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Research Paper

Essential oils of lavandin (*Lavandula x intermedia* Emeric ex Loisel.) of Spain: A case study on clones 'Grosso' and 'Super'

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ABSTRACT

Lavandin *(Lavandula x intermedia* Emeric ex Loisel.) is a sterile natural hybrid obtained from the crossing of lavender (*L. angustifolia* Mill.) and spike lavender (*L. latifolia* Medik.), and its cultivation has gained great importance in Spain. This work is aimed to study the chemical composition of essential oils (EOs) of lavandin from clones 'Grosso' and 'Super' cultivated in Spain. For this purpose, 215 EOs, obtained by steam distillation between 2016 and 2022 in agricultural farms located in 13 provinces of Spain, were analyzed by gas chromatography (GC) in three public R&D centers: the Regional Institute for Agrifood and Forestry Research and Development of Castilla-La Mancha (IRIAF), the Agrarian Technological Institute of Castilla y León (ITACyL) and the Agrifood Research and Technology Center of Aragon (CITA). The chemical composition of the EO of lavandin showed great inter- and intra-varietal variability, with the main compounds being linalool, linalyl acetate, camphor and 1,8-cineole. The results showed that the contents of camphor and 1,8-cineole were higher in 'Grosso' (7.2% *vs* 5.0% and 5.7% *vs* 3.8%, respectively), while those of linalool and linalyl acetate were higher in 'Super' (35.8% *vs* 34.2% and 33.7% *vs* 27.2%). Among minor compounds, the lavandin 'Super' exhibited a higher content of β-Z-ocimene and β-E-ocimene, and a lower content of terpinen-4-ol compared to 'Grosso'. Additionally, eleven compounds of lavandin 'Grosso' were compared within the ranges established by ISO 8902:2009 standard, revealing that only 18% of the samples complied with it for all of these compounds. In this sense, the contents of α-terpineol, linalool and lavandulyl acetate were above the upper limit of the standard in respectively 49%, 23% and 21% of the samples. On the contrary, 36% of the samples for β-Z-ocimene and 32% for linalyl acetate were below the range of this regulation. Unfortunately, these discrepancies with the ISO regulation exclude numerous EOs from the market despite their value in different sectors like perfumery, cosmetics, aromatherapy, phytosanitary or pharmacy. This work could serve as a guidance and/or reference study of Spanish essential oil of 'Grosso' and 'Super' clones for stakeholders involved in the lavandin EO market.

1. Introduction

The genus *Lavandula* belongs to the Lamiaceae family, and consists of more than 39 species, including lavandin (*Lavandula x intermedia* Emeric ex Loisel.), which is a sterile natural hybrid obtained by crossing *L. angustifolia* (lavender) and *L. latifolia* (spike lavender) (Garzoli et al.,

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2020). It is an evergreen shrub growing up to 90 cm tall, compact, reliable and well suited for large borders, with narrow greyish-green leaves and dense, fragrant, long-flowering spikes with a pungent aroma and violet to bluish color. The development and cultivation of this species has been closely linked to France throughout the 20th century in the search for a higher essential oil (EO) yield compared to lavender and spike lavender. This was specifically promoted in the region of Provenza, linked to a culture around lavender and lavandin crops encompassing tourism, gastronomy and perfumery. Since the beginning of the 21st century, this production model has expanded to other countries, notably Bulgaria and China. In Spain, the cultivated area of lavender and lavandin has experienced remarkable growth, increasing from 1600 ha in 2009 to 7200 ha in 2021 (Escudero et al., 2022). The cultivation is predominantly of 'Super' and 'Grosso' clones. In certain Spanish regions like La Alcarria (central Spain), a similar cultural phenomenon has emerged as in Provenza. Here, festivals, dinners, and concerts are held annually, drawing in numerous tourists. These events often showcase gastronomic delights associated with lavender and lavandin, including ice creams, liquors, and more. While lavandin and lavender EO possess distinct chemical compositions, with lavender primarily favored for high-value perfume production, their marketability hinges on a variety of terpenes. These include linalyl acetate, linalool, camphor, borneol, 1,8-cineole, and β-caryophyllene (Lafhal et al., 2016). Accordingly, different ISO standards have been developed for the EO sector, referring to the chromatographic profile among other aspects, establishing percentage ranges for each representative and characteristic component of an EO. In the case of lavandin, the ISO 8902:2009 (2009) standard is well suited for the EOs obtained by steam distillation of flowering tops of the 'Grosso' clone and mainly refers to a specific area in the south of France. Nevertheless, 'Grosso' EOs from plants grown outside that area may significantly differ from the ISO standard, as well as the EO composition of other lavandin clones as 'Super', not yet included in any ISO standard. Additionally, this composition may depend on various different genetic and abiotic factors, as previously observed in other related species of the Lamiaceae family, such as *Salvia lavandulifolia*, where differences were found depending on the growing area or harvest year (Sánchez-Vioque et al., 2022) but also due to the environmental conditions of the harvested plant, distillation parameters, EO storage, etc. (Kara and Baydar, 2013; Pokajewicz et al., 2022). Such discrepancies with ISO standards should not lead to consider a lavandin EO of lower quality since they can also be used in different industrial applications (agri-food, cosmetics, therapeutic, etc.) due to their demonstrated biological properties. For example, high contents of 1,8-cineole and camphor can negatively affect the aroma (Aprotosoaie et al., 2017; Détár et al., 2020; Tardugno et al., 2019) but may favor other uses like antimicrobial applications (Carrasco et al., 2016; Tardugno et al., 2019).

The aim of the present work is to analyze the chromatographic profile of different Spanish lavandin EOs from 'Grosso' and 'Super' clones as a result of a collaboration between the Regional Institute for Agrifood and Forestry Research and Development of Castilla-La Mancha (IRIAF), the Agrarian Technological Institute of Castilla y León (ITACyL), the Agrifood Research and Technology Center of Aragon (CITA), and the Spanish Interprofessional Association of Aromatic and Medicinal Plants (ANIPAM). This comprehensive database has been used for the comparison: (1) between the EO composition of the clones, and (2) with the ISO 8902:2009 standard.

2. Materials and methods

2.1. Essential oil collection

A total of 215 EOs of lavandin 'Grosso' (n=112) and 'Super' (n=103) were obtained by steam distillation from producers across 13 provinces of Spain and analyzed in the same harvest season during the period 2016–2022 (Table 1). Lavandin EOs were supplied to the IRIAF by members of ANIPAM as part of a collaborative effort in the Project of evaluation and improvement of the quality of EOs of native species of aromatic plants'. Samples collected by ITACyL corresponded to a collaboration with the University of Valladolid and farmers from the provinces of Valladolid and Palencia as part of the project "Cultivation and clonal selection of elite sage, rosemary, marjoram and lavender plants for their innovative application in the field of human health and nutrition". Additionally, samples obtained by CITA were collected by local partners and farmers who conducted field trials in collaboration with this center across various projects and years. In all cases, the EOs were identified as either 'Grosso' or 'Super' by their respective producers.

2.2. Essential oil analysis

The EOs supplied to the IRIAF were analyzed at the CIAF-Albaladejito using gas chromatography (GC) in a Varian 400-GC gas chromatograph operated with a split/splitless injector. Column (non-polar): VF-5MS (cross-linked phenyl-methyl siloxane) 60 m x 0.25 mm×0.25 μm film thickness (Varian Inc., Palo Alto, CA, USA). The temperature gradient begins at 70 ℃ and increases to 95 ℃ at a rate of 3 ℃ /min. Upon reaching 95 ℃, the rate is then increased to 4 ℃ /min until it reaches 240 ℃, where it is maintained for 5 minutes. Injection temperature: 250 ℃. Injection volume 0.5 μL. Carrier gas: Helium (1 mL/min). Injection mode: split (100:1). The temperature of Flame Ionization Detector (FID) was 300 ℃. Hydrogen flow: 35 mL/min; air flow: 300 mL/min; make up flow: 29 mL/min. Sampling rate: 50 msec. Relative retention times and Kovats Index of corresponding reference standards (Across Organics BVBA/SPRL, Fisher Scientific S.A. and Sigma Aldrich

Table 1
Number of samples of lavandin 'Grosso' (G) and 'Super' (S) essential oils from Spain provided by producers and analyzed between 2016 and 2022, categorized by province and season.

	2016		2017		2018		2019		2020		2021		2022		2016–2022	
Location	G	S	G	S	G	S	G	S	G	S	G	S	G	S	G	S
Albacete	2		2	3	4	2	1	1	5	2	6	1			20	9
Burgos			3	3	4	4	3	3	1	1	1	1	3	2	15	14
Cuenca			1	6	1	3	1	6	4	5	4	8	4	3	15	31
Guadalajara			1	1					1	6			2	3	4	10
Huesca								1	1	1	2	2			3	4
León										1	1	1	1	1	2	3
Murcia			1												1	0
Navarra									1				1		2	0
Palencia											28	8	1	1	29	9
Soria				1						2					0	3
Valencia			1										2		3	0
Valladolid			1	1	4	5	5	6	1	1	2	2	1	1	14	16
Zaragoza	1	1	1	1	1	1	1	1							4	4
TOTAL	3	1	11	16	14	15	11	18	14	19	44	23	15	11	112	103

Química A.) were used to identify the major components. Quantification of components (relative percentage abundance) was determined according to the area of the chromatographic peaks using the Galaxie® Chromatography software (Varian, Inc. 2002–2005).

The analyzes of EO samples supplied to CITA and ITACyL were carried out using an Agilent 6890 N series equipped with a 5973 N series mass selective detector and a 30 m, 0.25 mm id, 0.25 μm film thickness capillary column HP-5MS (Agilent Technologies, California, USA). The injection temperature was 250 ℃ and 1 μl of sample (previously diluted 1:33 in acetone) was injected in split mode (100:1). The carrier gas was helium at a flow rate of 1.2 mL/min and the oven column program was set to 70 ℃ (held for 5 min), raised to 100 ℃ at 2 ℃/min, raised to 154 ℃ at 3 ℃/min and raised to 280 ℃ at 100 ℃/min. Detection was in electron ionization (EI) mode (70 eV) and an ion source temperature of 200 °C. The interface temperature was 220 °C. The mass spectrometer scanning was recorded in full scan mode (35–350 *m/z*). A MSD ChemStation software was used for controlling the GC/MS system. Identification was carried out using spectra obtained from commercial standard compounds (provided by Sigma-Aldrich and Fluka) and by comparison of the mass spectra with mass spectral data from the Willey 275 and NIST MS Search Program 2.0 libraries and by comparison of previously reported Retention Index with those calculated using an n-alkane series under the same analysis conditions. Quantification of the EOs was done by integrating the area of total ion chromatogram.

2.3. Statistics

All statistical analyzes were performed using the IBM® SPSS® Statistics ver. 12.0.1 (Copyright © SPSS Inc. 1989–2003). A statistical description, including mean, standard deviation, minimum and maximum values of compounds of EO included in the ISO 8902:2009 was performed for both 'Grosso' and 'Super' clones. Analysis of Variance (ANOVA) was employed to identify statistically significant differences between the composition of EOs of clones. Principal Component Analysis (PCA) and two-step cluster analysis were conducted. PCA was performed on the correlation matrix, while the two-step clustering process utilized the automatic clustering method using the Schwarz Bayesian Criterion (BIC); log-likehood criterion was applied in the distance calculation and Student's t-test to measure the importance of variables in the formation of clusters.

3. Results and discussion

3.1. Differences in EOs of lavandin 'Grosso' and 'Super'

The chemical composition of the lavandin EOs from different locations in Spain, analyzed in this study over a 7-year period, presented great inter- and intra-varietal variability. Previous studies have also reported that the chemical composition of lavandin EOs may vary depending on several factors as genetics, age of plant, edaphoclimatic conditions, cultivation methods (Arabaci et al., 2007), harvest date (Baydar and Erbaş, 2009), distillation conditions (Kara and Baydar, 2013) or oil storage.

As observed in Table 2, the major compounds detected in the EOs of lavandin were linalool, linalyl acetate, camphor and 1,8-cineol. The mean content of camphor and 1,8-cineole was superior in 'Grosso' (7.2% *vs* 5.0% and 5.7% *vs* 3.8%, respectively) while the content of linalool and linalyl acetate was higher in 'Super' (35.8% *vs* 34.2% and 33.7% *vs* 27.2%, respectively). Likewise, there were other minor compounds such as the two ocimene isomers that had more than double the content in 'Super' and terpinen-4-ol which was much higher in 'Grosso'. In contrast, limonene and α-terpineol presented similar values for both clones.

As a result, differences can be observed in the representative chromatograms of EOs of lavandin 'Grosso' and 'Super' (Fig. 1). Such differences align with Melero-Bravo and Menegusso (2021), who observed the influence of the clone in a study on lavandin fertilization carried out in central Spain, and whose results are consistent with the present study: higher content of 1,8-cineole, camphor and terpinen-4-ol in 'Grosso', and a major content of linalool, linalyl acetate and β-E- ocimene in 'Super'. Similar results were obtained by Carrasco et al. (2016) when comparing two samples of each clone cultivated in farm fields in the South of Spain. Studies conducted in other countries such as Norway and the USA have found differences in the content of 1,8 cineole, camphor, and lavandulyl acetate between these clones (Aprotosoaie et al., 2017; Pokajewicz et al., 2023). However, our results are different from those obtained by Bombarda et al. (2008), Moon et al. (2007), and Renaud et al. (2001), where 'Super' presented higher contents than 'Grosso' in 1,8-cineole (2.6–15.9%) and camphor (6–12.2%).

Additionally, a Principal Component Analysis (PCA) was conducted to elucidate the variability of samples based on the chemical composition of their EOs. The first three principal components explained 73% of the total variance. PC1 (41.9%) was positively correlated with camphor (r= 0.92), lavandulyl acetate (r= 0.79) and terpinen-4-ol (r= 0.80) and inversely correlated with β-E- ocimene (r= -0.78). PC2 (16.5%) was positively associated with linalool (r= 0.83) and inversely associated with linalyl acetate (r= -0.74). Finally, PC3 (14.2%) was positively correlated with limonene (r= 0.68) and α- terpineol (r= 0.65). Fig. 2 represents in a 3D scatterplot (with each axis corresponding to one of the three principal components) with samples grouped by clone, highlighting the predominance of genetics over the environmental factors.

3.2. Compliance with ISO 8902:2009 standard

The comparison of lavandin Grosso EO compositions grown in Spain revealed that only 18% of samples met the ISO 8902:2009 standard for all the compounds analyzed in this study (Fig. 3). When considering each compound individually, more than 80% of the EOs exhibited a chemical content of limonene, β-E-ocimene, borneol, terpinen-4-ol,

Table 2
Composition of essential oils of lavandin 'Grosso' and 'Super' from Spain (2016–2022). Quantification of compounds is expressed as their relative peak areas (%).

Compound	*Lavandula x intermedia* Grosso'		*Lavandula x intermedia* Super'					
	Mean (%)	Minimum (%)	Maximum (%)	SD (%)	Mean (%)	Minimum (%)	Maximum (%)	SD (%)
Linalool	34.25b	23.90	43.77	4.2	35.78a	19.87	49.43	6.0
Linalyl acetate	27.24b	13.86	53.49	5.3	33.71a	19.03	50.11	6.7
Camphor	7.24a	4.89	10.27	0.9	4.99b	3.63	8.17	0.9
1,8-cineole	5.67a	1.75	12.66	1.7	3.83b	0.89	8.77	1.8
Borneol	2.60a	0.24	4.47	0.6	2.36b	0.19	6.13	0.9
Lavandulyl acetate	2.65a	0.73	4.87	0.8	1.59b	0.31	3.11	0.6
Terpinen-4-ol	3.49a	0.15	6.24	1.1	0.21b	0.00	1.28	0.3
α- terpineol	1.70a	0.07	4.55	0.9	1.52a	0.10	5.40	1.1
β-E-ocimene	0.61b	0.00	1.55	0.6	1.93a	0.00	3.73	0.6
β-Z-ocimene	0.73b	0.00	1.95	0.3	1.59a	0.51	4.59	0.5
Limonene	0.68a	0.13	1.57	0.2	0.70a	0.13	1.79	0.4

Different letters correspond to statistically significant differences between 'Grosso' and 'Super' clones for each compound in post-hoc Tukey's test ($\rho<0.05$).

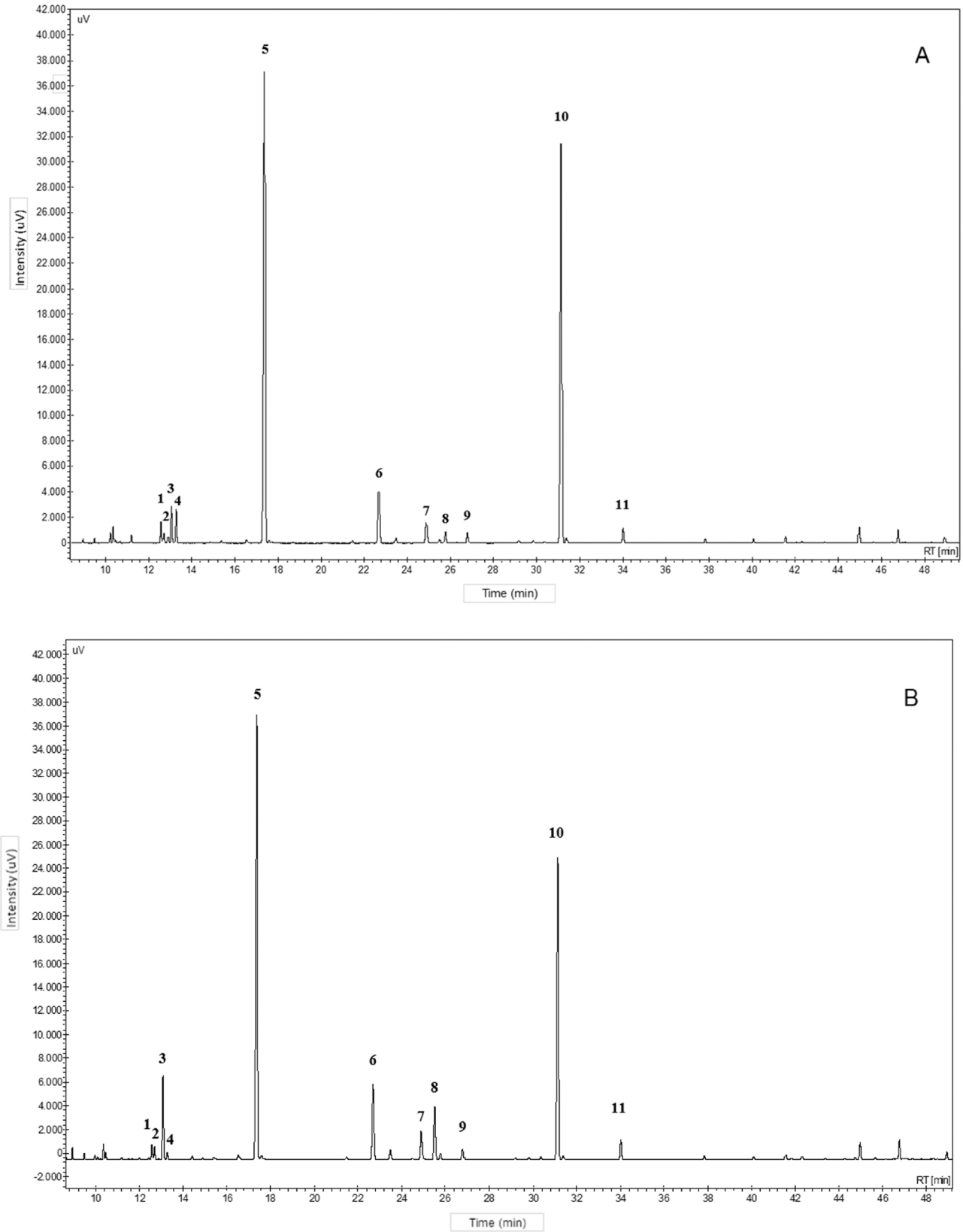

Fig. 1. Chromatograms of essential oils of lavandin Super´(1 A) and Grosso´(1B) of Spain, with representative major compounds (1: β-Z-ocimene; 2: limonene; 3: 1,8-cineole; 4: β-E-ocimene; 5: linalool; 6: camphor; 7: borneol; 8: terpinen-4-ol; 9: α-terpineol; 10: linalyl acetate; 11: lavandulyl acetate).

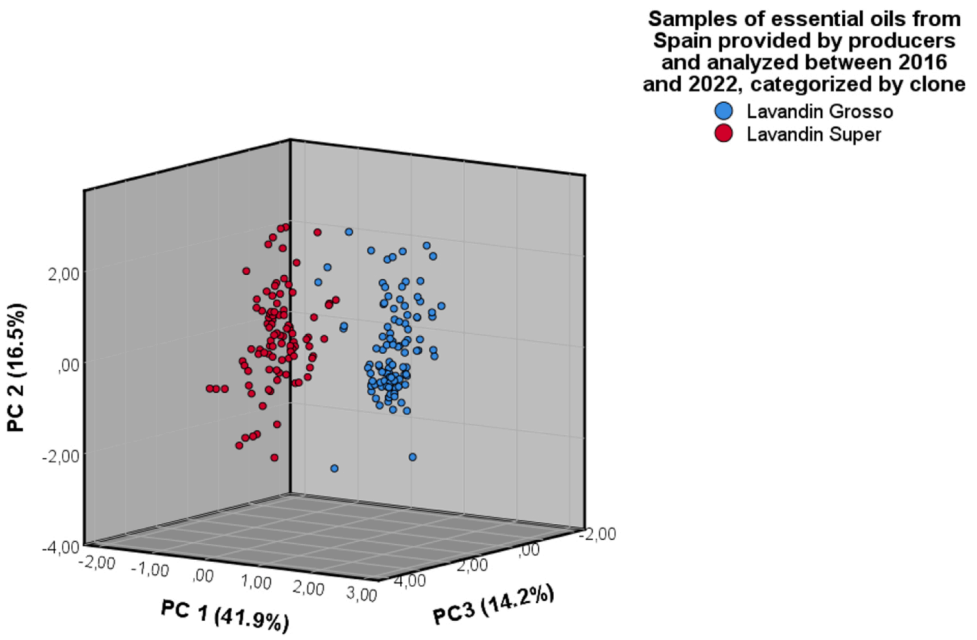

Fig. 2. Distribution of samples labelled by clone in a scatterplot of the three first principal components extracted with the PCA of samples based on the chemical composition of their EOs.

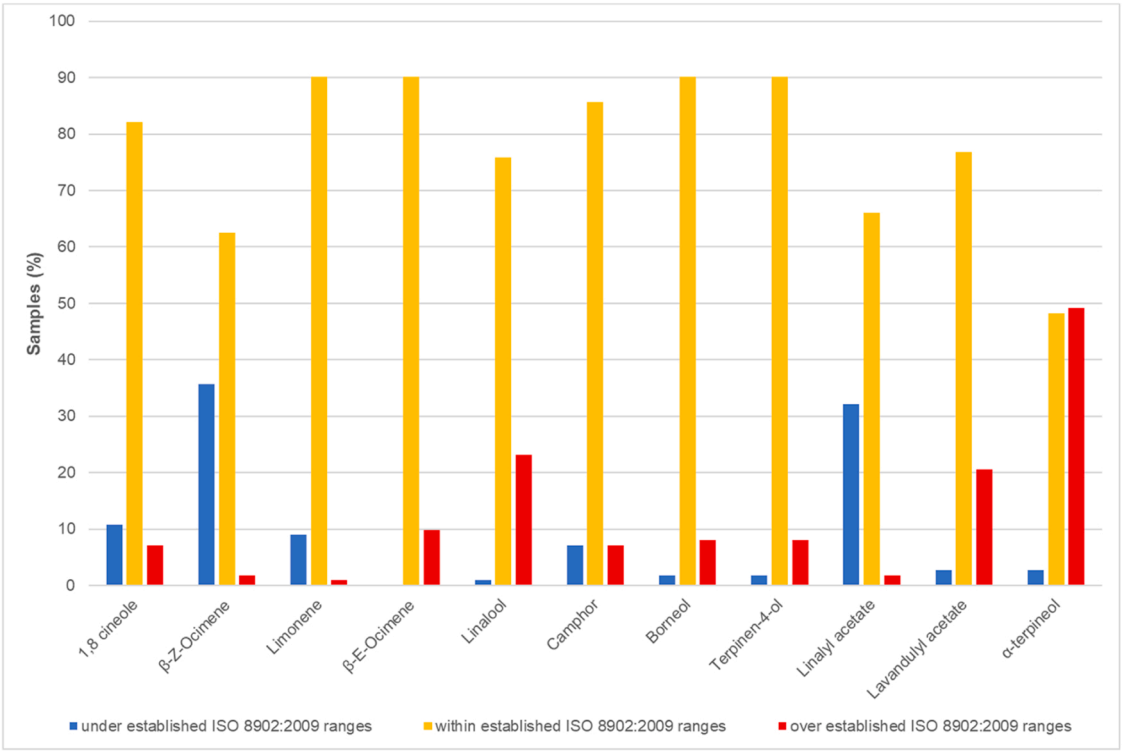

Fig. 3. Grouping of samples of EO from lavandin 'Grosso' grown in Spain (2016–2022), based on their deviation from ISO, (8902):(2009) standard ranges. Quantification is expressed as a percentage of total samples, for each compound.

camphor or 1,8-cineole within the standard range. However, concerning the major compounds, one out of three samples showed a content of linalyl acetate below the standard, while 23% had a linalool percentage exceeding the upper limit. Regarding the minor compounds, the contents of α-terpineol and lavandulyl acetate were above the range in 49% and 21% of the samples, respectively, whereas 36% of the EOs had a lower content of β-Z-ocimene compared to the standard.

The non-compliance of these samples of lavandin 'Grosso' EO could be attributed to the association of the ISO 8902:2009 standard with a specific geographic area, likely endorsed through samples of EO originating from that region, as is indicated by its tittle (French type) and by Terms and definitions (cultivated mainly in the south of France). In fact, it is used as a reference for all lavandin 'Grosso' EO, regardless of their origin. Such differences attributed to the location are common in other aromatic plants, as evidenced by wild Spanish populations of *Salvia lavandulifolia* (Sánchez-Vioque et al., 2022) or *Rosmarinus officinalis*

(Melero-Bravo et al., 2022). Furthermore, the ISO 3515:2002 (2002) standard, which targets cultivated *Lavandula angustifolia* EO, considers differences based on the country of origin by incorporating chromatographic profiles tailored to EOs from France, Bulgaria, Russia, Australia, and other origins. The differences in ranges according to origin established in this standard appear to indicate a regulatory framework and guidelines from major lavender essential oil-producing countries (Pokajewicz et al., 2023).

Focusing on scientific literature about lavandin 'Grosso', differences in their EOs emerged based on their origin. Lafhal et al. (2016) analyzed 30 samples of lavandin 'Grosso' EOs cultivated in French areas and most of them fell within the ranges of the ISO, (8902):(2009) standard. However, other studies conducted outside France have shown deviations from this regulation. For instance, contents of linalool, linalyl acetate, camphor, and 1,8-cineole have also been detected outside the limits established by the ISO, (8902):(2009) standard in Spanish EOs (Carrasco et al., 2016; Melero-Bravo and Menegusso, 2021). Similarly, Pistelli et al. (2017) showed that the content of linalyl acetate and α-terpineol in lavandin plants cultivated in Tuscany (Italy) was also out of range. These discrepancies are not limited to the Mediterranean basin. In this regard, Walasek-Janusz et al. (2022) reported a very high content of terpinen-4-ol (18%) in Polish lavandin 'Grosso', a compound responsible for the antimicrobial activity of the EO, and Lane and Mahmoud (2008) observed a lower content of linalyl acetate in the EO of plants grown in British Columbia (Canada). However, a natural EO of lavandin with a composition outside the limits of the standard may also be interesting for other applications. Several previous studies have reported antimicrobial capacities for lavandin EO against bacteria present in food, human and the environment (Martucci et al., 2015; Nikolić et al., 2014; Rota et al., 2004; Smigielski et al., 2018).

4. Conclusion

The study of the composition of 215 EOs from lavandin 'Grosso' and 'Super' cultivated in Spain revealed distinct chromatographic profiles between them, primary based on their contents of camphor, terpinen-4-ol, β-Z-ocimene and β-E-ocimene. Additionally, the EO composition of the 'Grosso' clone significantly deviates from the ISO 8902:2009 standard, potentially leading to perceived lower quality and subsequent market losses. While the existence of standards undoubtedly benefits the development of commercial activities, the ISO 8902:2009 standard, primarily oriented towards cultivation in the south of France, excludes a significant number of EOs from other origins that possess specific biological activities, and are therefore valuable in various sectors such as perfumery, cosmetics, aromatherapy, phytosanitary, or pharmacy. This work could serve as a guiding and/or reference study of Spanish EO of 'Grosso' and 'Super' clones for stakeholders involved in the lavandin EO market. It also highlights the importance of considering regional variations and specific biological activities when establishing standards for EOs to ensure inclusivity and promote the utilization of valuable resources across different sectors.

CRediT authorship contribution statement

Noemi Cerro-Ibáñez: Investigation, Formal analysis. **David Prieto-Blanco:** Investigation, Formal analysis. **David Herraiz-Peñalver:** Resources, Project administration, Investigation, Funding acquisition, Formal analysis. **Gonzalo Ortiz de Elguea-Culebras:** Writing – original draft, Formal analysis. **Mr. Enrique Melero-Bravo:** Writing – original draft, Resources, Project administration, Methodology, Investigation, Funding acquisition, Conceptualization. **Baudilio Herrero:** Writing – review & editing, Resources, Methodology, Investigation. **Silvia Pérez-Magariño:** Writing – review & editing, Validation, Methodology, Investigation. **M Carmen Asensio-S.-Manzanera:** Writing – review & editing, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Conceptualization. **María Ángeles Sanz:** Writing – review & editing, Validation, Methodology. **Juliana Navarro-Rocha:** Writing – original draft, Resources, Methodology, Investigation, Conceptualization. **Raúl Sánchez-Vioque:** Writing – original draft, Formal analysis.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: M Carmen Asensio-S.-Manzanera reports financial support was provided by National Institute for Agricultural and Food Research and Technology.

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