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RESEARCH ARTICLE

Chemical Composition of Almond Varieties Collected in the Valencian Region (Spain)

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ABSTRACT

Almonds (*Prunus amygdalus* Batsch) are consumed worldwide, with great economic importance in Spain. In the present work, six varieties collected in Valencia (Spain) were characterised: ‘Desmayo Rojo’, ‘Guara’, ‘Desmayo Largueta’ and ‘Marcona’ (Spanish varieties), and ‘Ferraduel’ and ‘Ferragnès’ (French varieties). The different varieties differed significantly in nutritional composition, fatty acid profile, and α-tocopherol content. ‘Desmayo Rojo’ (less known than ‘Desmayo Largueta’) presented the highest content of α-tocopherol and campesterol. β-Sitosterol was the major sterol, followed by campesterol and stigmasterol. Concerning sugars, sucrose, stachyose, and raffinose were quantified. The highest concentration of sucrose was found in ‘Ferragnès’, whereas stachyose was higher in ‘Desmayo Largueta’, ‘Desmayo Rojo’ and ‘Guara’. ‘Marcona’ presented the highest value of raffinose. It was possible to separate the six varieties into five groups, keeping the French varieties in the same group. Thus, this work showed the importance of maintaining and guaranteeing this genetic variability, as each variety has unique characteristics.

1 | Introduction

The almond tree (*Prunus amygdalus* Batsch) belongs to the Rosaceae family [1]. It is notable for reaching considerable heights, generally between 4 and 10 m, although some cultivars can exceed this height [1]. It is characterised by a straight trunk and spreading branches that form a broad crown. Its leaves are lanceolate and deep green in colour, with a very pronounced petiole, which varies in length depending on the cultivar, with small, serrated margins and a short mucro [1]. The almond tree flower is pentamerous with five sepals and five petals that vary in colour from white to pink and even reddish. With between 20 and 50 stamens, it has a single ovary, which is unilocular, mono- or biseminal and superior [1]. The almond is an edible seed found inside a hard, rough shell. It usually has a single seed, although some cultivars tend to produce double seeds. The seed has two enveloping teguments that are difficult to separate. The testa and tegmen are initially greenish and turn yellow and brown. This colour has taxonomic value for differentiating cultivars and is a good indicator of seed ageing.

Almonds, an important seed of a drupe, are among the most widely consumed nuts due to their flavour, nutritional and health properties, with beneficial impacts on cardiovascular diseases, diabetes and obesity [2]. In addition, almond ingestion is associated with improved cognitive performance, protection against skin photodamage and ageing [2]. They are very versatile because gluten-free flour and dairy alternatives can be produced from them, and they also serve as a protein source in vegetarian diets [3]. Recently, almond gum has been incorporated into biocomposite films, which have shown good mechanical and antimicrobial properties [4].

The worldwide production of almonds in shells reached 3.51 million tonnes in 2023 (latest value published by FAO) [5]. The USA and Spain were the main producing countries [5], representing 59% of the total production. The Spanish area occupied by almond orchards increased considerably from 534 100 ha in 2013 to 765 540 ha in 2020 [5]. The productions followed the same trend (149 000 tonnes in 2013 and 297 660 tonnes in 2023), showing the importance of this crop to the Spanish economy. Furthermore, almond production is mainly concentrated in the Mediterranean coast communities, like Andalusia, Murcia, Valencian Community, Aragón, Baleares (Mallorca) and Cataluña [6]. However, in Spain, the almond orchards present a great diversity of varieties. Still, two major commercial types dominate: (i) 'Marcona', 'Desmayo' and 'Guara', native Spanish; and (ii) foreign varieties, such as 'Ferraduel' and 'Ferragnès', originated from France. Concerning the 'Desmayo' variety, two types of fruits exist: 'Desmayo Largueta' and 'Desmayo Rojo' [7]. They differ in the fruit's form, yield and maturation stage. 'Desmayo Largueta' presents long and narrow fruits with a hard shell and a yield between 25% and 27% [7]. 'Desmayo Rojo' has long, wide fruits with a hard shell and yields between 23% and 25%. In this last variety, maturation is late [7]. Nevertheless, 'Desmayo Rojo' is practically unknown [8, 9].

According to our best knowledge, the chemical composition of the different varieties of almonds produced in Spain is poorly characterised. Some of the studies performed until now have focused on a few varieties and in a particular aspect, like the work conducted in the toasting of almonds from the 'Marcona' variety regarding volatile compounds and sensory attributes [10]; the influence of storage conditions on the quality of 'Marcona' and 'Desmayo Largueta' [11]; the determination of the oil content and fatty acid composition of 'Marcona', 'Desmayo Largueta' and 'Desmayo Rojo' [12], as well as the tocopherol characterisation of several almond cultivars [13, 14]. Thus, it is essential to verify if some of the almond varieties widespread in Spain have unique characteristics that are important to value in their marketing and usage.

Nevertheless, there are some studies on these varieties cultivated in different countries, such as 'Guara' in Australia [15]; 'Marcona' and 'Ferragnès' in Chile [16]; 'Marcona' and 'Ferragnès' + 'Ferraduel' in Morocco [17]; 'Ferragnès' in Portugal [18]; and 'Guara', 'Lauranne' and 'Ferragnès' (including hybrids) in Turkey [19, 20]. However, genetic and environmental factors may influence almonds' chemical composition. Thus, as almond is a fruit widely consumed worldwide, it is crucial to collect as much information about its chemical composition concerning varieties and places where it has been harvested. This information is essential for constructing food databases that are important and used for many purposes, such as in dietary intake surveys/studies, reference work, patient care, student education, product development, dietary assessment and clinical research [21]. In this sense, a better understanding of almond varieties' composition variability would help product development when compiling food composition data.

Thus, the main aim of the present work was to study the chemical composition of six varieties of almonds widespread in Spain, all collected in the Valencia Region: 'Desmayo Largueta', 'Desmayo Rojo', 'Ferraduel', 'Ferragnès', 'Guara' and 'Marcona'. The nutritional composition (lipid, protein, ash, carbohydrates and dietary fibre) and energetic value of these varieties were determined. Furthermore, some chemical components, namely fatty acids, sterols, α-tocopherol, squalene, individual sugars and minerals, were also determined. The novelty of the present study was to characterise these six varieties of almonds better and find potential compounds that could be used as a new and prospective source for the food industry.

2 | Results and Discussion

2.1 | Nutritional Composition

The nutritional composition and energetic value of the six varieties of almonds collected in Spain are shown in Table 1. Significant differences were found between varieties ($p < 0.05$). The main component was fat, varying between 54.9 and 59.7 g/100 g dry matter (DM) for 'Ferraduel' and 'Marcona'. 'Desmayo Rojo' presented the second-highest fat content (59.5 g/100 g DM); however, it was not significantly different from 'Marcona'. Similar values of oil contents were obtained when comparing our results with other Spanish varieties. In particular, ranges of 50.6% and 65.0% DM for 'Tejada-2' and 'Biota' varieties, 53.5% and 67.5% DM for 'Atocha' and 'Verdereta' varieties, and 49.9% and 58.6% for 'Desmeus' and 'Vivot' varieties were determined by Kodad et al. [12, 13] and Lipan et al. [22], respectively. Considering the varieties, the fat content determined in 'Desmayo Largueta' (57.7%) was similar to the values mentioned by Kodad et al. [13, 23] (52.6%–61.3% and 56.9%–64.8% during 2 and 3 years, respectively) and slightly lower than 62.5%–64.8% reported by Kodad et al. [12]. Similar values were determined in 'Desmayo Rojo' (our value equal to 59.5% compared with 57.0%–58.7% by Kodad et al. [12]), 'Guara' (57.0% vs. 55.8% [14]) and 'Marcona' (59.7% vs. 58.0% [14], 60.9%–65.3% [12] and 52.5%–65.3% [13]). Slightly lower values were determined in 'Ferraduel' (54.9% vs. 58.4%–61.2% mentioned by Kodad et al. [23]) and 'Ferragnès' (57.8% vs. 63.0% and 61.5%–63.2% noted by Kodad et al. [14, 23], respectively). However, Lipan et al. [22] determined a fat content of 56.1% for 'Ferragnès' grown on Mallorca Island.

The second major constituent in the almonds studied was protein (18.3–21.8 g/100 g DM), followed by carbohydrates (12.2–18.4 g/100 g DM). Our protein values were slightly lower than those determined by Lipan et al. [22], who mentioned a range of 26.4%–32.6%. However, similar protein values to ours were determined by Ahrens et al. [24], Barreira et al. [25] and Yada et al. [26] in varieties grown in the USA and Portugal (21.2–24.4, 21.9–25.0 and 21.0–23.6 g/100 g DM, respectively).

The lowest protein content was determined in 'Desmayo Rojo', while the highest was in 'Desmayo Largueta'. Our values for

TABLE 1 | Nutritional composition (g/100 g DM) of six almond varieties collected in the Valencian region.

Varieties	Lipid	Protein	Carbohydrates	Total dietary fibre	Ash	Energy[a]
'Desmayo Rojo'	59.5 ± 0.2^{d}	18.3 ± 0.1^{a}	15.3 ± 0.2^{c}	5.2 ± 0.2^{c}	1.68 ± 0.01^{a}	681 ± 1^{d}
'Ferraduel'	54.9 ± 0.4^{a}	19.0 ± 0.2^{b}	18.4 ± 0.3^{f}	5.8 ± 0.2^{d}	1.89 ± 0.02^{d}	655 ± 2^{a}
'Ferragnès'	57.8 ± 0.1^{c}	19.3 ± 0.2^{c}	16.3 ± 0.2^{d}	4.8 ± 0.1^{b}	1.81 ± 0.03^{c}	672 ± 1^{c}
'Guara'	57.0 ± 0.1^{b}	20.4 ± 0.1^{d}	16.7 ± 0.2^{e}	4.2 ± 0.1^{a}	1.82 ± 0.01^{c}	669 ± 1^{b}
'Desmayo Largueta'	57.7 ± 0.4^{c}	21.8 ± 0.3^{f}	12.7 ± 0.5^{b}	5.9 ± 0.3^{d}	1.88 ± 0.03^{d}	669 ± 2^{b}
'Marcona'	59.7 ± 0.6^{d}	21.4 ± 0.5^{e}	12.2 ± 0.8^{a}	4.8 ± 0.2^{b}	1.78 ± 0.05^{b}	682 ± 3^{d}

Note: Mean ± standard deviation. Values with a different letter in the same column are statistically different ($p < 0.05$).
[a]Results are expressed in kcal/100 g DM.

'Ferraduel', 'Ferragnès' and 'Marcona' (19.0, 19.3 and 21.4 g/100 g DM) were slightly lower than those reported by Barreira et al. [25] for these varieties grown in Portugal (22.9, 21.9 and 25.0 g/100 g DM, respectively).

Concerning carbohydrates, 'Marcona' was the least sweet, while 'Ferraduel' was the sweetest (12.2 vs. 18.4 g/100 g DM). Again, no carbohydrate values for varieties grown in Spain were found. Nevertheless, our values were slightly lower than those that Ahrens et al. [24] reported for USA varieties (24.7–28.1 g/100 g DM). 'Marcona' was also the less sweet variety studied by Barreira et al. (14.6 g/100 g DM) [25] in Portugal, while the sweetest was 'Ferragnès' (21.9 g/100 g DM).

As mentioned by Yada et al. [27], according to the American Association of Cereal Chemists (AACC), the term 'dietary fibre' commonly includes the complex mixture of indigestible polysaccharides (e.g. cellulose, hemicellulose, oligosaccharides, pectins, gums, waxes) and lignin found in plants, mainly as plant cell wall material. It promotes physiological effects that benefit human health. In the present study, the total dietary fibre content of almonds varied from 4.2 to 5.9 g/100 g DM ('Guara' and 'Desmayo Largueta', respectively). Yada et al. [26, 27] reported higher dietary fibre contents than ours, namely 11.5–14.2 and 12.8 g/100 g DM as listed in the USDA National Nutrient Database for Standard Reference and in the almond varieties 'Mission' and 'Fritz', respectively, grown in California (USA). On the contrary, almonds grown in Portugal showed similar fibre values to ours (5.1–11.8 g/100 g DM for 'Bonita São Brás' and 'Parada' varieties, respectively) [28].

Ash values varied between 1.68 and 1.89 g/100 g DM for 'Desmayo Rojo' and 'Ferraduel', respectively. However, 'Desmayo Largueta' also presented one of the highest ash contents (1.88 g/100 g DM). In general, the ash values obtained for almonds produced in other countries were higher than ours, namely in Portugal (3.37–3.93 g/100 g DM for 'Boa Casta' and 'Verdeal', respectively) [28]; California (3.86–4.77 g/100 g DM for 'Carmel' and 'Mission', respectively) [24]; and Turkey (2.74%–3.05% for 'Guara' and 'Ferragnès', respectively) [19]. This difference might be because plant tissues' mineral content can be affected by environmental factors and agronomic practices (geographic location, soil composition, irrigation and fertilisers) [27]. As expected, the almonds showed high energetic values of 655–682 kcal/100 g DM due to their high-fat content.

2.2 | Fatty Acids and α-Tocopherol

The contents of lipophilic compounds, namely fatty acids and α-tocopherol, present in the almond oil extracted are shown in Table 2. The five major fatty acids found in the six almond varieties were (in decreasing order): oleic (18:1), linoleic (18:2), palmitic (16:0), stearic (18:0) and palmitoleic (16:1) acids, with oleic and linoleic acids being the major ones. Oleic acid contents ranged between 64.2 and 73.2 mg/100 g of oil ('Guara' and 'Ferragnès', respectively), whereas linoleic acid values varied from 16.5 to 25.6 mg/100 g of oil ('Ferragnès' and 'Guara', respectively). Linoleic acid is an essential fatty acid; therefore, the ingestion of almonds has been shown to have positive effects in various diseases, including diabetes and cancer prevention [29]. The third most abundant fatty acid was palmitic acid, found between 5.44 and 7.07 mg/100 g of oil in 'Ferraduel' and 'Desmayo Rojo', respectively. Stearic and palmitoleic acids were detected in low amounts in oils extracted from the six almond varieties. Furthermore, the oils extracted from almonds showed low contents of saturated fatty acids (SFA) (7.53–9.14 mg/100 g of oil) and high contents of monounsaturated fatty acids (MUFA) (64.6–73.7 mg/100 g of oil). Furthermore, significant differences in SFA, MUFA and PUFA contents were also observed among varieties. The determined values are in accordance with the fatty acid compositions of several almond kernels described in the literature [13, 17, 19, 26]. Furthermore, according to Kester et al. [30], a high ratio of oleic to linoleic (O/L) acids is essential for maintaining oil stability. Almonds tend to rancify during storage and transport, losing quality [31]. In this order, 'Ferragnès' (4.44) and 'Ferraduel' (3.98) showed the highest values of O/L, indicating greater resistance to oxidation during processing, storage and transport. The SFAs (palmitic and stearic) are also considered to give more stability to the fat [32]. So, 'Desmayo Rojo', 'Guara' and 'Desmayo Largueta' varieties showed the highest SFAs values, showing oils more stable to oxidation reactions. In this order, combining the values of the O/L ratio and the SFAs, we can conclude that most of the varieties studied in the present work could produce high-quality oil.

Concerning α-tocopherol, almond oils are considered one of the richest food sources [3, 33]. The levels of this compound varied from 29.7 to 54.0 mg/100 g of oil for 'Guara' and 'Desmayo Rojo', respectively. So, 'Desmayo Rojo' variety showed the highest value of α-tocopherol, being an important component for protecting almond oil against oxidative deterioration [31]. Our results are in line with those reported by Kodad et al. [13, 14] and Garcıá-Pascual

TABLE 2 | Characterisation of fatty acids and tocopherol (mg/100 g of oil) of oil extracted from six varieties of almonds collected in the Valencian region.

Varieties	Fatty acids					Ratio oleic/linoleic	SFA	MUFA	PUFA	α-Tocopherol
	Palmitic acid (C16:0)	Palmitoleic acid (C16:1)	Stearic acid (C18:0)	Oleic acid (C18:1)	Linoleic acid (C18:2)					
'Desmayo Rojo'	7.07 ± 0.06^{e}	0.51 ± 0.03^{b}	2.07 ± 0.04^{a}	67.7 ± 0.5^{b}	22.9 ± 0.3^{d}	2.96	9.14 ± 0.06^{c}	68.2 ± 0.5^{b}	22.9 ± 0.3^{d}	54.0 ± 0.4^{d}
'Ferraduel'	5.44 ± 0.23^{a}	0.52 ± 0.06^{b}	2.09 ± 0.16^{a}	$69.0 \pm 9.0^{b,c}$	17.4 ± 0.3^{b}	3.98	7.53 ± 0.30^{a}	$69.6 \pm 9.0^{b,c}$	17.4 ± 0.3^{b}	30.1 ± 0.8^{a}
'Ferragnês'	$5.63 \pm 0.28^{a,b}$	0.52 ± 0.03^{b}	2.13 ± 0.07^{a}	73.2 ± 0.6^{e}	16.5 ± 0.4^{a}	4.44	$7.75 \pm 0.32^{a,b}$	73.7 ± 0.5^{d}	16.5 ± 0.4^{a}	33.7 ± 0.7^{b}
'Guara'	6.00 ± 0.12^{c}	0.43 ± 0.04^{a}	3.03 ± 0.09^{c}	64.2 ± 0.9^{a}	25.6 ± 0.4^{e}	2.51	9.03 ± 0.14^{c}	64.6 ± 0.9^{a}	25.6 ± 0.4^{e}	29.7 ± 0.7^{a}
'Desmayo Largueta'	6.73 ± 0.35^{d}	0.61 ± 0.04^{c}	2.25 ± 0.18^{b}	64.6 ± 0.8^{a}	23.2 ± 0.8^{d}	2.79	8.98 ± 0.36^{c}	65.2 ± 0.8^{a}	23.2 ± 0.8^{d}	38.8 ± 1.6^{c}
'Marcona'	5.66 ± 0.38^{b}	0.52 ± 0.07^{b}	2.32 ± 0.17^{b}	70.8 ± 0.5^{d}	20.7 ± 0.4^{c}	3.42	7.98 ± 0.47^{b}	71.4 ± 0.5^{c}	20.7 ± 0.4^{c}	30.0 ± 1.3^{a}

Note: Mean ± standard deviation. Values with a different letter in the same column are statistically different ($p < 0.05$).

et al. [11] for almonds cultivated in Spain, as well as by Melhaoui et al. [34] in Morocco. In particular, Kodad et al. [13] found values between 31.3 and 54.6 mg/100 g oil for 'Muel' and 'Vinagreta' in the year 2009, respectively; Kodad et al. [14] mentioned values that varied from 24.1 and 46.3 mg/100 g of oil for 'Bertina' and 'Marcona' in 2003, respectively; and Garcıá-Pascual et al. [11] detected levels between 33.8 and 40.5 mg/100 g of oil for 'Planeta' and 'Marcona', respectively. Melhaoui et al. [34] also determined α-tocopherol contents that varied between 40.1 and 47.4 mg/100 g of oil in 'Ferragnès', 'Ferraduel', 'Fournat', and 'Marcona' varieties grown in Morocco.

2.2.1 | Sterols and Squalene

Table 3 shows the amounts of sterols and squalene present in the six oils extracted from almonds. Significant differences between the varieties were detected ($p < 0.05$). Phytosterols are of great interest due to their antioxidant activity and impact on health [35]. In the present study, β-sitosterol was the major component found, followed by campesterol and stigmasterol. β-Sitosterol has been reported in the literature to have the ability to lower cholesterol levels and enhance immunity, with anti-inflammatory, antipyretic and anti-carcinogenic effects [36–38].

The amount of β-sitosterol varied between 72.4 ('Guara') and 103.2 mg/100 g of oil ('Ferragnès'), while campesterol values ranged from 11.7 ('Guara') and 27.6 mg/100 g of oil ('Desmayo Rojo'). Furthermore, stigmasterol showed values between 3.7 and 6.6 mg/100 g of oil for 'Ferragnès' and 'Desmayo Largueta', respectively. The sterols composition of almonds studied in the present work was in line with that detected in seven major California almond varieties [26] (β-sitosterol: 256–313 mg/100 g of oil; stigmasterol: 4.1–13.2 mg/100 g of oil; campesterol: 9.9–12.6 mg/100 g of oil), being our β-sitosterol values lower than the American samples.

Regarding squalene contents, the results varied between 8.2 and 17.6 mg/100 g of oil for 'Guara' and 'Ferraduel', respectively. Although there is scarce information regarding the content of squalene in almonds, our values were higher than those reported by Maguire et al. [39] (9.5 mg/100 g of oil), except when compared with the 'Guara' and 'Marcona' varieties. According to the revision done by Smith [40], squalene is a potential chemopreventive agent. So, almonds can contribute as a source of dietary squalene.

2.2.2 | Mineral Composition

Macro- and microelements detected in the six varieties of almonds are presented in Table 4. Almonds are an excellent source of minerals, especially potassium and phosphorus. The contents of these elements ranged from 702 (Desmayo Rojo) to 833 (Ferraduel) mg/100 g DM and from 560 ('Desmayo Rojo') to 593 ('Ferragnès' and 'Marcona') mg/100 g DM, respectively. However, calcium and magnesium were also detected in almonds. Calcium levels varied between 170 and 241 mg/100 g DM for 'Guara' and 'Ferraduel', respectively, while magnesium ranged from 219 to 244 for 'Desmayo Rojo' and 'Desmayo Largueta', respectively. Our values of macroelements were similar to those reported by

TABLE 3 | Sterols and squalene (mg/100 g of oil) of oil extracted from six varieties of almonds collected in the Valencian region.

Varieties	Sterols			Squalene
	β-Sitosterol	Campesterol	Stigmasterol	
'Desmayo Rojo'	87.7 ± 0.5^{b}	27.6 ± 1.9^{d}	6.4 ± 0.3^{d}	11.3 ± 0.4^{c}
'Ferraduel'	89.0 ± 0.7^{c}	21.2 ± 0.6^{b}	4.6 ± 0.2^{b}	17.6 ± 0.4^{f}
'Ferragnês'	103.2 ± 1.5^{e}	25.4 ± 0.7^{c}	3.7 ± 0.2^{a}	15.6 ± 0.2^{e}
'Guara'	72.4 ± 1.0^{a}	11.7 ± 0.4^{a}	4.7 ± 0.3^{b}	8.2 ± 0.1^{a}
'Desmayo Largueta'	87.3 ± 1.9^{b}	27.1 ± 1.3^{d}	6.6 ± 0.2^{e}	11.9 ± 0.4^{d}
'Marcona'	96.3 ± 1.9^{d}	21.1 ± 1.2^{b}	5.5 ± 0.3^{c}	10.0 ± 0.4^{b}

Note: Mean ± standard deviation. Values with a different letter in the same column are statistically different ($p < 0.05$).

Saura-Calixto et al. [41], who observed that average kernel contents consisted of 766 mg/100 g K, 364 mg/100 g P, 227 mg/100 g Mg and 185 mg/100 g Ca. On the contrary, Özcan et al. [19] showed higher values than ours for Ca (183–294 mg/100 g DM); Mg (298–404 mg/100 g DM); P (793–938 mg/100 g DM) and K (1314–1510 mg/100 g DM) for Spanish varieties. These differences were likely due to the region where almonds were produced, which affected the levels of minerals [27].

Regarding microelements, copper, iron, manganese and zinc were also determined. As expected, these minerals were present in much lower concentrations than macroelements. 'Desmayo Rojo' showed the lowest values of Cu (0.43 mg/100 g DM), Mn (1.10 mg/100 g DM) and Zn (3.77 mg/100 g DM), while 'Guara' presented the lowest Fe content (2.92 mg/100 g DM). On the contrary, 'Guara', 'Ferragnès', 'Marcona' and 'Desmayo Largueta' showed the highest values of Cu, Fe, Mn and Zn, respectively. Similar values to ours of Cu (1.0 mg/100 g DM) and Zn (4–6 mg/100 g DM) were detected by Özcan et al. [19] in varieties collected in Turkey. However, they reported higher values of Fe (20–27 mg/100 g DM) than ours [19].

2.2.3 | Sugars

Three sugars were identified in almonds: sucrose, stachyose and raffinose (Figure 1). Sucrose was the major sugar, ranging between 2.91 ('Guara') and 4.06 ('Ferragnès') g/100 g DM. Similar results were reported by Saura-Calixto et al. [41], who studied almond varieties from Spain and found that sucrose was the major sugar component in almond kernels. Raffinose was also present, varying between 0.14 and 0.62 g/100 g DM ('Ferragnès' and 'Marcona', respectively), while stachyose was found only in low levels (0.15 ['Ferragnès'] and 0.30 g/100 g DM ['Desmayo Largueta']). Kazantzis et al. [42] also detected sucrose and raffinose in 'Ferragnès' grown in Greece, but they did not mention the presence of stachyose. So, the six varieties studied in the present work showed a similar profile of sugars but in different amounts.

2.2.4 | Correlation Analysis

A high number of significant correlations ($p < 0.01$) equal to or greater than 0.6 were observed, specifically 48 correlations (Table S1). Of these, 25 presented correlation factors equal to or greater than 0.7. Lipids showed negative correlations with ash and potassium equal to −0.707 and −0.791, respectively. Dietary fibre showed a positive correlation with calcium ($r = 0.715$), and ash with potassium ($r = 0.865$), as would be expected since potassium is the element in the greatest proportion in ash.

Regarding individual sugars, sucrose showed a negative correlation with linoleic acid ($r = -0.764$) and copper ($r = -0.755$), and a positive correlation with sitosterol ($r = 0.783$). On the contrary, stachyose showed a positive correlation with linoleic acid ($r = 0.854$) and a negative correlation with sitosterol ($r = -0.759$). In addition, raffinose showed a negative correlation with squalene (−0.735).

Regarding fatty acids, linoleic acid showed a negative correlation with squalene ($r = -0.864$), and stearic acid showed a negative correlation with campesterol ($r = -0.806$) and a positive correlation with copper ($r = 0.914$).

α-Tocopherol showed a negative correlation with manganese ($r = -0.735$). Among the sterols, β-sitosterol and campesterol showed negative correlations with copper ($r = -0.767$ and −0.878, respectively). On the contrary, at the level of squalene, a positive correlation was observed with calcium ($r = 0.818$).

2.2.5 | Differentiation and Discrimination of Almond Varieties

After determining the chemical compositions (nutritional composition, fatty acids, α-tocopherol, sterols, squalene, mineral composition and individual sugars) of the six almond varieties, we attempted to distinguish and discriminate them using principal component analysis (PCA). As shown in Figure 2, these six almond varieties could be differentiated based on their chemical composition. The first two principal components accounted for 67.9 % (PC1 = 38.3% and PC2 = 29.6%, respectively) of the total variation. Sucrose, Fe, palmitoleic acid, campesterol, fibre and squalene positively contributed to PC1. On the contrary, PC1 was correlated negatively with Cu and linoleic and stearic acids. Regarding PC2, it was mainly correlated positively to the energy value, lipid content, palmitic acid, stachyose, raffinose, α-tocopherol and stigmasterol. On the other hand, PC2 was correlated negatively with some minerals (P, Ca, K, Mn, Mg, Zn),

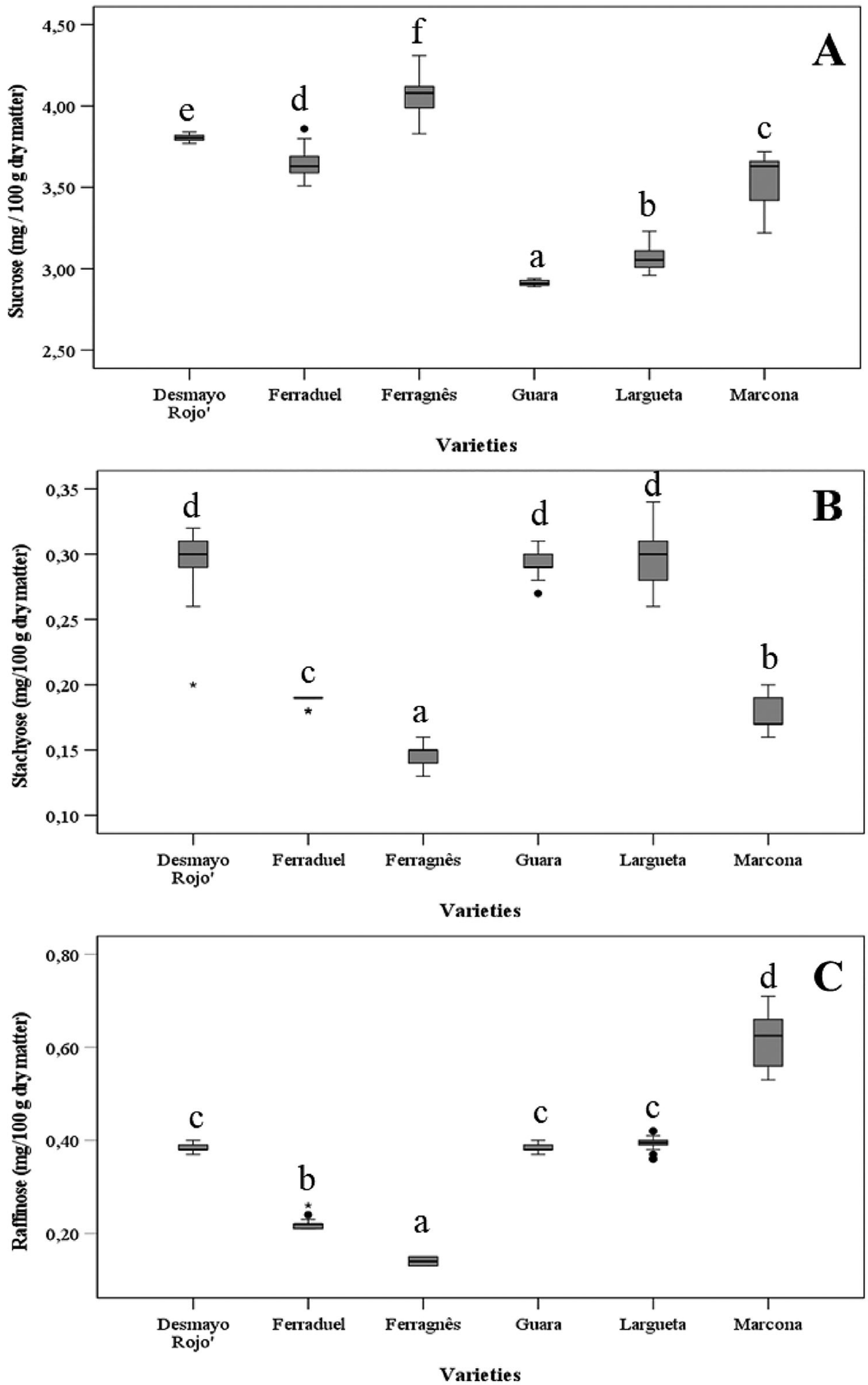

FIGURE 1 | Box plot graphs of the three main sugars identified in the six almond varieties collected in the Valencian region. A—Sucrose. B—Stachyose. C—Raffinose.

ash and carbohydrate contents, as well as oleic acid. Five groups can be observed in Figure 2. Group I was formed by the 'Guara' variety, with low scores in PC1 due to the high values of the stearic acid and Cu. Concerning 'Desmayo Rojo', corresponding to Group II, this variety presented the highest α-tocopherol and palmitic acid contents. Group III was constituted by 'Desmayo Largueta'. This variety presented the highest palmitoleic acid and fibre contents, whereas the 'Marcona' (Group IV) showed one of the highest values of β-sitosterol. Furthermore, 'Ferraduel' and 'Ferragnès' could also be separated from the other varieties (Group V) since they are varieties that are self-incompatible (insect-dependent cross-pollination). Thus, they are often planted

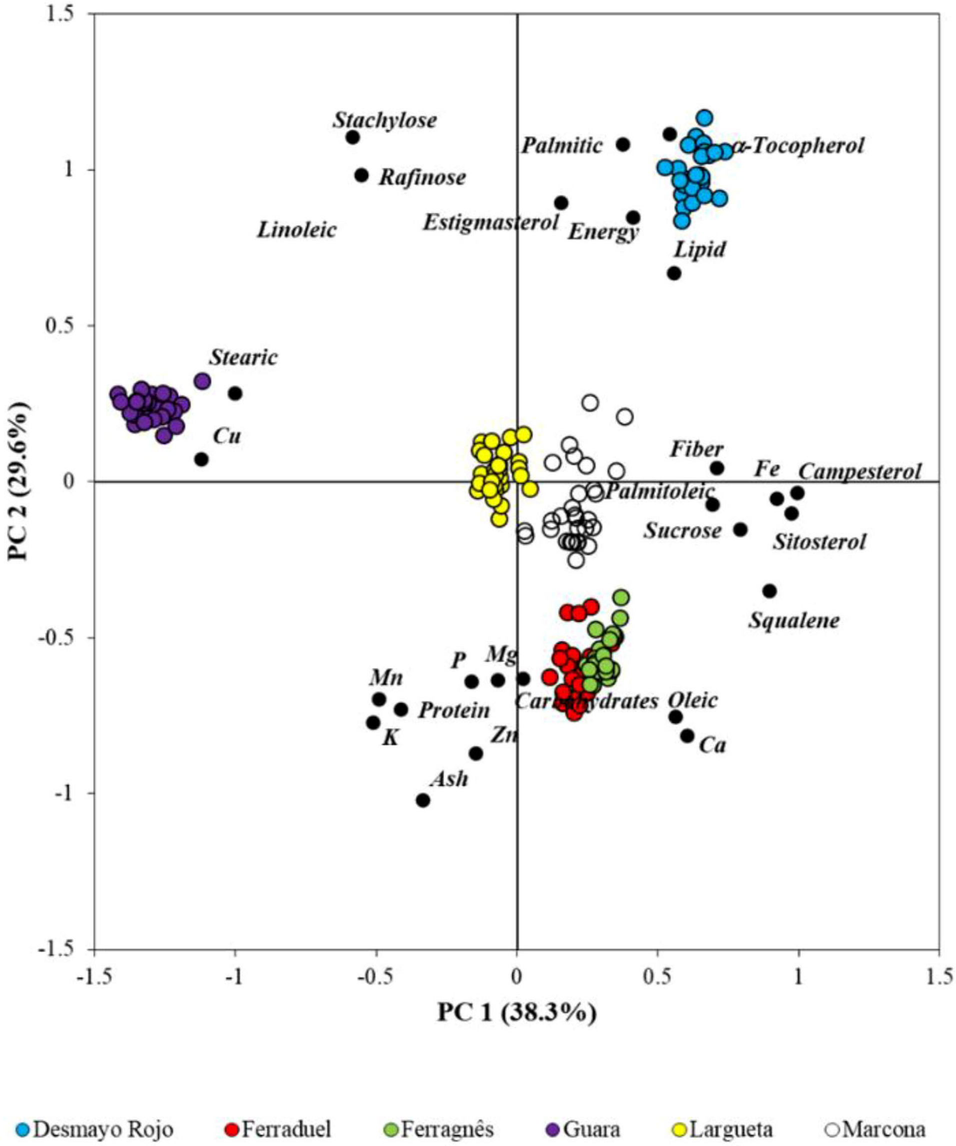

FIGURE 2 | Principal component analysis obtained by using nutritional composition, fatty acids profile, sterols, squalene, α-tocopherol, mineral composition and individual sugars for the six almond varieties collected in the Valencian region.

in combination in orchards [43]. Nevertheless, these two varieties showed the highest squalene values. Therefore, the chemical composition can be useful for identifying and differentiating almond varieties widespread in Spain. Furthermore, these chemical differences could properly select varieties and may be further used in breeding programs.

These results suggest that genetic factors may influence the chemical composition of almonds. Other nut-bearing or culinary nut species also exhibit variations in their chemical composition due to genetic diversity. Studies on hazelnut (*Corylus avellana* L.) [44] and sweet chestnut (*Castanea sativa* Mill.) [45, 46] highlight significant differences in nutritional profiles among varieties, which are strongly influenced by genetic variability. Nevertheless, in some cases, genotypic differences do not necessarily result in distinct chemical compositions, as observed in the case of walnut (*Juglans regia* L.) [47]. In such instances, morphological and biochemical data may be insufficient for identifying genetic diversity and relatedness and must be supplemented by molecular data [47]. Yu et al. [48] demonstrated that the *PdWRKY* genes are involved in almond resistance to various stresses, including low temperatures, drought, and salt. Furthermore, the expression levels of these genes change over time, indicating the occurrence of spatiotemporal expression patterns [48]. Thus, assessing genetic diversity using techniques such as the SSR molecular marker and examining relationships among almond genotypes, determining gene pools, developing conservation strategies, and identifying genetic resources, are of great importance [49].

3 | Conclusions

In conclusion, the present study revealed considerable chemical composition diversity among the six almond varieties collected in the Valencian region. The varieties studied showed high amounts of oleic and linoleic acids, α-tocopherol, β-sitosterol, K and P, compounds with beneficial health effects. Furthermore, the PCA showed that it is possible to discriminate and distinguish the different varieties. The lesser-known variety, 'Desmayo Rojo', exhibited interesting chemical properties, including high α-tocopherol and campesterol contents, highlighting the importance of preserving all varieties and not just opting for the most commercially viable ones. Furthermore, the determination of these parameters allowed distinguishing between the studied varieties, suggesting that these properties can be used to identify fruits whose varieties are unknown. Furthermore, the chemical variability attributed to almond varieties may contribute in the future to improvements in the nutrient composition of almonds by developing varieties with increased health-promoting compounds.

4 | Experimental Section

4.1 | Samples

Thirty samples of six varieties of almonds (*P. amygdalus*) ('Desmayo Largueta', 'Desmayo Rojo', 'Ferraduel', 'Ferragnès', 'Guara' and 'Marcona') were collected in the Valencian Community of Spain (39.9453° N, −0.629° W) by researchers from the Preservation and Improvement of Valencian Agro-diversity University Research Institute (COMAV). After harvest, almonds were immediately transported to the laboratory and stored at room temperature until analysis. To avoid sample degradation, the almonds were maintained intact. Immediately before analysis, the endocarps were removed and ground to obtain flour used for chemical analysis. The analyses were performed in triplicate.

4.2 | Nutritional Composition

The lipids, protein, crude fibre, ash, carbohydrates and energy values of the almond kernels were determined using AOAC procedures. Crude protein content was determined from the nitrogen content evaluated by the Kjeldahl method, using a conversion factor of 5.18 [26]. Total lipids were extracted in a Soxhlet apparatus at 40°C–60°C with petroleum ether as extracting solvent for 24 h. Total dietary fibre was measured by the enzymatic gravimetric AOAC method [50]. Ash content was measured by calcination at 550°C for a minimum of 2 h until achieving white ashes. The carbohydrate contents were calculated by difference, following Equation (1):

$$\text{Carbohydrates}\left(\frac{\text{g}}{100\ \text{g dry matter}}\right) = 100 - (\text{Protein} + \text{Lipids} + \text{Ash} + \text{Fibre}) \quad (1)$$

The protein, lipids, ash and fibre contents were expressed in g/100 DM.

The energy values of almonds were calculated following Equation (2):

$$\text{Energy} = (4 \times \text{Protein}) + (4 \times \text{Carbohydrates}) + (2 \times \text{Total Dietary Fibre}) + (9 \times \text{Lipids}) \quad (2)$$

The energetic values were expressed in kcal/100 g DM.

TABLE 4 | Content of macroelements and microelements (mg/100 g DM) in six varieties of almonds collected in the Valencian region.

Varieties	Macroelements				Microelements			
	Calcium (Ca)	Magnesium (Mg)	Phosphorus (P)	Potassium (K)	Copper (Cu)	Iron (Fe)	Manganese (Mn)	Zinc (Zn)
'Desmayo Rojo'	190 ± 7^{b}	219 ± 7^{a}	560 ± 7^{a}	702 ± 6^{a}	0.43 ± 0.01^{a}	3.50 ± 0.01^{d}	1.10 ± 0.01^{a}	3.77 ± 0.01^{a}
'Ferraduel'	241 ± 9^{e}	228 ± 6^{b}	$583 \pm 15^{b,c}$	833 ± 8^{e}	0.81 ± 0.01^{c}	3.19 ± 0.01^{b}	1.21 ± 0.01^{b}	4.51 ± 0.01^{e}
'Ferragnès'	218 ± 7^{d}	$233 \pm 6^{b,c}$	593 ± 26^{c}	761 ± 9^{c}	0.70 ± 0.01^{b}	4.15 ± 0.03^{f}	1.29 ± 0.01^{c}	3.97 ± 0.11^{b}
'Guara'	170 ± 6^{a}	$230 \pm 8^{b,c}$	579 ± 6^{b}	829 ± 6^{e}	2.59 ± 0.02^{f}	2.92 ± 0.01^{a}	1.42 ± 0.01^{d}	4.04 ± 0.02^{c}
'Desmayo Largueta'	217 ± 10^{d}	244 ± 9^{d}	$587 \pm 13^{b,c}$	817 ± 20^{d}	1.00 ± 0.03^{e}	3.84 ± 0.11^{e}	1.28 ± 0.03^{c}	4.83 ± 0.06^{f}
'Marcona'	207 ± 7^{c}	235 ± 7^{c}	593 ± 27^{c}	739 ± 28^{b}	0.87 ± 0.03^{d}	3.25 ± 0.13^{c}	1.46 ± 0.04^{e}	4.27 ± 0.06^{d}

Note: Mean ± standard deviation. Values with a different letter in the same column are statistically different ($p < 0.05$).

4.3 | Fatty Acids

Fatty acids were analysed in a gas chromatograph (GC) (GC 8000Top, CE Instruments Ltd.) with a flame ionisation detector (FID). Fatty acids separation was carried out on a Supelco WAX 10 column with the dimensions 30 mm × 0.25 mm (Sigma-Aldrich). Helium was the carrier gas at a flow rate of 1 mL/min. The injector and detector temperatures were 250°C and 270°C, respectively. An injection volume of 1 µL was used for sample analysis. The oven temperature was programmed to 120°C for the first 3 min, with a rate of increase of 4°C/min until 220°C. The relative percentage of each fatty acid was determined by conducting an internal normalisation of the chromatographic peak areas. The chromatographic peaks were identified by comparing the retention times of the sample peaks with those of a standard mixture of 37 fatty acid methyl esters (FAME Mix-37, Supelco). The results were expressed in g/100 g of oil.

4.4 | Sterols, Tocopherols and Squalene

The method used to determine the major tocopherols and sterols in oils extracted from almonds was described by Slover et al. [51]. An accurate lipid portion (100 mg) with a known amount of the internal standard (5,7-dimethyltocol) was saponified with aqueous KOH. The unsaponifiable fraction was extracted with cyclohexane. The solvent was removed with a stream of nitrogen. Then, trimethylsilyl ethers were formed using bis(trimethylsilyl)-trifluoroacetamide (BSTFA) plus 1% trimethylchlorosilane (TMCS) with pyridine. The total derivatised unsaponifiable fraction was injected using the same equipment to analyse the fatty acids. A Tracer TR Sterol column (30 m × 0.22 mm; Teknokroma, Spain) was used. The injector and detector temperatures were fixed at 290°C and 300°C, respectively. The oven temperature was maintained at 265°C, and a sample volume of 1 µL was injected into the equipment. Squalene was quantified simultaneously with tocopherols. The results were expressed in g/100 g of oil.

4.5 | Individual Sugars

The individual sugars of the six almond varieties were analysed using the method described by Soler et al. [52]. The results were expressed in g/100 g DM.

4.6 | Minerals

The mineral composition (Ca, Mg, P, K, Cu, Fe, Mn and Zn) of the almonds was determined by the method described by Yada et al. [26]. The results were expressed in mg/100 g DM.

4.7 | Statistical Analysis

Statistical analyses were performed using the Minitab software, v. 14 (Minitab Ltd., Coventry, United Kingdom). The normal distribution of the residuals and the homogeneity of variance were evaluated by Kolmogorov–Smirnov or Shapiro–Wilk's tests, and Levene's test, respectively. As the data followed a normal distribution and the variances were homogeneous, a one-way ANOVA was applied to determine the existence of significant differences between the almond varieties. When significant differences were detected, the Tukey post hoc test was performed. Differences were considered significant when the $p < 0.05$. A PCA was also done, considering the nutritional composition, fatty acids, α-tocopherol, sterols, mineral elements and sugars as independent variables. The PCA score plot was used to differentiate the six almond varieties.

Author Contributions

Isabel Lopéz-Cortés: performed the conceptualization, methodology, validation, formal analysis, investigation, writing – review and editing, and funding acquisition. **Domingo M. Salazar**: performed the conceptualization, methodology, validation, formal analysis, investigation, writing – review and editing, and funding acquisition. **Luana Fernandes**: performed the writing – original draft preparation. **Amparo Baviera-Puig**: performed the validation and formal analysis. **Nuno Rodrigues**: performed the methodology, validation, formal analysis, investigation and writing – review and editing. **José Alberto Pereira**: performed the methodology, validation, formal analysis, investigation and writing – review and editing. **Elsa Ramalhosa**: performed the formal analysis, investigation, writing – review and editing. All authors have read and agreed to the published version of the manuscript.

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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.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section.

Supporting File 1: cbdv70289-sup-0001-SuppMat.docx