration, or thermal treatment, as previously noted.

Regarding the occurrence of phenolic compounds in milk of animal origin and dairy products, it has been associated with different factors, namely the consumption of fodder crops by cattle and incorporation through the diet [52–55], the ruminal biotransformation of dietary phenolic compounds [56], process-related supplementation, or their intentional

addition as functional ingredients [57]. Similarly to PBMAs, although dairy milk has been found to contain phenolic compounds with antioxidant properties, the detailed profiling of these compounds has been poorly explored [52,53,55,58]. In milk obtained from ewes, cinnamic acid derivatives, such as caftaric acid, caffeic acid, ferulic acid, and *p*-coumaric acid, have been found in low quantities ranging from 0.01 to 0.13 μg/mL [55]. Benzoic acid derivatives were mainly found as traces, with low amounts of protocatechuic acid, vanillic acid, and *p*-hydroxybenzoic acid (0.01–0.06 μg/mL) [55]. Regarding flavonoids, epigallocatechin, kaempferol, luteolin, and naringenin were reported in similar quantities of 0.03–0.04 μg/mL and quercetin was found in higher concentration (0.59 μg/mL) [55]. Additionally, in a different report studying the transfer of polyphenols from forages to milks in the case of the most common cow diets, ferulic acid, quercetin, luteolin, and apigenin were estimated in concentrations ranging from 0.01 to 0.08 μg/mL, while *p*-hydroxybenzoic acid amounted to 0.25 μg/mL [53]. Comparing results from these reports to the PBMA samples studied herein, it is evident that phenolic content is richer and more diverse in the case of PBMAs, especially when compared to dairy milk that is obtained from animals receiving a standard diet.

5. Conclusions

In the present study, we employed a UHPLC–Orbitrap MS method for the extensive determination of phenolic compounds in PBMAs. This previously established method was applied in twenty-eight PBMAs obtained from the Greek market, encompassing a variety of almond-, soy-, coconut-, oat-, walnut-, and rice-based products. Almond-based milk products showed lower total concentrations but a broader distribution across compound classes, with *trans*-chlorogenic acid and neochlorogenic acid being the most prominent constituents. Soy-based milk samples were uniquely characterized by the presence of isoflavones including daidzein, genistein, and glycitein, while oat-based samples exhibited the highest overall phenolic concentrations, particularly for hydroxycinnamic acids, with *trans*-chlorogenic acid and neochlorogenic acid dominating the profile. Importantly, a suspect screening approach, using Exactive Plus Orbitrap, enabled the exploration and semi-quantification of three avenanthramides (A, B, C) in the studied oat-based milk samples and six isoflavonoids, namely daidzein and genistein derivatives, in soy-based samples. In summary, this study enhances our understanding of the occurrence of bioactive phenolic compounds in PBMAs, which constitute a rapidly growing segment of dairy alternatives, with increasing popularity worldwide.

Supplementary Materials: The following supporting information can be downloaded at https://www.mdpi.com/article/10.3390/separations12100282/s1. Table S1. Chromatographic and mass spectral data of the phenolic compound standards. Compounds are grouped by structural class and listed in order of chromatographic retention time (R_t) within each group. Table S2. Limits of detection (LODs, ng/mL) and limits of quantification (LOQs, ng/mL) of the phenolic compounds calculated based on the calibration curve. Compounds are grouped by structural class and listed in order of chromatographic retention time (R_t) within each group. Figure S1. Principal component analysis: graph depicting the contribution of the variables. Figure S2. Principal component analysis: scree plot of phenolic compounds in PBMAs. Figure S3. Chromatographic data, extracted ion chromatogram, and mass spectra of avenanthramides identified in oat-based milk samples using Orbitrap mass spectrometer. Figure S4. Chromatographic data, extracted ion chromatogram, and mass spectra of isoflavones identified in soy-based milk samples using Orbitrap mass spectrometer.

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