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Exploring the bioresource potential of beech nuts (*Fagus sylvatica* L.) from different regions of Spain: geographical origin impact on composition and antioxidant activity

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ABSTRACT

Beech nuts (*Fagus sylvatica* L.) are an underutilized resource with promising nutritional and functional potential. This study analysed physicochemical characteristics of nuts collected from three Spanish regions (Burgos, León, and Palencia) and applied chemometric tools to identify geographical biomarkers. All samples displayed antioxidant activity, with the highest levels observed in samples from Palencia. Although volatile compound profiling showed region-specific patterns dominated by aldehydes, the mean sensitivity achieved for repeated K-fold cross-validation (88%) led to several misclassifications, limiting its applicability as a non-invasive approach for geographical origin discrimination. The extracted oils (17–22% dw) were rich in unsaturated fatty acids, mainly oleic acid (35.2–39.3%) and linoleic acid (36.0–38.0%), with significant regional variation. Moisture and specific fatty acids ($C_{16:1}$, $C_{18:3}$, $C_{20:2}$, $C_{22:0}$, $C_{22:1}$) were identified as reliable markers of geographical origin, allowing a very satisfactory predictive performance (98% for repeated K-fold-CV). Beech nut oils were also rich in tocopherols (441–551 mg/kg), with γ-tocopherol as the predominant form. The identification of region-specific differences in composition and antioxidant capacity offers clear industrial advantages by enabling the targeted selection of raw materials with tailored nutritional, functional, and quality attributes, while also supporting traceability. Overall, the findings highlight beech nuts as a promising bioresource with potential applications as a functional ingredient in different industries.

1. Introduction

Nuts are widely recognized for their nutritional value and health-related benefits, as they are rich sources of lipids, proteins, dietary fiber, vitamins, minerals, and bioactive compounds, including phenolic compounds and tocopherols [1]. Regular consumption of nuts has been associated with improved cardiovascular health, reduced risk of type 2 diabetes, and anti-inflammatory effects [1,2]. Consequently, commonly consumed nuts such as walnuts, almonds, hazelnuts, and chestnuts, have been extensively studied, and their chemical composition and functional properties are well characterized. Walnuts, for example, are particularly rich in polyunsaturated fatty acids (52-70%), proteins (12–24%, especially in essential amino acids), and minerals (1.5–2.0%). They also contain a diverse range of flavonoids, polyphenols, and phenolic acids [2], while chestnuts, are distinguished by their high carbohydrate content and substantial dietary fiber levels while containing relatively low amounts of crude protein and, in contrast to conventional nuts (e.g., walnuts, almonds, and hazelnuts), minimal crude fat. Additionally, chestnuts are a valuable source of essential macronutrients (e.g., potassium, phosphorus, magnesium, calcium, and sodium) and micronutrients (e.g., manganese, iron, zinc, and copper) as well as some vitamins [3]. However, less common nuts, like beech nuts (*Fagus sylvatica* L.) have received less attention. Beech nuts are derived from the European beech, a more abundant and dominant tree species found throughout central and southern Europe [4]. In northwestern Spain, this species occupies approximately 40% of its total national area [5]. These trees can reach 30 to 45 m in height and produce beech nuts measuring between 12 and 18 mm in size [6]. Beech nuts have been consumed since

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ancient times by humans and animals in small quantities, as they contain low levels of trimethylamine, a toxic compound that is eliminated through roasting, allowing its subsequent use in confectionery or as a coffee substitute [6,7]. Traditionally, beech nuts have been primarily exploited for oil extraction, with the oil's chemical composition varying significantly depending on its geographical origin [8]. Similarly, Siger et al. [9] characterized the oil extracted from beech nut seeds, highlighting its high content of linoleic and oleic acid, while Kaliniewicz et al. [7] reported that beech nuts contained between 30 and 36% of fat, 25% of protein, saponins, malic acid, citric acid, vanillic acid, 6% of minerals, as well as sugars and starch. Beech nuts also provide significant amounts of essential minerals such as calcium, copper, iron, manganese, potassium, sodium, and zinc [10]. Their overall composition makes them highly comparable to conventional dried fruits in terms of nutritional quality.

Although beech nuts have a promising nutrient profile, their commercial use remains limited due to their seasonal availability and relatively small size compared to more widely consumed nuts. Despite these promising attributes, the available scientific literature on beech nuts remains limited and fragmented. Existing studies have largely addressed isolated aspects, such as lipid composition or selected physicochemical parameters, and have frequently relied on samples originating from a single geographical area [6,8,9]. Consequently, comprehensive studies integrating proximate composition, antioxidant activity, volatile profiles, fatty acid composition, and tocopherol content are still scarce. Moreover, data on the influence of geographical origin on the chemical composition and functional properties of beech nuts are particularly limited. Geographical origin is well known to affect the chemical profile of plant-derived products through interactions between environmental factors, including climate and soil conditions, and genetic variability. In recent years, chemometric and multivariate statistical approaches have been increasingly employed to identify chemical markers linked to geographical origin, thereby supporting traceability, authentication, and quality differentiation of agricultural and food products [11]. While these approaches have been successfully applied to various plant-based matrices, their use in beech nut studies remains largely unexplored, especially regarding the identification of robust physicochemical markers capable of discriminating samples according to origin.

Therefore, the present study aimed to conduct an integrated physicochemical characterization of beech nuts (*Fagus sylvatica* L.) harvested from three regions in northern Spain (Burgos, León, and Palencia) to evaluate the impact of geographical origin on their chemical composition and antioxidant properties. Proximate composition, antioxidant activity, volatile compounds, fatty acid profiles, and tocopherol composition were analysed using complementary analytical techniques, and chemometric tools were applied to identify chemical markers associated with geographical origin. This integrated approach provides new insights into the chemical variability, traceability potential, and valorization of beech nuts as an underutilized bioresource with promising applications in the food, cosmetic, and nutraceutical sectors.

2. Materials and methods

2.1. Sampling

European beech nuts (*Fagus sylvatica* L.) were collected from three distinct regions in northern Spain, namely Burgos, Palencia, and Léon (Fig. 1). Within the Burgos region, six collection sites were identified (Villamediana de San Román, Puras de Villafranca, Fresneda de La Sierra, Bezana, Valmala, and Pradoluengo). In the Palencia region, seven sites were selected (Polentinos, Piedrasluengas, Valcobero, Velilla del Río Carrión, Ventanilla, Camasobres, and Rebanal de Las Llantas). From Léon, four sites were included (Valdecastillo, Ciñera, Olleros de Sabero, and Cabornera). Approximately 150 fully mature whole fruits of beech nut seeds were hand-collected from each site during October 2022. The samples were subsequently transported to the laboratory for further analysis.

2.2. Sample preparation

The whole fruit of beech nut seed samples from three different regions of Spain were frozen at −21 °C and subsequently freeze-dried (CoolSafe Basic, 110 °C, 4L, 230V/50Hz, LaboGene, Denmark). The samples were then ground using a ZM200 ultra-centrifugal mill for 15 s to obtain flour. The flours obtained were placed in amber containers until further analysis. Each independent flour sample was subjected to different analytical procedures, with each parameter measured in duplicate.

2.3. Fruits content

2.3.1. Moisture content

The moisture content, given as a percentage, was measured in fresh beech nut seed samples by drying a ≈5 g portion of the whole fruit of beech nut seed samples in an oven at 105 °C (BINDER, Model ED 400, Tuttlingen, Germany) until a constant weight was achieved, following the method established by the Association of Official Analytical Chemists [12]. All measurements were performed in duplicate.

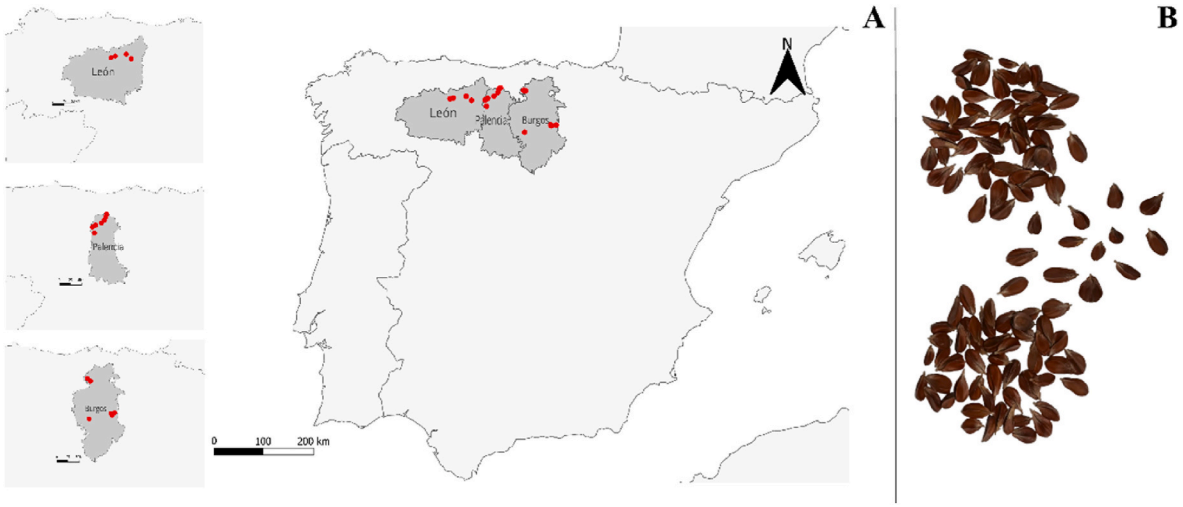

Fig. 1. – (**A**) Location of the three harvesting regions in northern Spain: Burgos, Palencia, and Léon; (**B**) European beech nut seeds (*Fagus sylvatica* L.).

2.3.2. Fat contents

The fat content, expressed as a percentage, was determined following the Association of Official Analytical Chemists [13] method. A known weight (≈5 g) of the whole fruit of beech nut seed samples was extracted using petroleum ether (40-60 °C) containing 0.01% BHT (di-tert-butyl methyl phenol). The extraction was carried out for 8 to 10 h using a Soxhlet apparatus (PSelecta®). Afterward, the solvent was evaporated and recovered using a rotary evaporator (Stuart®, RE300DB, Stone, UK) at 35 °C. The samples were then placed in an oven at 50 °C before being transferred to a desiccator. This process was repeated until a constant weight was achieved. The extracted fat was refrigerated for further analysis, as detailed below. All measurements were performed in duplicate.

2.3.3. Total phenols content and antioxidant activity

2.3.3.1. Extract preparation. The total phenol content and antioxidant activity were assessed in 100% methanolic extracts prepared from the freeze-dried beech nut samples (1.5 g). The mixture was left on a stirring plate (Labos, RS LAB 2C, Barcelona) at 500 rpm for 1 h with 50 mL of methanol in the dark. After filtration (Whatman paper n° 4), the extraction was repeated twice under similar conditions. At the end of the extraction process, the methanol was evaporated on a rotary evaporator (Stuart®, RE300DB, Stone, UK) at 35 °C. The total extract mass was recorded, and a subsample was made with methanol with a concentration of 0.25 mg/mL and stored in dark flasks at refrigerated temperature until further analysis.

2.3.3.2. Total phenols content. The total phenolic content (TPC) of the sample was determined from the combined extract. Following the methodology by Singleton et al. [14] as adapted by Rodrigues et al. [15] with some modifications: 1 mL of the 0.25 mg/mL methanolic extract solution was mixed with 1 mL of Folin–Ciocalteu reagent (Aldrich Chemistry), vortexed, and left to react for 3 min. Subsequently, 1 mL of saturated sodium carbonate solution (Na_2CO_3) and 7.5 mL of deionized water was vortexed and allowed to react for 90 min in the dark at room temperature. This procedure was performed in duplicate for each extract. The samples were then analysed using a UV-VIS spectrophotometer (UV-1280, Shimadzu, Kyoto, Japan) at 725 nm. TPC was calculated based on a calibration curve generated from the absorbance of standard methanolic solutions of gallic acid ($R^2 \geq 0.9999$). Results were expressed as milligrams of gallic acid equivalents per gram of extract, converted to sample based on the total extract mass (mg GAE/g).

2.3.3.3. Antioxidant activity

2.3.3.3.1. Radical scavenging activity. The radical scavenging activity of beech nuts extracts was evaluated using the DPPH• (2,2-diphenyl-1-picrylhydrazyl) method. In this method, as described by Hatano et al. [16] and adapted by Rodrigues et al. [15], with some modifications, 0.3 mL of the 0.25 mg/mL methanolic extract solution (0.3 mL of methanol was used for the blank) was combined with 2.7 mL of a methanolic DPPH• solution (0.06 mM), vortexed, and it was placed for 1 h in the dark at room temperature. After that, the absorbance was read at 517 nm using a UV-VIS spectrophotometer (UV-1280 Shimadzu, Kyoto, Japan). The radical scavenging activity was expressed as the percentage reduction of DPPH• radical activity.

2.3.3.3.2. Radical scavenging effect. The total antioxidant capacity of beech nuts was assessed using the ABTS•$^+$ (2,2′-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid) radical scavenging method, following the methodology described by Sánchez et al. [17]. The ABTS•$^+$ solution was prepared and adjusted to an absorbance of 0.700 ± 0.020 at 734 nm. For each analysis, 2 mL of the ABTS•$^+$ solution was mixed with 100 μL of the sample, methanolic extract solution (0.25 mg/mL). After being vortexed, the reaction was allowed to proceed for 6 min in the dark at room temperature, and the absorbance was measured using a UV-VIS spectrophotometer (UV-1280, Shimadzu, Kyoto, Japan). The ABTS•$^+$ scavenging activity was expressed as the percentage reduction of ABTS•$^+$ radical activity.

2.3.3.3.3. Reducing power capacity. The reducing power capacity of the extracts was evaluated using the method described by Berker et al. [18]. Briefly, 1 mL of the 0.25 mg/mL methanolic extract solution was mixed with 2.5 mL of 0.2 M sodium phosphate buffer solution (pH 6.6) and 2.5 mL of 1% potassium ferrocyanide ($K_3Fe(CN)_6$). The mixture was vortexed and incubated at 50 °C for 20 min in a water bath. After cooling, 2.5 mL of 10% (w/v) trichloroacetic acid was added, stirring the solution vigorously. Subsequently, 2.5 mL of the mixture supernatant was removed, 2.5 mL of distilled water added, and 0.5 mL of 0.1% iron (III) chloride. After 2 min of reaction, the absorbance of the mixture was measured at 700 nm using a UV-VIS spectrophotometer (UV-1280, Shimadzu, Kyoto, Japan). The reducing power was expressed as milligrams of trolox equivalents per gram of extract, converted to sample on the basis of the total extract mass (mgTrolox/g_{sample}).

2.3.4. Volatile compounds

The volatile compound profiles were analysed by headspace solid-phase microextraction gas chromatography coupled to mass spectrometry (HS-SPME-GC/MS) following the Malheiro et al. [19] method with some modifications as follows. Briefly, around 3.0 g weight of whole fruit of beech nut seeds sample was placed in 50 mL vials containing 5 μL of caryophyllene (0.227 μg/mL dissolved in methanol), which was used as an internal standard. The vials were heated in a water bath at 40 °C with shaking for 5 min to release volatile compounds. The SPME fiber (divinylbenzene/carbonex/polydimethylsiloxane (DVB/CAR/PDMS), 50/30 μm, Supelco) was then exposed to the headspace for 30 min under the same temperature and agitation conditions to adsorb volatile compounds. The adsorbed compounds were thermally desorbed at 220 °C in the gas chromatograph injection port for 1 min, with the fiber conditioned for an additional 10 min. The analysis was conducted on a Shimadzu GC-2010 Plus gas chromatograph equipped with a Shimadzu GC/MS-QP2010 SE mass spectrometer and a TRB-5MS column (30 m × 0.25 mm × 0.25 μm, Teknokroma). Helium was used as the carrier gas at a linear velocity of 30 cm/s and a 24.4 mL/min flow rate. The injector temperature was 220 °C, operating in splitless mode. The oven temperature program was set to 40 °C for 1 min, then increased by 2 °C/min until reaching 220 °C. Mass spectra were acquired via electron ionization (70 eV) in the *m/z* range of 35–500, with an ionization source temperature of 250 °C. Volatile compounds were identified based on their mass spectra and retention indices using NIST, PubChem, and ChemSpider databases. Retention indices were determined using a commercial n-alkane series (C7–C30, Sigma-Aldrich). The quantification of volatile compounds was semi-quantitative, calculated using the internal standard method by integrating total ion chromatogram (TIC) peak areas and expressing the results in ng per g of beech nut seeds of internal standard equivalents. All analyses were performed in duplicate.

2.4. Oil extracted

2.4.1. Fatty acids composition

The fatty acid composition of the extracted fat was determined by gas chromatography after conversion to fatty acid methyl esters via cold alkaline transesterification using a methanolic potassium hydroxide solution, following the EU standard protocols [20]. The analysis was determined with a Chrompack CP 9001 chromatograph equipped with a split-splitless injector, an FID detector, a Chrompack CP-9050 autosampler, and a 50 m × 0.25 mm i.d. fused silica capillary column (50 m × 0.25 mm i.d. × 0.25 μm; SelectFAME, Agilent, Santa Clara, CA, USA). Helium was the carrier gas at an internal pressure of 140 kPa. The injector and detector temperatures were set at 250–270 °C, with a 1:50 split ratio and 1 μL injection volume. An optimized temperature gradient ensured the complete separation of fatty acids, quantified as relative

percentages by internal normalization of the peak areas eluting between myristic and lignoceric methyl esters A certified fatty acid methyl ester standard mixture (Supelco 37 Component FAME Mix) was used for identification and FID calibration (Sigma).

2.4.2. Tocopherols composition

Tocopherols were determined using high-performance liquid chromatography (HPLC), following the ISO 9936 method [21], with some modifications [15]. Standards for α-, γ-, and δ-tocopherols were obtained from Sigma, while 2-methyl-2-(4,8,12-trimethyltridecyl) chroman-6-ol (tocol) was used as the internal standard (Matreya Inc., Pleasant Gap, PA, USA). For analysis, 50 mg of extracted lipids were mixed with 10 μL of tocol internal standard solution (100 μg/mL in n-hexane), diluted in hexane, and centrifuged for 5 min at 13000 rpm. The resulting supernatant was analysed using a Jasco HPLC system (Tokyo, Japan) equipped with an LC-NetII/ADC data unit, a PU-1580 Intelligent Pump, and an FP-920 fluorescence detector ($\lambda_{excitation}$ = 290 nm, $\lambda_{emission}$ = 330 nm). Chromatographic separation was performed on a Luna Silica column (3 μm, 100 × 3.0 mm; Phenomenex, USA) with a constant temperature of 23 °C. The mobile phase consisted of n-hexane and 1,4-dioxane (97:35) at a 1.0 mL/min flow rate. Data analysis was conducted using the ChromNAV Control Center software (Jasco, Japan). Tocopherols were identified based on standards, co-elution retention times, and UV spectra. Quantification was achieved using the internal standard method with fluorescence signal responses and calibration curves for individual tocopherols. Total tocopherol content was calculated as the sum of all identified tocopherols and expressed as mg/kg of extracted fat.

2.5. Statistical analysis

One-way ANOVA was applied to assess the effect of geographical origin in the analyses carried out on the whole fruit of beech nut seeds (moisture, fat, volatile compound profile, total phenolics, antioxidant activity) and the oil from the whole fruit of beech nut seeds (fatty acids, tocopherols). Tukey's post-hoc test was used when significant differences were found. For pairwise comparisons between two regions, the t-Student test was applied. Linear Discriminant Analysis (LDA) combined with the Simulated Annealing (SA) variable selection algorithm was used as a multivariate supervised classification tool to evaluate the possibility of using physicochemical data for geographic origin discrimination. The predictive performance of the selected LDA-SA models was evaluated by leave-one-out cross-validation (LOO-CV) and repeated K-fold cross-validation (4 folds × 10 replicates). Classification accuracy was assessed by the percentage of correct predictions and visualised using class membership ellipses for the first two discriminant functions (DFs) calculated based on Bayes' theorem [11]. Additionally, variable importance evaluation was carried out using model-based information, allowing to incorporate the correlation structure between the predictors into the importance calculation. The predictor variable importance for each class was computed being all measures of importance scaled to have a maximum value of 100. Data analysis was performed using R statistical software (version 3.6.2) and appropriate libraries.

3. Results and discussion

3.1. Fruits content

3.1.1. Moisture and fat contents

The moisture content of whole fruit of beech nut seeds sample was analysed in three regions (Burgos, Léon, and Palencia). The results showed no statistically significant differences among the regions (Table 1), with Burgos presenting the highest value (37 ± 4%), followed by Palencia (34 ± 5%) and Léon (33 ± 6%). Concerning fat content, the results revealed no statistically significant differences among the regions (Table 1). However, Léon exhibited the highest fat content (22 ± 3%), followed by Palencia (19 ± 8%) and Burgos (17 ± 7%). Values of moisture are generally consistent across the studied regions, Obranović et al. [8] reported a moisture content of 25% for beech nuts collected in Croatia, while Elisovetcaia et al. [22] found moisture levels of 21% and 20% in samples from Romania and the Republic of Moldova, respectively. Concerning fat content Siger et al. [9] reported a higher fat content of 27% for beech nuts from Poland, whereas Obranović et al. [8] documented a significantly lower fat content of 13% in beech nuts from the northwest region of Croatia. The observed differences between regions' results may be influenced by crop year and environmental factors, including climate conditions, agroecological practices, and post-harvest handling methods.

Table 1
Moisture contents (%), fat contents (%), total phenols content (mg GAE/g), and antioxidant activity (DPPH assay, ABTS assay, and reducing power) of whole fruit of beech nut seeds from different regions (mean ± standard deviation).

Parameters	Whole fruit of beech nut seeds			
	Burgos	Léon	Palencia	P-value (one-way ANOVA)
Moisture (%)	37 ± 4[a]	33 ± 6[a]	34 ± 5[a]	0.405
Fat (%)	17 ± 7[a]	22 ± 3[a]	19 ± 8[a]	0.525
Total phenols content and antioxidant activity				
TPC (mg GAE/g)	45 ± 13[a]	45 ± 13[a]	54 ± 18[a]	0.538
DPPH assay (inhibition%)	47 ± 11[a]	66 ± 24[ab]	72 ± 15[b]	0.043
ABTS assay (inhibition%)	50 ± 12[a]	66 ± 23[ab]	75 ± 17[b]	0.056
Reducing power (mg Trolox/g)	84 ± 17[a]	135 ± 60[a]	133 ± 35[a]	0.061

*Different lowercase letters in the same row mean a statistically significant effect (P-value <0.05) of the geographical origin on the evaluated parameter (one-way ANOVA followed by the Tukey's test).

3.1.2. Total phenols content and antioxidant activity

3.1.2.1. Total phenols content. The TPC did not show any statistically significant differences across the studied regions (Burgos, Léon, and Palencia), as shown in Table 1. Palencia had the highest average TPC value (54 ± 18 mg GAE/g), followed by Léon and Burgos (45 ± 13 mg GAE/g). Given the limited number of studies addressing the phenolic content of beech nuts, comparison with other nuts, such as walnuts, was performed allowing a possible contextual insight. Walnut kernels have been reported to present total phenolic contents ranging approximately between 15 and 30 mg GAE/g, depending on cultivar and extraction conditions [23], which are lower than the values observed in the present study for beech nuts. In addition, studies on other tissues of the beech tree have reported TPC values of 36 ± 3 mg/g for bark [24] and 70 ± 3 mg GAE/g for leaves [25]. These findings indicate that the TPC of beech nuts is higher than that of beech bark but lower than that of beech leaves. Such differences are likely related to the distinct physiological roles of phenolic compounds in these tissues, with leaves accumulating higher levels due to their direct exposure to environmental stressors, while bark primarily fulfills structural and protective functions. Collectively, these results highlight the antioxidant potential of beech nuts, supporting their relevance as a meaningful source of phenolic compounds when compared with other nuts and different parts of the beech tree. The regional differences observed may reflect the combined influence of environmental conditions, including climate and soil characteristics, which are known to modulate metabolic pathways involved in lipid biosynthesis and the accumulation of antioxidant compounds in plant seeds.

3.1.2.2. Antioxidant activity. The antioxidant activity of beech nut seeds, evaluated using the DPPH assay, exhibited clear regional

differences (Table 1), indicating that geographical origin influences their antioxidant potential. Samples from Palencia showed the highest radical scavenging activity (72 ± 15%), followed by León (66 ± 24%) and Burgos (47 ± 11%). The comparatively lower activity observed in Burgos may reflect differences in TPC or variations in the profile of other bioactive compounds, such as tocopherols. Although the differences in TPC were not statistically significant, the trend corresponds with the DPPH assay results, suggesting that Palencia and León seeds may possess a stronger ability to neutralize free radicals, potentially due to environmental or genetic factors that promote antioxidant accumulation.

Similarly, the ABTS assay revealed apparent regional variation, with Palencia again showing the highest inhibition (75 ± 17%), followed by León (66 ± 23%) and Burgos (50 ± 12%) (Table 1). This pattern is a copy of the DPPH results and reinforces the notion that seeds from Palencia may experience environmental conditions that may promote the synthesis of antioxidant compounds, enhancing their radical-scavenging capacity. The concordance between TPC, DPPH assay, and ABTS assay results supports the impact of geographical origin on the bioactive profile of beech nut seeds.

The reducing power, expressed as mg Trolox equivalents per gram of sample, also varied among regions, although differences were not statistically significant. León exhibited the highest reducing power (135 ± 60 mg Trolox/g), closely followed by Palencia (133 ± 35 mg Trolox/g), while Burgos showed the lowest value (84 ± 17 mg Trolox/g). This trend aligns with other antioxidant measures, indicating that seeds from León and Palencia generally possess higher antioxidant potential. The lower reducing power in Burgos may again be associated with regional differences in phenolic composition, tocopherol content, or other

Table 2
Volatile profile of whole fruit of beech nut seeds (ng/g of whole fruit of beech nut seeds) from different regions of Spain, namely Burgos, Léon, and Palencia (mean ± standard deviation).

Compounds	Whole fruit of beech nut seeds						
	Ret. time (min.)	LRI	LRI Lit	Burgos (ng/g)	Léon (ng/g)	Palencia (ng/g)	P-value (one-way ANOVA)
Aldehydes							
3-methyl-butanal	4.74	751	665	n.d.	3.20 ± 3.60^{a}	1.00 ± 3.08^{a}	0.486
Hexanal	7.88	869	822	19.02 ± 20.60^{a}	21.65 ± 22.12^{a}	9.95 ± 17.96^{a}	0.343
Heptanal	12.95	955	904	0.79 ± 0.83^{a}	0.67 ± 0.68^{a}	0.38 ± 0.67^{a}	0.363
(Z)-2-heptenal	16.32	1001	936	0.17 ± 0.24^{a}	0.12 ± 0.11^{a}	0.05 ± 0.09^{a}	0.181
Benzaldehyde	16.51	1003	1003	0.03 ± 0.03^{b}	0.09 ± 0.09^{a}	0.02 ± 0.02^{b}	0.002
Octanal	19.48	1051	1036	1.15 ± 1.53^{a}	1.01 ± 0.98^{a}	0.41 ± 0.87^{a}	0.252
(E)-2-octenal	23.28	1100	1047	0.06 ± 0.08^{a}	0.11 ± 0.17^{a}	0.07 ± 0.12^{a}	0.624
Nonanal	26.62	1151	1130	0.72 ± 0.99^{a}	0.63 ± 0.62^{a}	0.28 ± 0.42^{a}	0.266
Decanal	33.83	1253	1209	0.06 ± 0.10^{a}	0.03 ± 0.02^{a}	0.01 ± 0.01^{a}	0.208
(E)-2-decenal	37.68	1306	1270	0.01 ± 0.01^{a}	n.d.	0.01 ± 0.02^{a}	0.907
ΣAldehydes				22.00 ± 24.42^{a}	27.51 ± 28.39^{a}	12.17 ± 23.28^{a}	0.818
Alcohols							
2-methyl-1-butanol	5.80	818	765	0.95 ± 2.22^{a}	0.31 ± 0.31^{a}	0.11 ± 0.15^{a}	0.276
1-pentanol	6.71	833	801	2.80 ± 3.14^{a}	3.46 ± 3.53^{a}	1.59 ± 2.79^{a}	0.367
1-hexanol	11.19	919	896	2.79 ± 4.18^{a}	2.38 ± 2.37^{a}	1.02 ± 1.73^{a}	0.295
1-heptanol	17.33	1013	980	0.34 ± 0.41^{a}	0.39 ± 0.37^{a}	0.15 ± 0.23^{a}	0.211
1-octen-3-ol	17.94	1020	998	0.21 ± 0.19^{a}	0.33 ± 0.32^{a}	0.15 ± 0.30^{a}	0.343
1-octanol	24.30	1112	1082	0.23 ± 0.48^{a}	0.15 ± 0.15^{a}	0.05 ± 0.08^{a}	0.320
Octanoic acid	29.93	1195	1190	0.03 ± 0.11^{a}	0.02 ± 0.04^{a}	n.d.	0.599
ΣAlcohols				7.35 ± 10.72^{a}	7.04 ± 7.09^{a}	3.07 ± 5.28^{a}	0.530
Alkanes							
Dodecane	26.37	1147	1200	0.01 ± 0.02^{a}	0.01 ± 0.02^{a}	0.00 ± 0.00^{a}	0.258
Undecane	26.42	1148	1115	0.02 ± 0.02^{a}	n.d.	0.00 ± 0.01^{a}	0.126
Tetradecane	46.93	1453	1413	0.01 ± 0.03^{a}	n.d.	0.00 ± 0.00^{a}	0.274
ΣAlkanes				0.04 ± 0.07^{a}	0.01 ± 0.02^{ab}	0.01 ± 0.01^{b}	0.031
Carboxylic acids							
Hexanoic acid	18.54	1026	1013	0.52 ± 0.83^{a}	1.24 ± 1.49^{a}	0.44 ± 0.86^{a}	0.202
ΣCarboxylic acids				0.52 ± 0.83	1.24 ± 1.49	0.44 ± 0.86	0.202
Esters							
Methyl hexanoate	14.42	977	921	0.31 ± 0.33^{a}	0.39 ± 0.41^{a}	0.24 ± 0.48^{a}	0.715
Methyl octanoate	28.15	1172	1130	0.04 ± 0.05^{a}	n.d.	0.01 ± 0.04^{a}	0.307
ΣEsters				0.35 ± 0.37^{a}	0.39 ± 0.41^{a}	0.26 ± 0.52^{a}	0.946
Furans							
2-pentyl-furan	18.74	1029	1040	0.06 ± 0.14^{a}	0.27 ± 0.32^{a}	0.24 ± 0.52^{a}	0.386
5-ethyldihydro-2(3H)-furanone	22.87	1095	1081	0.04 ± 0.05^{a}	0.10 ± 0.13^{a}	0.04 ± 0.11^{a}	0.308
ΣFurans				0.10 ± 0.20^{a}	0.37 ± 0.45^{a}	0.28 ± 0.63^{a}	0.521
Ketones							
2-heptanone	11.67	925	892	0.74 ± 0.62^{a}	0.00 ± 0.00^{b}	0.37 ± 0.77^{ab}	0.044
2-octanone	12.34	933	947	n.d.	0.99 ± 1.12	n.d.	——
ΣKetones				0.74 ± 0.62^{a}	0.99 ± 1.12^{a}	0.37 ± 0.77^{a}	0.847
Terpenes							
3-octen-2-one	21.97	1084	1035	0.14 ± 0.18^{a}	0.12 ± 0.12^{a}	0.03 ± 0.07^{a}	0.080
L-menthol	31.50	1214	1165	0.03 ± 0.10	n.d.	n.d.	——
α-humulene	50.30	1505	1482	0.06 ± 0.03^{a}	0.07 ± 0.02^{a}	0.06 ± 0.02^{a}	0.292
ΣTerpenes				0.23 ± 0.30^{a}	0.20 ± 0.14^{a}	0.09 ± 0.09^{a}	0.546

*Different lowercase letters in the same row mean a statistically significant effect (P-value <0.05) of the geographical origin on the concentration of the evaluated volatile compound. When the volatile compound was present in all three regions, a one-way ANOVA followed by Tukey's test was performed. When it was detected in only two regions, a Student's t-test was applied.
Ret. Time – Retention time.
LRI – Linear retention index obtained in a TRB-5MS column.
LRI Lit – Linear retention index reported in the literature.
n.d. – not detected.

antioxidant compounds influenced by environmental factors such as climate, soil nutrients, or tree genetics. Overall, the consistency across TPC, DPPH assay, ABTS assay, and reducing power analyses underscores the impact of geographical origin on the functional properties of beech nut seeds, highlighting their potential as a natural source of antioxidants.

3.1.3. Volatile compounds

The volatile profiles of whole beech nut seeds from Burgos, León, and Palencia are presented in Table 2. Eight major classes of volatile compounds were identified: aldehydes (10 compounds), alcohols (7 compounds), alkanes (3 compounds), carboxylic acids (1 compound), esters (2 compounds), furans (2 compounds), ketones (2 compounds), and terpenes (3 compounds). Aldehydes exhibited the most pronounced regional differences. For instance, 3-methyl-butanal was not detected in samples from Burgos, whereas higher concentrations were observed in León (3.20 ± 3.60 ng/g) and intermediate levels in Palencia (1.00 ± 3.08 ng/g), although these differences were not statistically significant. This compound has previously been reported in chestnuts [26] and is associated with malty, roasty, and cucumber-like aroma notes. In contrast, benzaldehyde showed significant regional variation, with higher concentrations in León (0.09 ± 0.09 ng/g) compared to Burgos (0.03 ± 0.03 ng/g) and Palencia (0.02 ± 0.02 ng/g). Benzaldehyde has been identified in chestnuts and is linked to floral and green aromas [26], as well as fruity odor notes [27]. Additionally, (E)-2-decenal was not detected in samples from León, while the remaining aldehydes did not show statistically significant regional differences. A similar trend was observed for alcohols, which did not differ significantly among regions. Nevertheless, 1-pentanol and 1-hexanol were the most abundant alcohols detected, consistent with previous reports in chestnuts [26]. Specifically, 1-pentanol is associated with sweet and vanilla-like aroma notes [28,29], whereas 1-hexanol contributes to herbaceous and woody olfactory characteristics [27–29]. Octanoic acid, belonging to this group, was not detected in samples from Palencia. Within the alkane group, only minor regional variation was observed; however, undecane and tetradecane were not detected in samples from León. Hexanoic acid was the sole carboxylic acid identified and did not exhibit statistically significant differences among regions, although higher mean concentrations were observed in León (1.24 ± 1.49 ng/g) compared to Burgos and Palencia. Two esters, methyl hexanoate and methyl octanoate, were detected without significant regional differences; however, methyl octanoate was absent in samples from León. This compound has been associated with fruity, green, and citrus aroma notes in chestnuts [27–29]. Furans, including 2-pentyl-furan and 5-ethyldihydro-2 (3H)-furanone, were detected at low concentrations and did not differ significantly among regions, although slightly higher levels were observed in León. Regarding ketones, 2-heptanone was detected in Burgos (0.74 ± 0.62 ng/g) and Palencia (0.37 ± 0.77 ng/g) but was absent in León, whereas 2-octanone was exclusively detected in León (0.99 ± 1.12 ng/g). The terpene profile showed modest regional variation, with 3-octen-2-one and α-humulene being the most abundant compounds. Notably, L-menthol was detected only in samples from Burgos.

Overall, the large standard deviations observed for certain volatile compounds indicate substantial variability in their measured concentrations across independent samples, likely reflecting natural sample heterogeneity and the low abundance of these compounds.

3.2. Oil extracted

3.2.1. Fatty acids composition

The fatty acid composition of beech nut oils extracted from seeds collected in three distinct regions of Spain was analysed. The relative abundances of individual fatty acids and the total content of saturated (SFA), monounsaturated (MUFA), and polyunsaturated (PUFA) fatty acids are summarized in Table 3. Significant differences were observed among regions, suggesting that geographical and probably environmental factors influence the lipid profile. Nevertheless, oleic acid ($C_{18:1}$) and linoleic acid ($C_{18:2}$) were the predominant fatty acids across all regions.

Table 3
Fatty acid profile (%) and tocopherols contents (mg/kg_{oil}) of beech nut seeds oil from different regions (mean ± standard deviation).

Parameters	Beech nut oils			
	Burgos	Léon	Palencia	P-value (one-way ANOVA)
Fatty Acid Profile (%)				
Myristic acid ($C_{14:0}$)	0.1 ± 0.0^{a}	0.1 ± 0.0^{a}	0.1 ± 0.0^{a}	0.488
Palmitic acid ($C_{16:0}$)	7.6 ± 0.3^{a}	7.8 ± 0.6^{a}	7.4 ± 0.2^{a}	0.283
Palmitoleic acid ($C_{16:1}$)	0.3 ± 0.0^{a}	0.3 ± 0.0^{a}	0.2 ± 0.0^{a}	0.055
Stearic acid ($C_{18:0}$)	3.2 ± 0.5^{a}	2.5 ± 0.1^{b}	2.8 ± 0.2^{ab}	0.015
Oleic acid ($C_{18:1}$)	39.3 ± 2.9^{a}	35.2 ± 1.6^{b}	36.8 ± 1.2^{ab}	0.022
Linoleic acid ($C_{18:2}$)	36.0 ± 1.7^{a}	38.5 ± 0.8^{b}	38.0 ± 0.8^{b}	0.012
Linolenic acid ($C_{18:3}$)	3.1 ± 0.6^{a}	4.0 ± 0.7^{a}	3.6 ± 0.4^{a}	0.078
Arachidic acid ($C_{20:0}$)	0.6 ± 0.1^{a}	0.5 ± 0.0^{a}	0.5 ± 0.0^{a}	0.151
Eicosenoic acid ($C_{20:1}$)	7.2 ± 0.6^{a}	8.0 ± 0.5^{a}	7.6 ± 0.4^{a}	0.097
Eicosadienoic acid ($C_{20:2}$)	0.2 ± 0.1^{a}	0.3 ± 0.0^{b}	0.3 ± 0.0^{b}	0.009
Behenic acid ($C_{22:0}$)	0.8 ± 0.0^{a}	0.8 ± 0.1^{a}	0.7 ± 0.0^{a}	0.829
Erucic acid ($C_{22:1}$)	1.3 ± 0.2^{a}	1.6 ± 0.2^{a}	1.4 ± 0.2^{a}	0.171
Docosadienoic acid ($C_{22:2}$)	0.0 ± 0.0^{a}	0.1 ± 0.0^{b}	0.0 ± 0.0^{a}	0.004
Lignoceric acid ($C_{24:0}$)	0.2 ± 0.0^{a}	0.2 ± 0.0^{b}	0.2 ± 0.0^{a}	0.004
Lignoceroleic acid ($C_{24:1}$)	0.1 ± 0.0^{a}	0.1 ± 0.0^{a}	0.1 ± 0.0^{a}	0.699
ΣSFA	12.4 ± 0.7^{a}	12.0 ± 0.6^{a}	11.9 ± 0.2^{a}	0.160
ΣMUFA	48.1 ± 2.1^{a}	45.1 ± 1.5^{b}	46.1 ± 0.8^{ab}	0.022
ΣPUFA	39.3 ± 2.3^{a}	42.8 ± 1.4^{b}	41.9 ± 0.9^{b}	0.010
Tocopherols content (mg/kg_{oil})				
α – Tocopherol	8.5 ± 4.6^{ab}	10.1 ± 1.9^{a}	4.9 ± 1.2^{b}	0.028
γ – Tocopherol	328.6 ± 76.1^{a}	442.1 ± 66.1^{b}	346.2 ± 30.0^{a}	0.023
δ – Tocopherol	103.8 ± 18.8^{a}	99.0 ± 19.7^{a}	163.7 ± 38.0^{b}	0.002
Total tocopherols	441.0 ± 61.6^{a}	551.2 ± 50.7^{b}	514.7 ± 22.5^{b}	0.006

*Different lowercase letters in the same row mean a statistically significant effect (P-value <0.05) of the geographical origin on the content of the evaluated parameter (one-way ANOVA followed by the Tukey's test).

Oleic acid relative abundance was highest in Burgos (39 ± 3%), followed by Palencia (37 ± 1%) and León (35 ± 2%), indicating that oils from Burgos are the richest in MUFA. These results are generally consistent with previous reports with abundances varying from 32 to 39% [6,8,9]. In contrast, linoleic acid ($C_{18:2}$) was more abundant in León and Palencia (38 ± 1%) than in Burgos (36 ± 2%), reflecting a higher PUFA proportion in these regions. These values align closely with Siger et al. [9] (38%) and are slightly lower than those reported by Obranović et al. [8] (41%), whereas Prasad et al. [6] observed a substantially higher percentage (49%).

Overall, MUFA levels were significantly higher in Burgos (48 ± 2%) compared to Palencia (46 ± 1%) and León (45 ± 2%), exceeding the values reported in previous studies, namely by Obranović et al. [8] (43%) and Siger et al. [9] (40%). Conversely, PUFA abundance was greater in León (43 ± 1%) and Palencia (42 ± 1%) than in Burgos (39 ± 2%), although Obranović et al. [8] reported even higher PUFA (46%). The variations between regions and with literature values likely reflect a

combination of environmental influences—such as climate, soil composition, and local cultivation conditions—as well as genetic differences among beech nut populations. Methodological analytical differences across studies may also contribute to the observed discrepancies.

These findings indicate that geographical origin not only affects the relative abundance of individual fatty acids but also shapes the overall MUFA/PUFA balance, which may have implications for both nutritional quality and industrial applications of beech nut oils. In the present study, regional variations in fatty acid composition are of particular significance, as oils with higher MUFA contents are generally associated with enhanced oxidative stability, whereas oils richer in PUFA provide essential fatty acids and additional nutritional benefits. These differences underscore the relevance of geographical origin when considering specific industrial applications. Temperature regimes are known to influence fatty acid desaturation in oilseed crops, with cooler growing conditions typically favoring higher PUFA proportions. In this context, the higher relative abundance of linoleic and linolenic acids observed in samples from León and Palencia may be partially attributable to regional temperature patterns, although direct climatic data were not evaluated.

3.2.2. *Tocopherols composition*

The tocopherol composition of beech nut oils varied significantly among Burgos, León, and Palencia, reflecting regional differences in antioxidant profiles (Table 3). The content of γ-tocopherol, the predominant tocopherol across all regions, was highest in León (442 ± 66 mg/kg), followed by Palencia (346 ± 30 mg/kg) and Burgos (329 ± 76 mg/kg), consistent with previous reports for this beech nut variety [8,9].

In contrast, δ-tocopherol concentrations were greatest in Palencia (164 ± 38 mg/kg), substantially exceeding levels in Burgos (104 ± 19 mg/kg) and León (99 ± 20 mg/kg). This high δ-tocopherol content may confer distinctive oxidative stability and functional properties to oils from Palencia. Regarding α-tocopherol, León exhibited the highest concentration (10.1 ± 1.9 mg/kg), followed by Burgos (9 ± 5 mg/kg) and Palencia (5 ± 1 mg/kg). In which concerns β-tocopherol, it was not detected in any of the samples, in agreement with observations reported by Siger et al. [9].

Overall, these results highlight pronounced regional differences in tocopherol composition, suggesting that both environmental conditions and genetic variability contribute to the antioxidant profile of beech nut oils. Consequently, oils from different regions may offer distinct nutritional and functional benefits, which could be leveraged in food,

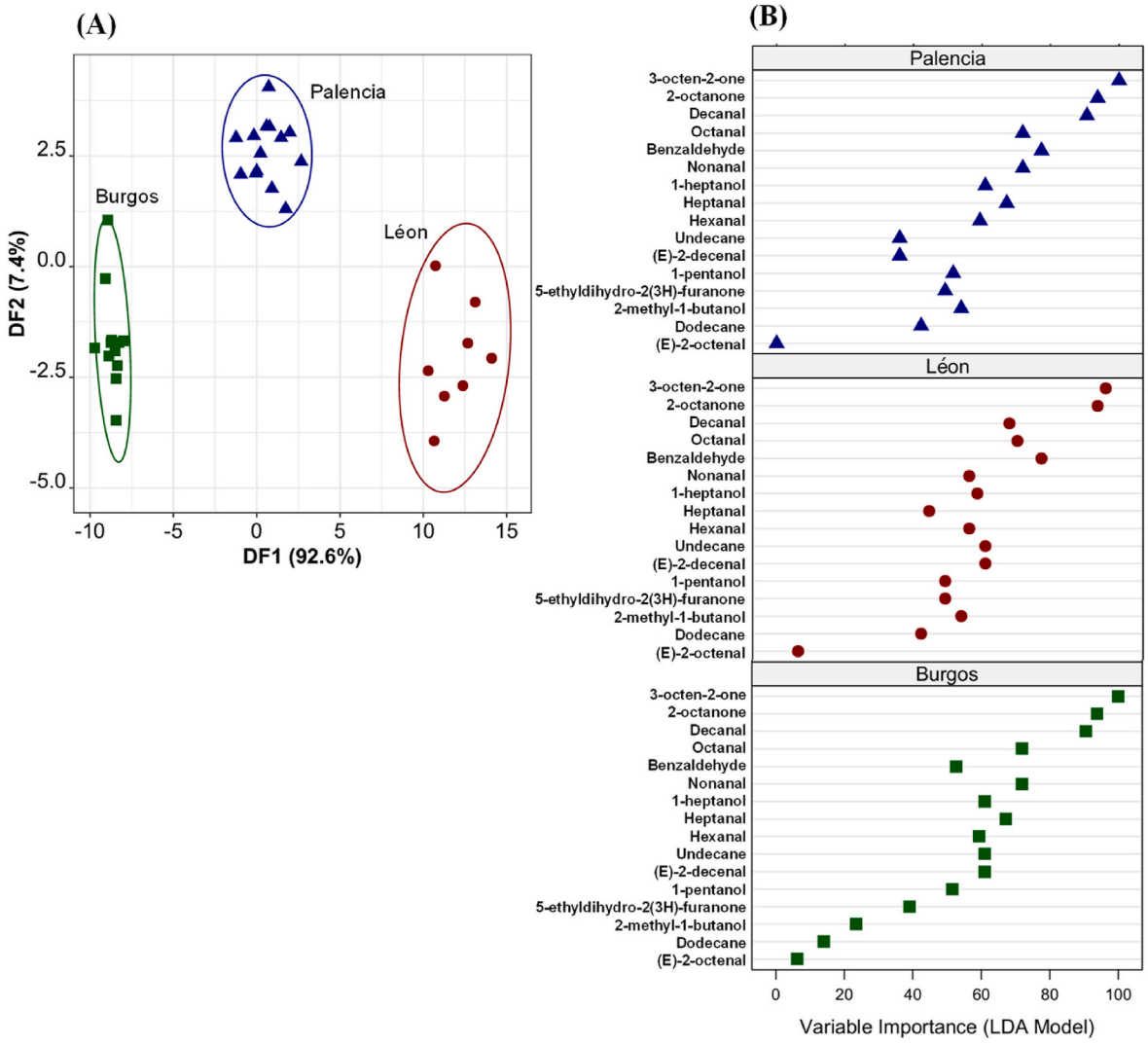

Fig. 2. Supervised discrimination of whole fruit of beech nut seeds according to the geographical origin, based on LDA-SA model established using 16 compounds (2-methyl-1-butanol; 1-heptanol; 1-pentanol; 5-ethyldihydro-2(3H)-furanone; (E)-2-decenal; 2-octanone; (E)-2-octenal; 3-octen-2-one; benzaldehyde; decanal; heptanal; hexanal; nonanal; octanal; undecane and dodecane): (**A**) 2D discrimination plot (training); and, (**B**) Variable importance analysis highlighting the contribution of each variable to the established classification model by geographical origin (all measures of importance are scaled to a maximum value of 100). ■ Burgos; ● Léon; and ▲ Palencia.

cosmetic, or nutraceutical applications. Solar radiation and sunlight exposure play an important role in regulating the synthesis of antioxidant compounds in plants, including tocopherols. The higher tocopherol levels observed in samples from Palencia may therefore be related to regional differences in light exposure or other environmental conditions that induce oxidative stress, promoting greater accumulation of these compounds.

3.2.3. Geographical origin discrimination based on physicochemical data

The LDA-SA approach was applied to the volatile compound profiles obtained from the GC analysis of whole beech nut seeds collected across the three under study regions of Spain. The resulting multivariate classification model comprised two discriminant functions (DFs), which explained 92.6% and 7.4% of the total variability, respectively (Fig. 2). Through the SA algorithm, sixteen volatiles were identified as having the highest discriminant power, namely: 2-methyl-1-butanol, 1-heptanol, 1-pentanol, 5-ethyldihydro-2(3H)-furanone, E−2-decenal, 2-octanone, E−2-octenal, 3-octen-2-one, benzaldehyde, decanal, heptanal, hexanal, nonanal, octanal, undecane, and dodecane. These compounds enabled a satisfactory differentiation of the geographical origin of the samples. The model achieved 100% accuracy in the training phase (Fig. 2A), correctly classifying seeds according to their region of origin. Variable importance analysis (Fig. 2B) highlighted 3-octen-2-one, 2-octanone, and decanal as the main predictors for Palencia and Burgos regions, whereas 3-octen-2-one and 2-octanone played the strongest role in differentiating Léon samples from those of the other two regions. Nonetheless, predictive performance decreased under validation: LOO-CV yielded 91% accuracy, with misclassifications between Burgos and Palencia, and repeated K-fold CV further reduced average sensitivity to 88 ± 12%, with errors observed across all regions, with a higher misclassification rate between Burgos and Palencia. Despite this reduction, the results confirm the effectiveness of LDA-SA in identifying volatile compounds as potential non-invasive, non-destructive markers for the geographical discrimination of beech nut seeds, supporting the formation of distinct, non-overlapping regional groupings (Burgos, Léon, and Palencia).

The LDA-SA technique was also applied to the other previously evaluated physicochemical parameters, namely moisture content, fat content, fatty acid relative abundance, tocopherol composition, TPC, DPPH assay, ABTS assay, and reducing power, to assess their discriminative potential among the three geographical regions under study. The selected LDA-SA model consisted of two DFs. DF1 explained 98.7% of the total variance, underscoring its dominant role in discriminating samples from the three geographical regions, with Burgos beech nuts appearing particularly distinct. Although DF2 accounted for only 1.3% of the variance, it further contributed to differentiating samples from Léon and Palencia. The SA algorithm identified six key variables with the strongest origin-discriminating capacity: moisture content and five fatty acids ($C_{16:1}$, $C_{18:3}$, $C_{20:2}$, $C_{22:0}$, and $C_{22:1}$). Using these biomarkers, the model achieved 100% correct classifications in both the training phase (Fig. 3A) and LOO-CV, confirming their effectiveness in distinguishing beech nut seeds by geographic origin. Moreover, the variable importance analysis (Fig. 3B) revealed that the relative abundance of $C_{16:1}$, followed by $C_{20:2}$ and $C_{18:3}$, were the predictors that contributed most to distinguishing the Palencia and Léon regions. In contrast, for the Burgos region, $C_{20:2}$, together with $C_{22:1}$ and again $C_{18:3}$, were identified as the variables with the strongest contribution in differentiating this region from the other two under study.

Lastly, the repeated K-fold CV results showed that the LDA-SA model based on six selected parameters (moisture and five fatty acids) achieved a high average sensitivity (98 ± 8%), enabling robust prediction of geographical origin. Misclassifications were limited to samples from Léon and Palencia. The clear grouping and absence of overlap among regions highlight the versatility of this chemometric approach and confirm the potential of LDA-SA to identify physicochemical markers (e.g., moisture, $C_{16:1}$, $C_{18:3}$, $C_{20:2}$, $C_{22:0}$, and $C_{22:1}$) for differentiating beech nuts by geographical origin. These findings support its application for geographic traceability of beech nuts and provide insights into how environmental and regional factors influence their chemical and functional traits. However, future validation using larger and more diverse datasets is required. Interestingly, a comparison with results based on volatile profiles revealed a shift in regional positioning. While for the physicochemical-based model (Fig. 3) Léon samples clustered between the other two regions and closer to the DF1 origin, in the volatile-based model (Fig. 2) Palencia occupied this central position. The lower classification performance with volatiles (88 ± 12%), compared to moisture and fatty acids (98 ± 8%), may be attributed to their higher intra-group variability of the former data. Nevertheless, volatile profiling offers the advantage of being non-invasive, as it directly analyses whole fruits without requiring oil extraction, unlike fatty acid analysis, which involves an invasive procedure. Despite the lower predictive classification performance, this non-destructive feature makes the volatile-based

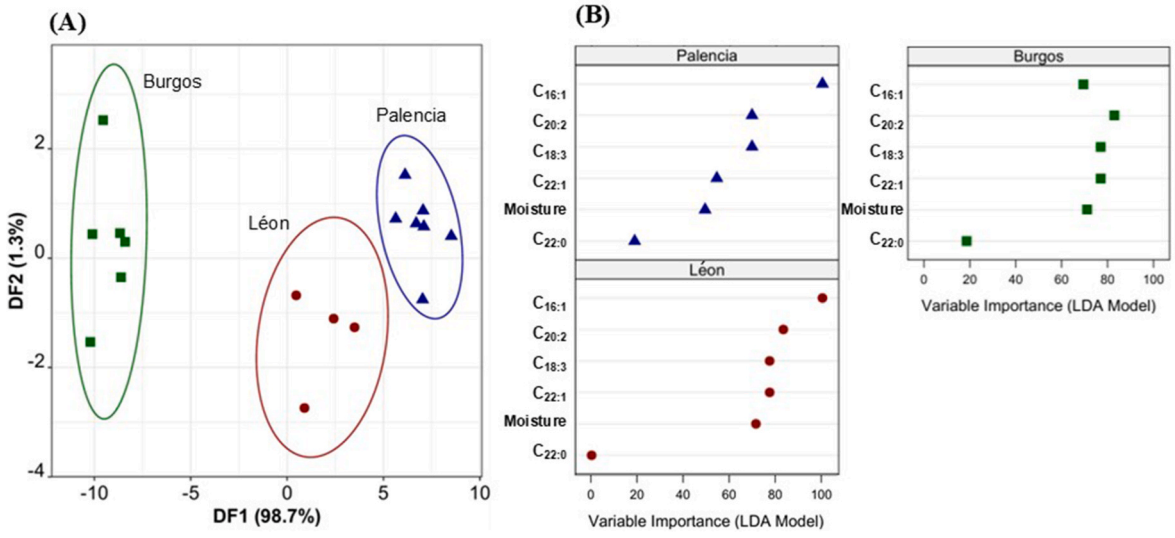

Fig. 3. Supervised discrimination of whole fruit of beech nut seed and beech nut oils according to the geographical origin based on LDA-SA model established using six parameters (Moisture, $C_{16:1}$, $C_{18:3}$, $C_{20:2}$, $C_{22:0}$, and $C_{22:1}$): (**A**) 2D discrimination plot (training); and, (**B**) Variable importance analysis highlighting the contribution of each variable to the established classification model by geographical origin (all measures of importance are scaled to a maximum value of 100). ■ Burgos; ● Léon; and ▲ Palencia.

approach particularly attractive for applications requiring sample preservation or minimal processing. It should be emphasized that, while these associations are biologically plausible and supported by previous studies, the lack of site-specific environmental measurements limits direct correlation of the observed compositional differences to individual environmental factors. Future studies integrating climatic, edaphic, and compositional data would be required to elucidate these relationships more precisely.

4. Conclusions

This study presents a comprehensive physicochemical characterization of beech nuts (*Fagus sylvatica* L.) from three Spanish regions, highlighting their nutritional value and antioxidant activities thus, the potential applications in the food, cosmetic, and pharmaceutical industries. The beech nuts were found to be particularly rich in oleic and linoleic acids and exhibited high antioxidant capacity, reinforcing their relevance as a functional bioresource. Chemometrics enabled effective discrimination of samples according to geographic origin, with volatile compounds and, in particular, fatty acids serving as key regional discriminant biomarkers, thereby demonstrating the influence of origin on chemical composition. From an industrial perspective, these regional differences provide a valuable tool for the targeted selection of raw materials with specific compositional and antioxidant profiles, enabling the optimization of product formulation, quality consistency, and functional performance. Moreover, the identification of geographical biomarkers supports authenticity, traceability, and potential valorization strategies based on origin. These findings provide new insights into an underutilized species, although further research with broader and more diverse sampling is required to confirm regional trends and to advance the potential of beech nuts for commercial exploitation in functional foods, cosmetics, and pharmaceuticals.

CRediT authorship contribution statement

Sandra Lamas: Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis. **Nuno Rodrigues:** Writing – review & editing, Writing – original draft, Supervision, Methodology, Investigation, Formal analysis, Conceptualization. **Baudilio Herrero:** Writing – review & editing, Investigation, Conceptualization. **Susana Casal:** Writing – review & editing, Investigation, Formal analysis. **Rebeca Cruz:** Writing – review & editing, Methodology, Formal analysis. **António M. Peres:** Writing – review & editing, Software, Investigation. **José Alberto Pereira:** Writing – review & editing, Supervision, Resources, Investigation, Funding acquisition, Conceptualization.

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Declaration of competing interest

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

Data availability

Data will be made available on request.

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