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Genotypic and Environmental Influences on the Composition of Lavender Essential Oils

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Abstract

Essential oils are defined as secondary metabolites, produced by plants for various ecological functions, in response to biotic and abiotic stimuli. Genetic variability and instability, due to polyploidy or hybridisation, together with variations in temperature, water supply, altitude, season, geography and agronomic management, strongly influence the chemical composition of the oil.

The project aims at evaluating the influence of genotypic and environmental factors on the chemical composition of lavender essential oils.

A collection of 34 essential oil samples of *Lavandula angustifolia* and *Lavandula x intermedia* varieties has been obtained from 7 locations. Gas chromatographic analyses detected 133 components. A comparative analysis has highlighted that several samples exceeded the quality standards ISO 3515:2002 for *Lavandula angustifolia* essential oil and ISO 8902:2009 for *Lavandula x intermedia Grosso* essential oil. Estimations of the oils' average chemical composition have supported the role of linalool and linalyl acetate in characterising lavender essential oils. The Multiple Variable Analysis for correlations detected a negative correlation between linalool and linalyl acetate, besides a generally high number of significant correlations among all molecules, thus suggesting a cross-talk between biosynthetic pathways. The Principal Components Analysis (PCA) and Hierarchical Cluster Analysis (HCA) have verified the major contribution of linalool and linalyl acetate to the broad differentiation of samples, although groupings have been observed in relation to common or recurring features.

Evidence on the influence of the environment on the composition of lavender essential oils has been provided. Furthermore, this research reviewed and supported the association of genetic and biochemical studies, to assess the inter- and intraspecific differentiation in essential oils' composition. This characterisation acquires a central role in breeding programs for essential oil quantity and quality, but also in the preservation of germplasm collections and natural biodiversity.

Eventually, a selection of 4 essential oil pairs has been included in a triangle test, administered within the Herbs & Spices Global Expo at Macfrut 2022, in order to explore the consumers' perception of lavender essential oils variability.

The experiment encouraged a multidirectional exchange of knowledge among stakeholders, in light of the recent update in the institutional framework of medicinal plants cultivation and transformation.

In this context, the study lays the foundations for the constitution of a global database collecting essential oils of *Lavandula* species and varieties.

Riassunto

Gli oli essenziali sono identificati come metaboliti secondari, prodotti dalla pianta per una varietà di funzioni ecologiche, in risposta a stimoli biotici e abiotici. Variabilità e instabilità genetica, dovute da poliploidia e ibridizzazione, assieme a variazioni di temperatura, apporto idrico, altitudine, stagione, geografia e gestione agronomica, influenzano notevolmente la composizione chimica dell'olio.

Il presente progetto è finalizzato a valutare l'influenza dei fattori genotipici e ambientali sulla composizione chimica dell'olio essenziale di lavanda.

Una raccolta di 34 campioni di oli essenziali di *Lavandula angustifolia* e *Lavandula x intermedia* è stata ottenuta da 7 diversi siti. L'analisi gascromatografica ha rilevato 133 componenti. Un'analisi comparativa ha evidenziato che molti campioni non rientrano negli standard qualitativi ISO 3515:2002 per l'olio essenziale di *Lavandula angustifolia* e ISO 8902:2009 per l'olio essenziale di *Lavandula x intermedia Grosso*. Una valutazione della composizione media dei campioni ha supportato il ruolo centrale di linalolo e acetato di linalile nella caratterizzazione degli oli essenziali di lavanda. L'analisi delle correlazioni ha riscontrato una correlazione negativa tra linalolo e acetato di linalile, oltre ad un numero generalmente elevato di correlazioni tra tutte le molecole, suggerendo quindi un *crosstalk* tra vie biosintetiche. L'analisi delle componenti principali (PCA) e l'analisi gerarchica dei cluster (HCA) hanno confermato la primaria influenza di linalolo e acetato di linalile sull'ampia differenziazione dei campioni, sebbene alcuni raggruppamenti siano stati osservati in relazione a caratteristiche comuni o ricorrenti.

L'influenza dell'ambiente sulla composizione chimica dell'olio essenziale di lavanda è stata confermata dai risultati conseguiti. Inoltre, questa ricerca ha esaminato e supportato l'associazione di studi genetici e biochimici per valutare la differenziazione inter- e intraspecifica in relazione alla composizione dell'olio essenziale. Questa caratterizzazione acquisisce un ruolo centrale nei programmi di miglioramento genetico per qualità e quantità di olio essenziale, ma anche nella conservazione delle collezioni di germoplasma e della biodiversità naturale.

Infine, una selezione di 4 coppie di oli essenziali è stata inserita in un test discriminante triangolare, somministrato in occasione di Herbs & Spices Global Expo presso Macfrut 2022, con l'obiettivo di valutare la percezione del consumatore della variabilità degli oli essenziali di lavanda.

L'esperimento ha incoraggiato uno scambio di conoscenze multidirezionale tra soggetti operanti nel settore, in luce del nuovo Testo unico in materia di coltivazione, raccolta e prima trasformazione delle piante officinali.

In questo contesto, lo studio pone le fondamenta per la costituzione di un database globale che raccolga oli essenziali di diverse specie e varietà di *Lavandula*.

1 Introduction

1.1 Classification of lavender species and cultivars

Within the Lamiales order, the genus *Lavandula* L. (Synonyms – *Stoechas* Mill., *Fabricia* Adans., *Chaetostachys* Benth., *Sabaudia* Buscal. and Muschl., *Isinia* Rech. f.) belongs to the Lamiaceae (Labiatae) family, subfamily *Nepetoideae*, and includes 39 species (Upson, 2002), with about 400 registered cultivars (Aprotosoaie et al., 2017). In the *Nepetoideae* subfamily, the tribe *Lavanduleae* (Endl.) Boiss is currently considered a distinct group (Upson, 2002).

The taxonomic classification is organised into six sections. Each section includes species and intraspecific taxa, discriminated based on morphological characters. In particular, sections are distinguished according to habit, leaf shape, and arrangement of the flowers on the verticils, bract, calyx and corolla characters. For the present study, the first section *Lavandula* is further examined. This section boasts a large commercial value since it includes the major taxa cultivated in Europe for essential oil production and horticultural and ornamental usage: *Lavandula angustifolia* and *Lavandula x intermedia* (Upson, 2002).

L. angustifolia Miller subsp. *angustifolia* (*L. spica L.* var. α, *L. officinalis* Chaix, *L. fragrans* Jord., *L. vera* DC) is native of South West and South-Central Europe, mainly Italy, France and Spain. This species is naturally found in mountainous areas, over 1.500 m a.s.l., even though its cultivation has been extended to lower altitudes. Plants are characterised by shrubs of 50 cm in height. At first, lanceolate leaves are grey and tomentose when young and become greener over time. Inflorescence stalks are 10-25 cm long and usually unbranched. Spikes normally measure 4-8 cm in length and sometimes exhibit a lower flower cluster. Over 50 cultivars of *L. angustifolia* are currently acknowledged (Aprotosoaie et al., 2017). A common variety of *L. angustifolia* is represented in Figure 1.1a. The flowering period usually goes from mid-June to July (Upson, 2002).

L. x intermedia Emeric ex Loisel. (*L. angustifolia* x *L. latifolia*) (*L. hybrida* Reverchon; *L. x hortensis* Hy) is a natural sterile hybrid of *L. angustifolia* x *L. latifolia*, originating from Spain, France and Italy. This hybrid, commonly named "lavandin", exhibits a vigorous shrub, of 60-

150 cm in height. Leaves are grey and tomentose, with a lanceolate or spathulate shape. Inflorescence stalks are branched, with lax spikes (Upson, 2002). A common variety of *L. x intermedia* is represented in Figure 1.1b. The flowering period usually goes from late June to August. This species has been widely selected for essential oil production. It produces higher yields as compared to *L. angustifolia*, but lower quality oils, with higher camphor content (Charlesworth, 2002). Higher productivity of *L. x intermedia* is mainly related to distinctive morphological traits. Hybrid plants are characterised by a larger storage capacity and a higher density of secretory glands, as compared to lavender (Aprotosoaie et al., 2017). Being a sterile plant, lavandin can only be subjected to vegetative propagation (Voltolina, 2017).

Figure 1.1 *L. angustifolia Hidcote Blue* (a) and *L. x intermedia Grosso* (b).
(Lavandeto di Arquà Petrarca, n.d.) https://www.lavandetodiarqua.it/collections/lavande

1.2 Lavender cultivation for essential oil production

Lavender is a pluriennial crop, characterised by a variable development throughout the crop cycle, in relation to genotypic and environmental conditions (Lis-Balchin, 2002a). After an initially moderate growth, the crop reaches full productivity in the third year and can be maintained for up to 6 years for lavender and 8-10 years for lavandin (Voltolina, 2017).

L. angustifolia can be propagated by seed or by cuttings, while the sterile hybrid *L. x intermedia* can only be subjected to vegetative propagation. In the case of reproduction by seeds, sowing can be performed directly in open field conditions (Lis-Balchin, 2002b). However, seeds are commonly grown in nurseries for about one year, after a period of vernalisation at 2°C for one week or 10°C for one month. In the case of vegetative propagation, clones are produced from 10-15 cm cuttings of selected mother plants. They are initially grown in nurseries, with a density of 200-300 cuttings per m^2, and subsequently transplanted in open fields. Whenever woody cuttings are used, transplantation can be carried out after one year. Shorter time frames are required if green cuttings are employed, but usually rooting hormone solutions are necessary (Voltolina, 2017).

Planting is performed on even soil, in autumn or spring, according to environmental conditions. *L. angustifolia* can be spaced 160-180 cm between rows and 50-60 cm on the rows, giving 1,1-1,6 plants per m^2. *L. x intermedia* is normally planted at a distance of 160-200 cm between rows and 60-80 cm on the row, with 0,7-1,1 plants per m^2. Indeed, lavandin is generally grown due to the high vigour, productivity and uniformity of the cultivation (Voltolina, 2017).

Interventions throughout the seasons include tillage operations for weed removal and fertiliser application. Among fertiliser applications, nitrogen, phosphorous and magnesium play a major role. Nitrogen availability is fundamental for vegetative development and the accumulation of essential oil. Phosphorous regulates flowering. Potassium favours the formation of the basal structure and the plant vigour. Magnesium activates the physiological processes and contributes to the quality and quantity of essential oil yields (Voltolina, 2017).

Harvest can be performed from the second year, in June or July. Timing is crucial when harvesting for essential oil production. It has been observed that lavender is normally ready for distillation when, on average, half of the petals on flowers have wilted. Additionally, Aprotosoaie et al. (2017) highlighted that the optimal harvest period is characterised by a minimum temperature of 26°C and no precipitations in the previous ten days. In general, flowers are cut with 6 to 10 cm of stalk, which favours an even steam flow through the herb charge in the still. Harvesting of flower heads can be executed by hand, by adapted instrumentation, usually requiring an operator, or by mechanical harvesters. After harvest, lavender is either directly subjected to distillation or dried on the field for a few days before undergoing distillation. Modern mechanical harvesters are arranged to load the flowers directly into large distilling vessels, thus avoiding additional manipulation of the herbs (Denny, 2002).

Full productivity is achieved from the fourth growing season. Fresh flower yields have been estimated at 500-1.000 kg/1.000 m^2 for *L. angustifolia* and 800-1.500 kg/1.000 m^2 for *L. x intermedia*. After drying, yields decrease to 80-150 kg/1.000 m^2 (Voltolina, 2017). Oil yield has been estimated at 40 kg/ha for *L. angustifolia*, and 120 kg/ha for *L. x intermedia* (Lis-Balchin, 2002b). Performing distillation of fresh product, *L. angustifolia* essential oil yields have been reported between 0,5 and 1,5%, with some varieties reaching up to 6,8% in the USA. Yields of *L. x intermedia* have been reported around 3% in recent literature (Aprotosoaie et al., 2017).

1.3 The chemistry of lavender essential oil

L. angustifolia and *L. x intermedia* are largely important for essential oil production. Studies on the phytochemistry of the species have identified over 100 constituents in lavender oil, with a high variability both in terms of composition and substances' concentration. Nevertheless, all oils of a certain species can be characterised due to the presence of some major components, mainly terpenoids. A set of ISO quality standards specifies quality requirement for the chemical composition of lavender essential oils. The standard ISO 3515:2002 establishes that *L. angustifolia* essential oil is mainly characterised by linalyl acetate, linalool, β-ocimene and lavandulyl acetate. The standard ISO 8902:2009 defines that *L. x intermedia Grosso* essential oil contains high amounts of linalool, linalyl acetate, camphor and 1,8-cineole (Aprotosoaie et al., 2017). The main constituents of lavender and lavandin essential oils are represented in Figure 1.2.

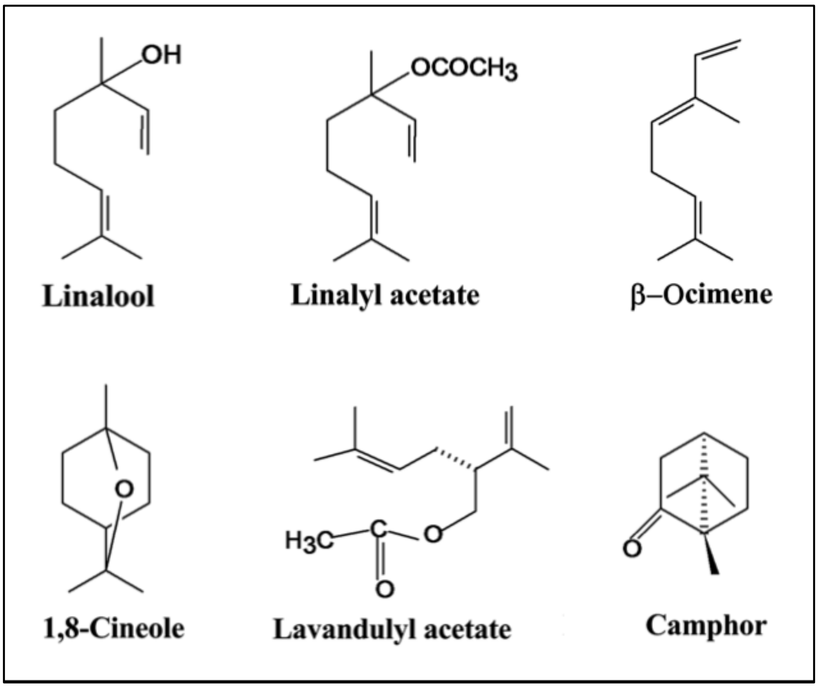

Figure 1.2 Chemical structure of the main constituents of *L. angustifolia* and *L. x intermedia* essential oils (Aprotosoaie et al., 2017)

Terpenoids are a group of naturally occurring compounds deriving from isopentenyl diphosphate (IPP) and dimethylallyl diphosphate (DMAPP), produced by the 2-C-methyl-D-erythritol-4-phosphate (MEP) and mevalonate (MVA) pathways. These substances are composed of isoprene (2-methylbutane) units. Isoprene (C_5) combines in different structures, to form hemiterpenoids (C_5), monoterpenoids (C_{10}), sesquiterpenoids (C_{15}), diterpenoids (C_{20}), and so on. Baser and Buchbauer (2020) stated that only hemiterpenoids, monoterpenoids and sesquiterpenoids are characterised by a sufficient degree of volatility to be constituents of essential oils. Degradation products of terpenoids with higher numbers of carbon atoms per molecule can be present in essential oils.

Monoterpenoids (C_{10}) can exceed 90% of *L. angustifolia* and *L. x intermedia* essential oil (Aprotosoaie et al., 2017). These molecules are synthesised in the plastid from geranyl pyrophosphate (GPP), by geranyl pyrophosphate synthase (GPPS), and can be distinguished into different groups. Terpenoid hydrocarbons include myrcene, α-ocimene, β-ocimene, limonene, p-cymene, α-phellandrene, β-phellandrene, α-pinene, β-pinene, carene, camphene. Monoterpenoid alcohols are further discriminated into acyclic or cyclic. Acyclic monoterpenoid alcohols include geraniol, nerol, citronellol and linalool. Linalool is a naturally occurring molecule produced by allylic hydrolysis of geranyl pyrophosphate, and is further modified by the addition of an acetyl group, leading to the formation of linalyl acetate (Woronuk et al., 2011). Together with its acetate form, linalyl acetate, linalool contributes to the characteristic scent of lavender oils. Cyclic monoterpenoid alcohols involve α-terpineol, terpinen-4-ol, isopulegol, borneol, isoborneol. Moreover, 1,8 cineole is classified as monoterpenoid ether. Geranial, neral and citronellal are monoterpenoid aldehydes. Finally, carvone, D-isomenthone, pulegone, piperitone, camphor and fenchone are grouped as monoterpenoid ketones (Başer & Buchbauer, 2020). Despinasse et al. (2017) reported that borneol and camphor are synthesised in lavender from bornyl diphosphate (BPP), derived in turn from geranyl pyrophosphate (GPP). Functional characterisation has shown that bornyl diphosphate synthase of lavender (LaBPPS) catalyses the production of bornyl diphosphate (BPP), which undergoes dephosporylation leading to the production of borneol, further oxidised in camphor by a borneol dehydrogenase (LiBDH).

Sesquiterpenoids (C_{15}) are synthesised from farnesyl pyrophosphate (FPP), formed by the action of farnesyl pyrophosphate synthase (FPPS) in the cytosol. The process leads to a variety of structural rearrangements and cyclisations. Classification of sesquiterpenoids can be structured based on the biosynthetic pathway used for the cyclisation reactions from either (*Z*,*E*)-farnesyl pyrophosphate or (*E*,*E*)-farnesyl pyrophosphate. Sesquiterpenoids derived from (*Z*,*E*)-farnesyl pyrophosphate biosynthetic pathways include β-caryophyllene, bisabolol, α-santalol and β-santalol. Sesquiterpenoids derived from (*E*,*E*)-farnesyl pyrophosphate biosynthetic pathways comprise *trans*-humulene, α-humulene, guaiol. Among sesquiterpene alcohols, farnesol, nerolidol and bulnesol can be recognised. Eventually, α-gurjunene is identified among sesquiterpenoid hydrocarbons (Başer & Buchbauer, 2020).

1.4 Ecological functions of lavender essential oils

Essential oils are complex mixtures of chemicals produced in the plant's biosynthetic pathways as secondary metabolites.

Li et al. (2021) investigated the role of terpenoid biosynthesis in lavender, focusing on the ecological functions of the main volatile compounds produced by flowers, leaves and stems. Lavender secondary metabolites are produced and stored within glandular trichomes (GTs), consisting of eight secretory cells and one secretory cavity. These secretory structures are present more abundantly on flowers, but also on leaves and stems. As a matter of fact, analyses performed through SPME coupled with GC-MS detected variable levels of terpenoids among different plant organs and during distinct developmental stages (Li et al., 2021).

Moreover, Li et al. (2021) recognised the ecological functions of secondary metabolites as a "chemical language" for the plant's interaction with the surrounding environment. In particular, linalool, linalyl acetate, and lavandulyl acetate are produced in flowers for the attraction of pollinators. This function is of primary importance for lavender reproduction since insect pollinators are necessary to perform cross-pollination. Moreover, α-pinene, β-pinene, β-ocimene, germacrene D, *(E)*-β-farnesene and limonene, produced in flower buds, are induced upon herbivores' and predators' attacks. Finally, in leaves and stems, borneol, camphor, 1,8-cineole, camphene, and bornyl acetate act as repellents to insect pests, due to their spicy smell (Li et al., 2021).

In conclusion, the previous considerations suggest that an array of ecological mechanisms influence the chemical composition of lavender essential oils, by altering the plant's biosynthetic pathways. It has been established that variations are induced by biotic stresses, by interactions among plants or by communication between plants and other organisms.

1.5 The distillation process for lavender essential oil production

The term “distillation” derives from the latin word “destillare”. This word is composed of “de”, meaning “downward”, and “stillare”, from “stilla”, meaning “drop”. The etymology of this term recalls the meaning of trickling down a liquid drop by drop (Başer & Buchbauer, 2020). Distillation is defined as the process of extracting and slowly purifying a liquid, through a mechanism of evaporation and subsequent condensation.

Purification, or separation, of a liquid occurs through the processes of evaporation and condensation. First, evaporation involves the transformation of liquid into vapour, through heating. In relation to the specific boiling temperature, liquids evaporate to a greater or lesser degree when subjected to an increase in temperature. Subsequently, condensation entails the transition from gas to liquid phase, under cooling conditions.

The isolation of volatile essential oils from aromatic plants is performed by distillation with steam or boiling water, i.e. hydrodistillation. In this particular case, distillation involves two immiscible liquids, that form two separate phases (Günther, 1948). The process is based on specific properties of the substances, such as volatility, boiling temperature and vapour pressure.

The theory of distillation relies on Dalton’s law, which states that, considering a mixture of two immiscible liquids, the total vapour pressure of the system is equal to the sum of the partial vapour pressures of the two individual liquids. As the mixture is gradually heated, the vapour pressure of each component increases, thus leading to an increase in the total vapour pressure. When the total vapour pressure is equal to the pressure around the liquid, boiling begins. The boiling point of a liquid is defined as the temperature at which the vapour pressure of the liquid equals the pressure around the liquid. In the case of immiscible liquids, the boiling temperature of the mixture is lower than the boiling temperature of the two individual components. Therefore, boiling begins when the temperature of the mixture reaches the boiling temperature of the component with the lowest boiling point (Günther, 1948).

For instance, a mixture of water vapour and linalyl acetate starts boiling at a temperature of 99.6°C, under normal atmospheric pressure. If considered singularly, the boiling temperature of water is 100°C, while the boiling temperature of linalyl acetate is 226°C.

Concurrently, at 99.6°C, water exerts a vapour pressure of 748 mmHg, while linalyl acetate exerts a vapour pressure of 12 mmHg. As these two immiscible substances are mixed, the boiling point of the mixture lies below the boiling temperature of water, which is the component with the lowest boiling temperature. Indeed, the mixture exerts a total vapour pressure of 748 + 12 = 760 mmHg. Considering that the atmospheric pressure is considered equal to that at the bottom of a column of mercury 760 cm tall, thus measuring 760 mmmHg, the mixture of water and linalyl acetate starts boiling at 99.6°C. For this reason, hydrodistillation has been widely applied for essential oil extraction. Indeed, maintaining a lower temperature allows preventing damage and degradation of essential oils caused by overheating (Denny, 2002).

The composition of the vapour phase is given by the molecular weights and the vapour pressure of each substance. However, in the case of essential oils, the proportionality between the quantity of oil and water is nearly impossible to calculate. Essential oils are, in fact, a mixture of different components in variable proportions (Başer & Buchbauer, 2020). These components are associated with different values of partial vapour pressure and, therefore, with different levels of volatility. Volatility is defined as the tendency of a liquid to vaporise and is positively related to the vapour pressure of the substance. Volatile substances are characterised by higher vapour pressure and lower boiling points (Günther, 1948). In the course of the distillation process, essential oil compounds vaporise according to their specific volatility. First, lighter and more volatile compounds undergo earlier vaporisation; gradually, compounds characterised by lower volatility are converted into vapour towards the latter stages of distillation.

The processes of steam distillation can be further classified into two distinct methods: water and steam distillation and direct steam distillation (Fig. 1.4). In the process of water and steam distillation, both water and plant material are placed inside the still. A perforated grid is placed within the still. The bottom part of the still is filled with water, up to 1-2 centimetres below the level of the grid, and the plant material is distributed above. Differently, the direct steam distillation method requires a separated boiler, to generate steam. Indeed, water is not present within the still, but the steam flow is introduced from the bottom of the still and proceeds beyond the supporting grid and throughout the charge.

Steam distillation is one of the most frequently used methods for lavender essential oil production. Several types of devices are currently available, from more traditional and essential methods to more complex and technologically advanced equipment (Başer & Buchbauer, 2020). Variations in size, capacity and material must be evaluated according to the extent of the operation and the distillation method to be adopted. The basic design of a device for steam distillation generally consists of three parts: the still, the condenser, and the receiver for the condensate. In the case of direct steam distillation, a boiler for generating steam is also necessary, together with a steam coil that directs the steam flow below the still. Instead, for water and steam distillation, a heat source is required (Günther, 1948).

The still pot, including a perforated grid, is generally a cylindrical container or tank, variable in diameter and height, closed by a lid. The plant material is distributed randomly above the grid and carefully pressed. An irregular and tight arrangement of the plant material ensures a homogeneous distribution of the steam through the plant material. The lid, equipped with a water seal, is characterised by a gooseneck outlet, connected to a condenser (Başer & Buchbauer, 2020).

Condensers are available in different types and shapes. These devices are designed for cooling the vapour mixture exiting the still, based on the surface available for heat removal and the temperature difference between the vapour and the cooling medium. The internal part of a condenser includes one single tube or a series of long pipes, of tubular or spiral shape, with vapours entering from the top and leaving from the bottom. Cooling water is introduced from the bottom of the condenser, flows opposite the vapour direction and is then discarded (Günther, 1948). Upstream water flow ensures that the temperature differential between the cooling water and the condensing vapour remains constant along the condenser.

The final major component of a distillation system is the receiver of the condensate, or oil separator, such as Florentine flasks. The two products of distillation, essential oil and aromatic water, or hydrolat, separate within the receiver due to differences in specific gravities. Since most essential oils have a higher boiling point and a lower vapour pressure as compared to water, the ratio between water and essential oil in the condensate collected from distillation is relatively high. In the case of water and steam distillation, the condensed water can be redirected into the still, to be recycled as a water supply. In the process of direct steam distillation, this procedure is not recommended. However, distillation water can be recovered through the method of cohobation, i.e. redistillation of the condensed water (Günther, 1948).

Extraction of surface essential oils from plant material is strongly influenced by the degree of solubility of the oil components in water. This mechanism is defined as hydrodiffusion. In particular, due to the higher temperatures, essential oil dissolves in the water within the oil glands and permeates by osmosis through the plant membranes. Once the volatile substances reach the surface, they are progressively vaporised by the steam flow and replaced by water particles (Günther, 1948). This mechanism, together with the elaborate shape of lavender flowers, promotes hydrodiffusion and therefore facilitates the extraction of essential oil from the plant material.

Ultimately, attention should be drawn to the vapour characteristics, with regard to lavender essential oil extraction. Lavender essential oils are classified as superficial oils as they are produced on absorptive surfaces. As mentioned in the previous section, glandular trichomes are located on the plant surface tissues and characterised by a hairy structure, that makes the herb highly absorptive. At the beginning of the distillation process, steam particles condense on the herb surfaces. Concurrently, the oil glands burst and create patches of homogeneous oil on the surface of the plant tissues. Since water and oil are immiscible, they can come in contact exclusively in the perimetral area surrounding the oil patches. At this oil-water interface, the plant oil and the condensed water intermingle by capillary action and the oil can be vaporised. Appropriate moisture conditions have to be achieved to optimise the extraction process. On one side, a sufficient amount of water is required on the herb surface, to recover the surface oil patch as the oil is vaporised. On the other side, water excess has to be avoided, in order to maintain the capillary action without hindering the plant's absorptive capacity. Simultaneously, lavender moisture contents vary throughout the distillation period. Flowers tend to be sappy early in the season, becoming drier and very absorptive towards the end. For this reason, the possibility of adjusting steam's moisture content might improve substantially the efficiency of the distilling operations (Denny, 2002).

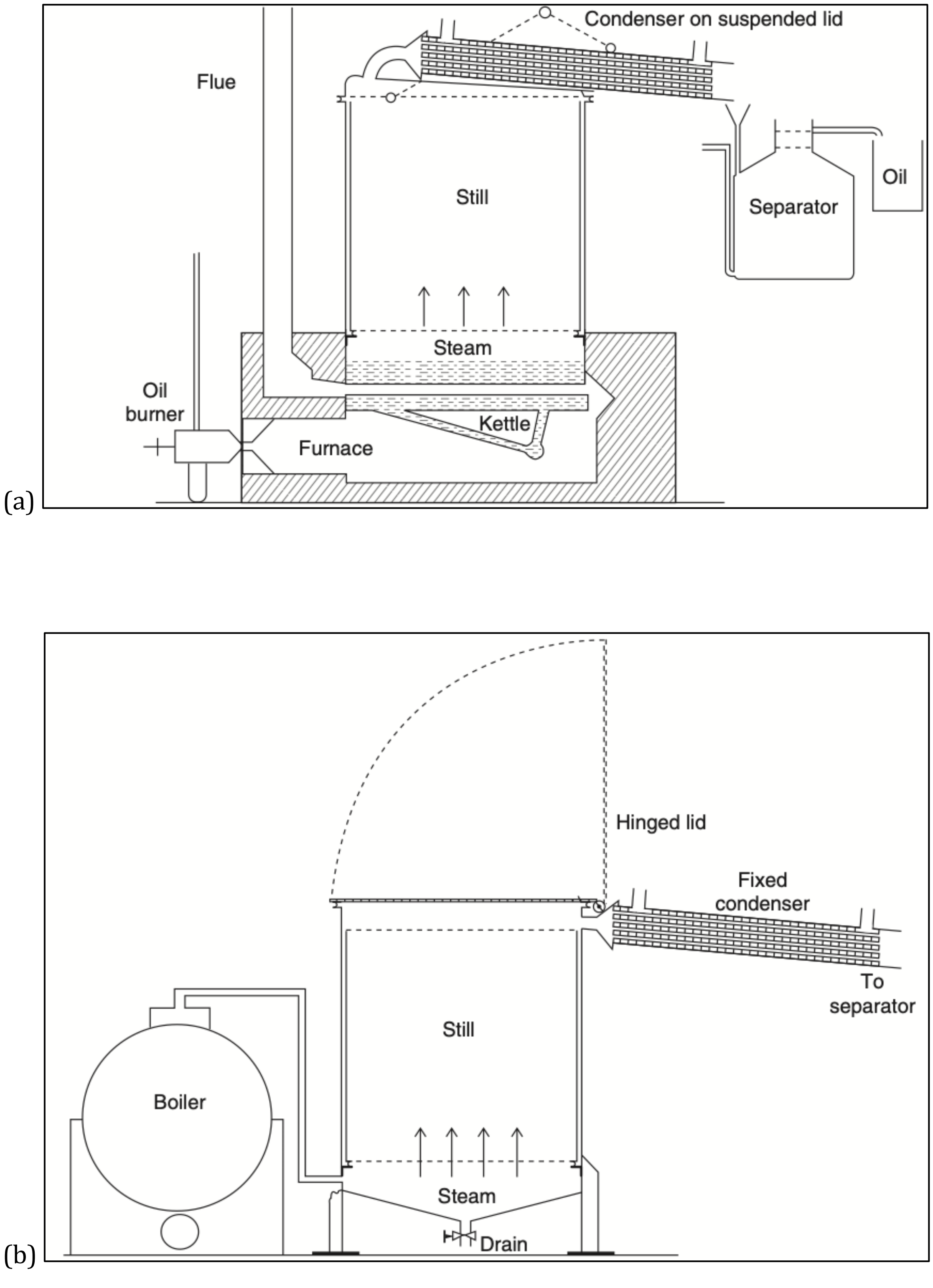

Figure 1.4 Scheme of distillation equipment for water and steam distillation (a) and direct steam distillation (b) (Denny, 2002)

1.6 Lavender essential oil usage and properties

Thanks to their therapeutic properties, essential oils of *L. angustifolia* and *L. intermedia* are widely adopted in the pharmaceutical industry, but also in aromatherapy and herbal medicine (Buchbauer, 2002). They are implemented in the cosmetics and perfumery industries, for perfumes, hygiene and cleaning products. Their use has also been documented within the food industry (Lis-Balchin, 2002d). Essential oils and essential oil-based products are marketed worldwide by wholesalers, extraction laboratories, cosmetics companies, perfumeries and pharmacies (Voltolina, 2017).

L. angustifolia essential oil is characterised by a floral, sweet and fresh scent, with a pleasant, balsamic undertone. It is almost colourless, usually acquiring light yellowy shades. Besides, this pleasant oil is largely used in the perfume industry, often blended with other oils such as bergamot or rosemary, or with other aromachemicals such as citronellol, geraniol and musks. *L. x intermedia* essential oil expresses a more herbaceous odour, with a camphene-cineole top note. This oil ranges from light yellow to almost colourless. It is commonly used in the perfume and cosmetics industries, blended with other products such as clove oil, citronella and patchouli (Lis-Balchin, 2002c).

Essential oils extracted from *Lavandula* species are generally characterised by antimicrobial, antifungal, and antioxidant effects, with minor acaricidal and insecticidal action. Lavender essential oils are known universally for their calming properties (Buchbauer, 2002). Experiments verified that *L. angustfolia* essential oil has a relaxing and spasmolytic effect, by acting on the cardiovascular and central nervous systems. Moreover, lavender oils show low toxicity. In fact, researchers demonstrated that undiluted lavender essential oil could be used for delicate skin issues, with minor events of allergic reactions (Hart & Lis-Balchin, 2002). Furthermore, recent studies investigated the neuropsychological effects of lavender essential oils, mainly related to their calming and sedative properties (Kirk-Smith, 2002).

1.7 Variability of essential oils

Variability in the chemical composition of lavender essential oil is due to a series of genetic and environmental factors. Genetic variability and instability, together with variations in temperature, water supply, altitude, season, geography and agronomic management, strongly influence the chemical composition of the oil (Lis-Balchin, 2002c).

Genetic diversity within the genus *Lavandula* has been widely investigated and breeding programs have been created for oil yields and quality (Van Oost et al., 2021). Registration of *Lavandula* varieties is currently regulated by the European Community Plant Variety Office (CPVO) CPVO/TP-194/1-Rev protocol, which establishes morphological descriptors mainly directed to the sections *L. angustifolia*, *L. stoechas*, *L. pinnata* and to intersectional hybrids. However, the combination of morphological and molecular marker, together with biochemical profiles might overcome the limits of phenotypic characterisation (Scariolo et al., 2021). Several studies investigated the phylogenetic relationship among and within *Lavandula* species (Moja et al., 2016; Scariolo et al., 2021; Van Oost et al., 2021). Codominant molecular markers, among which Simple Sequence Repeat (SSR) and Single Nucleotide Polymorphism (SNP) markers, have been successfully implemented for the investigation of genomic regions and biosynthetic pathways associated to the synthesis of essential oils (Scariolo et al., 2021). Moreover, Van Oost et al. (2021) analysed genetic distance, genome size and chromosome numbers of a collection of 82 *Lavandula* genotypes, employing Amplified Length Polymorphism (AFLP) markers. Sources of genetic variation within the *Lavandula* genus have been highlighted. Specifically, the study demonstrated that the occurrence of frequent hybridisation events resulted in a considerable variation in genome size and chromosome number, and that polyploidy was detected in several genotypes, confirming its major role in the evolution of the genus. For the purpose of the present experiment, the phylogenetic analysis of *L. angustifolia* varieties has been considered, with particular attention to *L. angustifolia Munstead* and *L. angustifolia Little Lady*. At the genus level, clusters related to different species were clearly identified, but a certain degree of variability was detected by Principal Coordinate Analysis (PCoA). Within the main cluster of *L. angustifolia* varieties, *Munstead* and *Little Lady* were pinpointed in close proximity to one another, thus confirming a low genetic variability between those samples (Van Oost et al., 2021). Further study on lavender genetic variability was conducted by Zagorcheva et al. (2020), who specifically investigated genetic diversity among 10 Bulgarian and 5 foreign varieties by means of Sequence-Related Amplified Polymorphism (SRAP) markers. Among foreign varieties, *Munstead* and *Melissa Lilac* have been identified for the purpose of the present experiment. The phylogenetic tree constructed from SRAP data gathered all foreign varieties in one single cluster, suggesting a low level of genetic diversity among these samples (Zagorcheva et al., 2020). Abreast of phylogenetic studies, associations between genotypes and chemotypes have been identified, but further research would endorse markers' identification for breeding selection (Scariolo et al., 2021).

Furthermore, variations in essential oil chemical compositions and substance concentrations have been associated with different environmental influences (Harborne & Williams, 2002). Agronomically relevant factors, such as climate, soil type, drought, water stress, biotic stress, propagation method and cultivation practices, have been discussed in relation to their effect on the production essential oils (Başer & Buchbauer, 2020).

Climate affects the plant's physiology and biochemistry, according to specific light, temperature and humidity requirements. It has been reported that the cultivation of lavender in Provence has been challenged by drastic temperature changes in the latter years. Too cold weather at the beginning of the growth period followed by very hot weather and lack of water led to a decrease by 1/3 in essential oil yield. Also, too cold weather at flowering negatively affects pollination and plant metabolism, thus decreasing essential oil yields (Başer & Buchbauer, 2020). Appropriate water supply is essential for ensuring metabolic functions while hindering fungal growth in the root zone (Başer & Buchbauer, 2020). However, a study performed at the Gorgan University of Agricultural Science investigated the influence of drought stress on the plant metabolism, including the production of secondary metabolites. In this experiment, *L. angustifolia Munstead* and *Hidcote* resulted in high essential oil yields and dry weight of aerial part under drought conditions. Moreover, changes in essential oil compositions have been observed in relation to genotype and drought level (Gorgini Shabankareh et al., 2021). The influence of soil texture, structure and pH on lavender cultivation and essential oil production has been widely assessed. For instance, prior research documented the effect of different soil types on the production of different lavender essential oils in Provence and Bulgaria. As illustrated in the previous section, lavender essential oils, as secondary metabolites, have a role in the eco-physiological behaviour of the plant (Başer & Buchbauer, 2020). Some compounds are induced upon herbivores' and predators' attacks and act as repellents to insect pests (Li et al., 2021). Therefore, variation in essential oil composition can be detected in plants under biotic stress. Similarly, the presence of pollinating agents influences both quality and quantity of essential oils, since flowers enhance the production of volatile compounds in order to attract pollinators. Intraspecific differences in chemical composition have been related to adaptation processes in terms of natural selection (Başer & Buchbauer, 2020).

Cultivation practices, mainly related to planting and harvest, might also influence the production of essential oil. Planting has been generally associated with alterations in oil yield and composition, in relation to timing and crop establishment decisions. Breeding and propagation methods might influence the composition of the oil, in relation to the use of seeds or cuttings. In the case of *L. angustifolia*, plants grown from seeds might produce larger genetic variation within the progeny. On the contrary, asexual propagation by cuttings, which is necessarily applied to *L. x intermedia*, leads to the generation of clones. In the first case, the essential oil composition might differ from one genotype to another. In the second case, progeny plants produce essential oils which result very similar to those of the parental lines. As previously explained, the timing and method of harvesting have to be properly determined, according to the plant life cycle, especially with the purpose of distillation. Simultaneously, drying before distillation might lead to significant modifications in essential oil yield and composition. Although lavandin flowers used to be dried in the field before distillation, in order to obtain a pleasant, floral scent, current harvesting techniques and immediate distillation result in higher yields. The odour that lavender essential oils acquire after immediate processing is characterised by greener notes but adjusts after a period of maturation (Başer & Buchbauer, 2020).

Ultimately, an interaction between genotypic and environmental condition further influences the essential oil quantity in lavender. As reported by Aprotosoaie et al. (2017), varieties of *L. angustifolia* from four locations in Bulgaria produced high essential oil yields only in optimal pedo-climatic conditions, while other cultivars achieved constant production despite less favourable environmental conditions.

2 Objectives

The project aims at evaluating the influence of genotypic and environmental factors on the chemical composition of lavender essential oils.

The primary objective is to assess the effect of distinct locations, varieties and years of production on the variation in the chemical composition of lavender essential oils.

Further investigation explores the consumers’ perception of lavender essential oils variability.

This study lays the foundations for the constitution of a global database collecting essential oils of *Lavandula* species and varieties.

3 Materials and methods

3.1 Experimental layout

The experimental layout of the present project has been designed to compare and evaluate simultaneously a series of variables, related to the samples that have been analysed. Variables have been identified and gathered into a table including all sample details. All samples have been collected, numbered and subjected to gas chromatography and statistical analysis.

Aa series of variables have been identified to compare and evaluate simultaneously the different characteristics of the samples. These variables have been gathered into a table including all sample details (Table 3.1). A complete description of all variables is available in the following paragraphs. Furthermore, an identification code has been assigned to each sample, to easily summarise all sample details.

3.1.1 Location

The site of Arquà Petrarca has been selected as the main location for the present experiment. Additional locations have been selected, based on similar cultivation practices and distillation methods.

a. Arquà Petrarca, Padova, Veneto, Italy. Lavandeto di Arquà Petrarca is located in the village of Arquà Petrarca, on the Euganean hills, at an altitude of 20 m a.s.l. This lavender farm boasts a collection of more than 100 lavender varieties. A selection of 20 varieties, both of *L. angustifolia* and *L. x intermedia*, included in this experiment, have been grown in separate plots. Harvest has been performed with the aid of hedge trimmers.
b. Asiago, Veneto, Italy. One sample has been obtained from a lavender cultivation in Asiago. In this particular case, lavender has been subjected to distillation in Arquà Petrarca after drying.
c. Bovolenta, Padova, Veneto, Italy. Azienda Agricola Va Oltre is located in the village of Bovolenta, near Padova, at an altitude of 6 m a.s.l. *L. x intermedia Grosso* has been

planted in rows shaped in the form of a labyrinth. Harvest has been performed with the aid of hedge trimmers. Distillation has been performed in Arezzo.

d. Arezzo, Toscana, Italy. One sample has been obtained from a lavender cultivation in Arezzo, located at 296 m a.s.l.
e. Kinross, Scotland, UK. Scottish Lavender Oils is registered at Tarhill Farm, in Kinross, located at 500 m a.s.l. Essential oils of 4 lavender varieties have been obtained from this farm.
f. Santa Maria in Stelle, Verona, Veneto, Italy. Pernigo Creative Organic Farm is a multifunctional farm located in the village of Santa Maria in Stelle, in a hilly area close to Verona. Two varieties of *L. angustifolia* have been grown organically. Harvest has been performed with the aid of hedge trimmers.
g. San Canzian d'Isonzo, Gorizia, Friuli-Venezia Giulia, Italy. Le Officinali di Eleonora is a medicinal plant farm located near Gorizia, at 8 m a.s.l. Samples received from this farm include 3 essential oils of both *L. angustifolia* and *L. x intermedia* varieties.
h. Porto Tolle, Rovigo, Veneto, Italy. Lavanda Polesana is a lavender farm located at 1 m a.s.l. One sample of *L. angustifolia* has been obtained from this location.

N.	Location	Species	Variety	Year	CODE
1	Arquà Petrarca	Lavandula angustifolia	Maillette	2021	A21AP Maillette
2	Arquà Petrarca	Lavandula angustifolia	Blue Mountain White	2021	A21AP Blue Mountain White
3	Arquà Petrarca	Lavandula angustifolia	Miss Katerine	2021	A21AP Miss Katherine
4	Arquà Petrarca	Lavandula angustifolia	Elizabeth	2021	A21AP Elizabeth
5	Arquà Petrarca	Lavandula angustifolia	Royal Purple	2021	A21AP Royal Purple
6	Arquà Petrarca	Lavandula angustifolia	Melissa Lilac	2021	A21AP Melissa Lilac
7	Arquà Petrarca	Lavandula angustifolia	Imperial Gem	2021	A21AP Imperial Gem
8	Arquà Petrarca	Lavandula angustifolia	Hidcote Blue	2021	A21AP Hidcote Blue
9	Arquà Petrarca	Lavandula angustifolia	Contrast	2021	A21AP Contrast
10	Arquà Petrarca	Lavandula angustifolia	Loddon Blue	2021	A21AP Loddon Blue
11	Arquà Petrarca	Lavandula angustifolia	Bleue Velours Charles	2021	A21AP Bleue Velours Charles F
12	Arquà Petrarca	Lavandula angustifolia	Bleue Velours Paul	2021	A21AP Bleue Velours Paul D
13	Asiago	Lavandula angustifolia	Bleue Velours Paul	2021	A21AP Bleue Velours Paul
14	Arquà Petrarca	Lavandula angustifolia	Mix	2021	MA21AP
15	Arquà Petrarca	Lavandula x intermedia	Grosso	2021	H21AP Grosso
16	Arquà Petrarca	Lavandula x intermedia	Provence	2021	H21AP Provence
17	Arquà Petrarca	Lavandula x intermedia	Hidcote Giant	2021	H21AP Hidcote Giant
18	Arquà Petrarca	Lavandula x intermedia	Silver Dwarf	2021	H21AP Silver Dwarf
19	Arquà Petrarca	Lavandula x intermedia	Mix	2021	MH21AP
21	Arquà Petrarca	Lavandula angustifolia	Mix	2020	MA20AP
22	Arquà Petrarca	Lavandula angustifolia	Mix	2020	MA20AP
23	Bovolenta	Lavandula x intermedia	Grosso	2020	H20BO Grosso
24	Bovolenta	Lavandula x intermedia	Grosso	2021	H21BO Grosso
25	Arezzo	Lavandula x intermedia	Grosso	2021	H21AR Grosso
26	Scozia	Lavandula angustifolia	Munstead	2021	A21SC Munstead
27	Scozia	Lavandula angustifolia	Little Lady	2021	A21SC Little Lady
28	Scozia	Lavandula angustifolia	Folgate	2021	A21SC Folgate
29	Scozia	Lavandula x intermedia	Grosso	2021	H21SC Grosso
30	Verona	Lavandula angustifolia	Buena Suerte	2019	A19PE Buena Suerte
31	Verona	Lavandula angustifolia	Hidcote Superior	2021	A21PE Buena Suerte
32	Gorizia	Lavandula angustifolia	Maillette	2020	A20GO Maillette
33	Gorizia	Lavandula angustifolia	Maillette	2021	A21GO Maillette
34	Gorizia	Lavandula x Intermedia	Grosso	2020	H20GO Grosso
35	Porto Tolle	Lavandula angustifolia	Buena Suerte	2021	A21FR Buena Suerte

Table 3.1 Variables for each sample included in the experiment.

3.1.2 Species and variety

Essential oils from a total of 20 varieties of *L. angustifolia* and *L. x intermedia* species have been analysed and compared.

Within the *angustifolia* species, 17 varieties have been included. Details about these varieties are reported below, along with a brief morphological description and, if available, place of origin, originator and probable date of introduction.

a. *Blue Mountain White.* Height, 50 cm. Robust and vigorous plant, characterised by white flowers, with light green calyx. Probably selected in New Zealand (Fig. 3.1a).
b. *Bleue Velours Charles.* Height, 50 cm. Flowers are characterised by bright violet calyx and lighter petals, which create a beautiful colour contrast. Bushes are small and compact (Fig. 3.1b).
c. *Bleue Velours Paul*. Height, 40 cm. This cultivar is similar to the *Bleue Velours Charles* but shows darker flowers (Fig. 3.1c).
d. *Buena Suerte.* This cultivar has been found in two locations included in the present research. In both cases, plants were purchased as *L. angustifolia*. However, classification has been ambiguous due to the presence of branched flowers (Fig. 3.1d).
e. *Contrast.* Height, 60 cm. The name is related to the contrasting colour of the flowers, characterised by bright purple calyx and light lilac petals (Fig.2.2e).
f. *Elizabeth*. Height, 70 cm. Characterised by very deep purple flowers and an extended flowering period (Fig. 3.1f).
g. *Folgate*. Height, 60 cm. Purple blue flowers. This cultivar has been introduced in 1933 (Fig. 3.1g).
h. *Hidcote Blue* (UK, before 1950, L Johnson). Height, 50 cm. Very deep violet-blue flowers, with bright calyx and slightly lighter petals (Fig. 3.1h).
i. Hidcote Superior (Fig. 3.1i).

"Hidcote" consists of a group of cultivars, originating from the Hidcote Gardens in London. This well-known group of lavender varieties has been introduced in 1950.

j. *Imperial Gem* (UK, 1980s, Norfolk Lavender). Height, 70 cm. Characterised by very intense flower colour and abundant flowering (Fig. 3.1j).
k. *Little Lady*. Height, 50 cm. This cultivar is characterised by pale lilac-blue flowers and compact shrubs. It is also marketed under the name “Batlad” (Fig. 3.1k).
l. *Loddon Blue* (UK, before 1963, T. Carlile). Height, 70 cm. Very dark violet flowers and silver, aromatic leaves. This cultivar was introduced by Thomas Carlile in the Loddon Nursery, in England, in 1963, together with the *Loddon pink* cultivar (Fig. 3.1l).
m. *Maillette* (France, P. Grosso). Height, 40 cm. Medium size and compact bush, characterised by bright blue calyx, violet petals and silver leaves. Widely used in the perfume industry due to its intense scent (Fig. 3.1m).
n. *Melissa Lilac*. Height, 60 cm. Soft, velvety, indigo blue flowers, with lilac calyx. This cultivar is native to New Zealand (Fig. 3.1n).
o. *Miss Katherine*. Height, 70 cm. This very robust and productive plant, with large and compact spikes and dark pink flowers, is characterised by star markings when opening. This cultivar has been introduced in 1992 (Fig. 3.1o).
p. *Munstead* (UK, 1916, Barr). Height, 45 cm. Medium spikes with light blue-lilac flowers and thin stem (Fig. 3.1p).
q. *Royal Purple* (UK, 1940s, Norfolk Lavender). Height, 70 cm. Long and delicate spikes with violet flowers (Fig. 3.1q).

Figure 3.1 *L. angustifolia* varieties. (a) *Blue Mountain White*, (b) *Blue Velours Charles*, (c) *Blue Velours Paul*, (d) *Buena Suerte*, (e) *Contrast*, (f) *Elizabeth*, (g) *Folgate*, (h) *Hidcote Blue*, (i) *Hidcote Superior*, (j) *Imperial Gem*, (k) *Little Lady*, (l) *Loddon Blue*, (m) *Maillette*, (n) *Melissa Lilac*, (o) *Miss Katherine*, (p) *Munstead*, (q) *Royal Purple*. Pictures from Lavandeto di Arquà Petrarca (n.d) (https://www.lavandetodiarqua.it/collections/lavande) and Downderry Nursery (n.d) (https://downderry-nursery.co.uk/)

Among the hybrids, 4 cultivars have been considered. Examined cultivars are reported below, along with a brief morphological description and, where available, place of origin, originator and probable date of introduction.

a. *Grosso* (France, *c*. 1972). Height, 100 cm. Dark violet flowers. This cultivar has been discovered in the French district of Vaucluse and is currently of the most cultivated hybrids, especially in Provence, for essential oil production. Selection has been performed for obtaining a single abundant flowering, suitable for mechanical harvest. This cultivar has been introduced in 1972 (Fig. 3.2a).
b. *Hidcote Giant* (UK, before 1958, L. Johnson). Height, 90 cm. Small, compact bushes, with big, aromatic lilac spikes. Leaves are big, characterised by a light green, hairy surface (Fig. 3.2b).
c. *Provence* (France). Height, 90 cm. Packed spikes, with large lilac flowers. While Lis-Balchin (2002) reports that this cultivar originates from France, the collection of Lavandeto di Arquà Petrarca illustrates that this cultivar has been selected in California, mainly for ornamental use, thanks to its intense scent (Fig. 3.2c).
d. *Silver Dwarf*. Height, 120 cm. Grey bush, with elongated lilac spikes (Fig. 3.2d).

Figure 3.2 *L. x intermedia* varieties. (a) *Grosso*, (b) *Hidcote Giant*, (c) *Provence*, (d) *Silver Dwarf*.

Information related to the examined varieties has been collected from different sources, including Lavandeto di Arquà Petrarca collection, UK Royal Horticultural Society catalogue and works by Charlesworth, Lis-balchin and Upson (2002).

3.1.3 Year of production

All samples from the main site of Arquà Petrarca have been distilled in 2021. Samples from different years of production have been included for comparison. In particular, 28 samples from 2021, 5 from 2020 and 1 from 2019 have been analysed.

3.1.4 Distillation method

The essential oils analysed in the present study have been extracted by means of steam distillation, a mechanism that relies on the theoretical considerations provided in the introduction. Although different distillation equipment has been applied in different locations, all devices operate a similar distillation process and their essential oil products can be correctly compared. In the main location of Arquà Petrarca, distillation has been performed in July 2021. The same still has been used for extracting all essential oil samples, that have been separately subjected to the distillation process. Albrigi essential oil extractor, 12 lt capacity, has been adopted for the experiment. Essential oil yields of each variety have been measured and noted.

3.1.5 Sample preparation

After distillation and maturation, small samples of essential oils have been collected from each location between December and January 2022. Samples have been numbered and stored at low temperatures, in order to avoid degradation.

3.2 Gas chromatographic analysis

The chemical composition of the essential oils has been analysed through gas chromatography by a GC 7890B equipped with an Agilent J&W DB-5MS column of 60 m or 30 m in length, 320 μm internal diameter, 0,50 μm film thickness (Fig. 3.3). Samples 1-21 have been analysed with a 60 m column. Samples 22-35 have been analysed with a 30 m column. Analysis of samples 14-17 has been repeated with the 30 m column. The column has been changed since the analyses of some samples were not performed correctly, and results were not suitable to a proper interpretation.

The equipment is provided with software for GC system control, capillary columns support, split-splitless injection system with 120:1 split ratio. Injection system has been set at 250°C. The programmable gas chromatography oven has been set up with the following program: initial temperature 40°C, 1 min hold time, 2°C increase up to 100°C, 7 min hold time, 4°C increase up to 250°C, 4.5 min hold time, for a total run time of 70 min, hydrogen carrier gas with 2 ml/min constant flow. The GC equipment is connected to a VUV (Vacuum Ultraviolet) detector, VGA-101, by VUV Analytics. This vacuum ultraviolet detector features an expanded wavelength spectrum of 120-430 nm and a higher operating temperature, reaching as high as 430°C.

Single chromatograms, produced for each sample, have been visualised and elaborated by VUVision Data Analysis Software by VUV Analytics. Chromatograms depict a two-dimensional representation of all detected components, with the retention time (RT) on the x-axis and the concentration on the y-axis. The area of each peak represents the concentration of the corresponding molecule within the sample. After measuring the area of each peak, the software compares the absorbance of the detected substance with the spectral data present in the library, in order to recognise and name the molecule. In case of incomplete or insufficient correspondence, the Kovats index has been integrated for further comparison. Moreover, final recognition has been based on the elution time (min) of the molecule. On the basis of these indices, peaks have been identified and named after the corresponding molecule.

3.3 Chemometric analysis

3.3.1 Data analysis

Data collection and preliminary processing have been performed with Microsoft Excel (v. 2112). A series of Excel tables has been constructed to select and order data in preparation for the statistical analysis.

Values of the peaks' areas have been collected on a first table. Since two distinct columns have been implemented for the gas chromatographic analyses, data have been collected separately according to the method used. The total area has been reported for each sample. Molecules lacking identification, but present repeatedly within different samples, have been labelled as "Unk", i.e. Unknown, and have been numbered progressively. A limited number of peaks with insufficient identification has been excluded from the final results. Subsequently, the concentration of each molecule has been calculated as a percentage of the total area for each sample. Once weighed concentrations have been obtained, tables related to each of the two gas chromatography columns have been merged, to operate a proper comparison among all samples.

3.3.2 Statistical analysis

The statistical analysis has been performed by StatGraphics 19. Multiple Variable Analysis, Principal Component Analysis (PCA) and Hierarchical Cluster Analysis (HCA) have been applied for investigating associations between molecules and relationships among all essential oil samples, based on their chemical compositions.

Through a Multiple Variable Analysis, correlations have been computed on the complete set of molecules, except for the water content value. All data produced in the Multiple Variable Analysis have been downloaded from Statgraphics 19 and further elaborated in Excel. Significance levels of correlation coefficients (r) have been evaluated with $p<0,05$ and significant values have been highlighted. Interpretation has been focused on those molecules included in the ISO standard 3515:2002 for *L. angustifolia* essential oils, and ISO 8902:2009 for *L. x intermedia*.

Principal Component Analysis (PCA) and Hierarchical Cluster Analysis (HCA) have been widely implemented in chemometric studies, as exploratory unsupervised methods for detecting similarities among samples. The main aim of these multivariate techniques is to assess either presence or absence of clusters, within the dataset, without including information on class membership in the calculations (Granato et al., 2018).

The Principal Component Analysis (PCA) is a statistical test based on factor analysis. This tool has been applied to represent variations in the dataset. In the PCA, principal components are iteratively calculated. The variance explained by each component is progressively computed, with each component explaining a lower percentage of variation than the previous one (Granato et al., 2018). All values of essential oil molecules have been included in the Data Input interface, and sample codes have been set as Point Labels. After a first run, the Minimum Eigenvalue has been increased from 1 to 5, since more than 95% of the variance was explained by the first 5 components, with components 1 and 2 accounting for about 85% of the total variance. For a visual representation, a two-dimensional Scatterplot and a two-dimensional Plot of Component Weights have been selected. Both graphs are constructed with component 1 at the x axis and component 2 at the y axis. The Scatterplot pinpoints all samples as dots on the Cartesian plane, with larger or lesser clustering. The Plot of Component Weights shows a projection of all molecules on the Cartesian plane. Each molecule is connected to the origin by a segment, whose length is an indicator of the influence of each molecule on sample differentiation. The longer the segment, the stronger is the molecule's influence on sample differentiation.

Data concerning component 1 and 2 of the Table of Component Weights and Table of Principal Components have been extracted from Statgraphics 19 and further processed in Excel. Values of Component Weights have been used to calculate the distance of each point from the origin, i.e. the value of the segment length. Then, molecules have been arranged in descending order based on the segment length. Molecules listed in ISO 3515:2002 and ISO 8902:2009 have been identified among the first 28. Therefore, the 28 molecules with larger segment values have been pinpointed in two Plots of Component Weights, with different scales for proper evaluation. With values from the Table of Component Weights, a Scatterplot has been reproduced and all dots have been labelled with the corresponding sample code.

The Hierarchical Cluster Analysis (HCA) is a method of multivariate statistics designed to assess the hierarchical organisation of samples into groups, i.e. clusters, based on the level of similarity or dissimilarity (Granato et al., 2018). A cluster analysis has been carried out on the complete dataset, with sample codes as Point Labels. The analysis has been computed with Nearest Neighbour method and Squared Euclidean Distance Metric. Results have been represented in a dendrogram, a tree diagram that represents the connections among samples (Granato et al., 2018). At the base of the graph, the sample codes correspond to each of the vertical lines. Horizontal lines connect samples based on the distance present between them. The longer the line, the greater is the distance between samples, in terms of the chemical composition of essential oils.

3.4 Sensory analysis

The final stage of the experiment has been focused on the consumers' perception of the essential oil variability. A sensory activity has been conducted within the Spices & Herbs Global Expo, at Macfrut 2022, in Rimini, 4th-6th May 2022. Three sensory activities have been designed in order to test visitors on a voluntary basis. Tests have been carried out every day with the following procedure, for the entire exhibition period.

Test results have been collected anonymously in a single form (See Appendix 1) and later gathered on a summary Excel table for further statistical analysis. During the experiment, all participants were asked to fill the questionnaire with some personal data and with answers for each of the three activities. Locating the experiment in the context of a sectorial event led to the participation of more or less specialised panellists, of different ages and backgrounds, thus increasing the variability of the sample.

The first test had a central role within the present project since it has been centred on lavender essential oils. The experimental design has been based on a triangle test. Triangle tests are widely used for food and non-food products, as discriminatory methods that assess whether a perceptible sensory difference exists between two products. This test is applicable to similar products that differ for only one subtle character (Sinkinson, 2017). Indeed, the experiment has been designed to determine whether differences in the chemical composition of lavender essential oils have a perceptible influence on the oils' scent. In compliance with the test principles and applications explained by Sinkinson (2017), the experimental designed has been developed with the following procedure. Samples of lavender essential oils have been identified within the complete set of oils. Essential oils differing for only one variable have been selected and coupled. Four series of oils have been created and assigned with a distinctive colour, for immediate recognition. In a triangle test, each series includes three samples, two of which are the same. The odd sample is identical to the others for each of the variables, except for one (Sinkinson, 2017). In this experiment, discriminant variables included year of production, location and variety. Details of the experimental layout can be consulted in Table 3.3.

Series	Species	Variety	Location	Year	Code
Red	Lavandula angustifolia	Maillette	Gorizia	2020	**652**
	Lavandula angustifolia	Maillette	Gorizia	2021	473
	Lavandula angustifolia	Maillette	Gorizia	2021	892
Blue	Lavandula x intermedia	Grosso	Bovolenta	2020	**169**
	Lavandula x intermedia	Grosso	Bovolenta	2021	548
	Lavandula x intermedia	Grosso	Bovolenta	2021	478
Green	Lavandula x intermedia	Grosso	Bovolenta	2021	**294**
	Lavandula x intermedia	Grosso	Arezzo	2021	859
	Lavandula x intermedia	Grosso	Arezzo	2021	317
Yellow	Lavandula angustifolia	Munstead	Scotland	2021	**726**
	Lavandula angustifolia	Little Lady	Scotland	2021	319
	Lavandula angustifolia	Little Lady	Scotland	2021	752

Table 3.2 Experimental layout of the triangle test on lavender essential oils. Codes related to the odd sample have been highlighted.

Sample preparation was carried out every day in advance, using brand-new dark glass bottle of 20 ml capacity. Essential oils were diluted in alcohol, with a ratio of 2 drops per 10 ml of alcohol. Each sample was marked with three-digit random codes.

Test administration was performed with the aid of fragrance test strips. The four series were administered separately. For each series, one drop of each mixture was placed on separate paper strips. The visitors were asked to smell each of the three samples in random order. They were also advised to have a brief rest between one sample and the other, to reset the olfactory sense by smelling coffee grains or their own skin. Participants were asked to recognise the odd sample within each series and report the corresponding code on the questionnaire.

A second test has been included in the experiment, in order to assess the consumers' ability in using the sense of smell for odour recognition. A total of 10 essential oils extracted from different medicinal plant species was kindly supplied by Laboratori Biokyma srl. Mixtures of alcohol and essential oil, with a dilution ratio of 2 drops of oil per 10 ml of alcohol, were prepared every day in advance, using brand-new 20 ml dark glass bottles. Each sample was numbered with a three-digit random code. Mixtures were administered singularly in a random order, and proper recommendations for resetting the sense of smell were provided again.

Participants were asked to recognise the essential oil, identify the species of the medicinal plant from which it was extracted and record their answers on the questionnaire.

A third test has been developed to evaluate the consumers' knowledge of medicinal plants. A total of 15 dry herbs were kindly supplied by Laboratori Biokyma srl and placed into separate bowls. Each bowl was numbered with a three-digit random code. Visitors were allowed to watch, smell and touch the herbs, to match each dry herb to the corresponding name of the species. In order to avoid that participants answered by exclusion, two additional plant species have been included in the table. Detailed information about the herbs included in the activity was available by scanning a QR code.

Data have been subsequently gathered into an Excel sheet, and a Chi-square test of independence has been applied. This non-parametric statistic is used to verify whether there is an association between variables. Therefore, the null hypothesis (H_0) implies no relationship between variables (Turhan, 2020), while the alternative hypothesis (H_1) states that there is statistical significance between measured variables. In the present experiment, panellists have been categorised into mutually exclusive categories, based on age class, education level, gender, smoker/non-smoker, expertise in using the sense of smell, customary subjection to strong odours, and recent consumption of coffee. With regards to the age classes, the sample has been divided into four classes with the same number of individuals, in order to ensure homogeneity. The first class includes panellists up to the age of 22, the second class ranges from 23 to 28, the third class goes from 29 to 44, and the fourth class includes panellist beyond the age of 44. Details of the other sample categories have been represented in Figure 3.4, with features and size of each subcategory.

The influence of each category on the ability to detect the difference in essential oils' scent has been analysed by computing a chi-square test for each series of essential oils as a function of each sample category. The following formula has been employed:

$$\chi^2 = \frac{(Obs - Exp)^2}{Exp}$$

where:

Obs = observed values, for each subcategory in each series

Exp = expected values, calculated for each subcategory and each series over the total number of observations

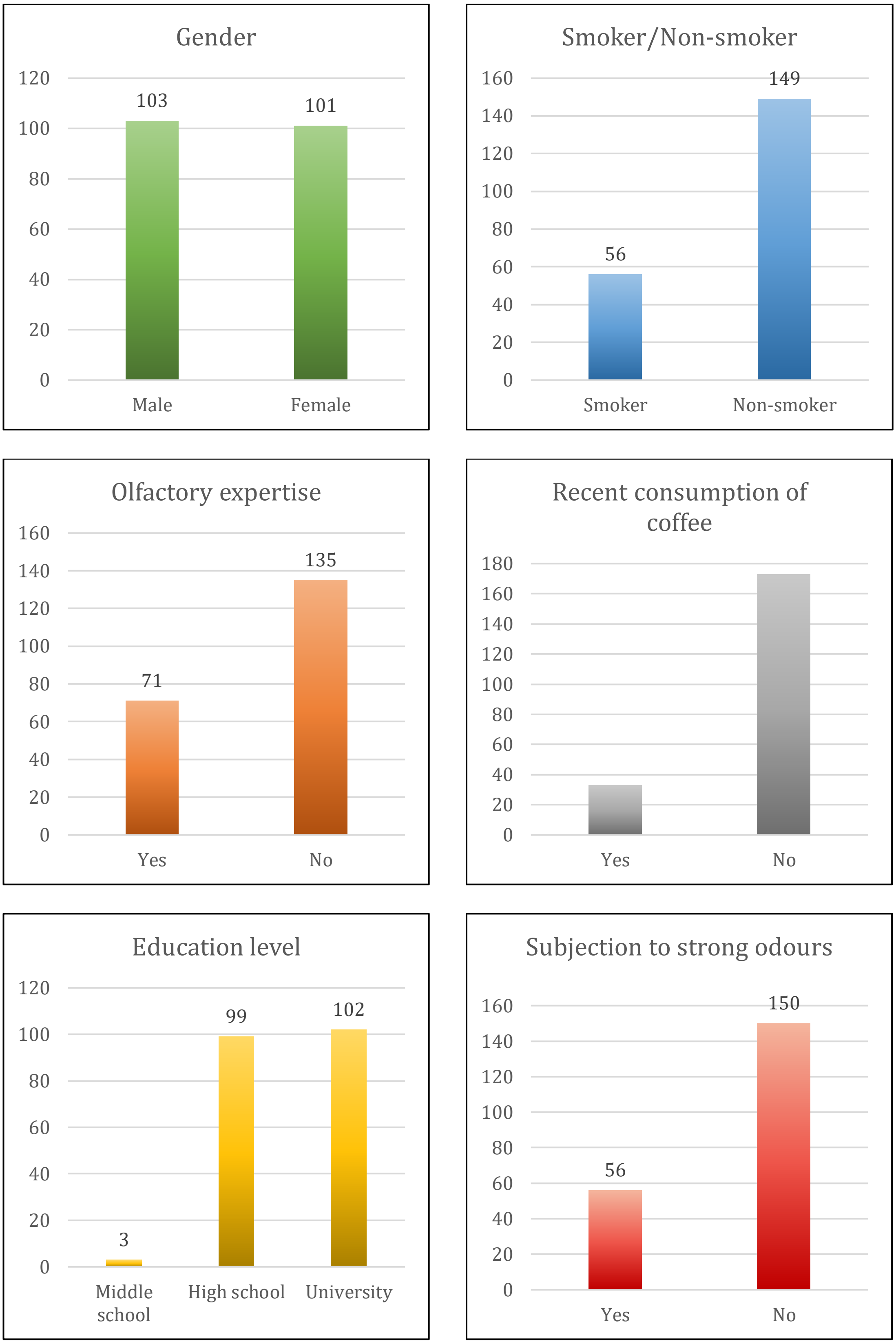

Figure 3.3 Sample categories for the chi-square of goodness of fit test, with features and size (n. of panellists) of each subcategory.

4 Results and discussion

4.1 Results

4.1.1 Yields

Annotations of essential oil yields, as reported in table 4.1, have provided reference values for a practical evaluation of the level of productivity and aptitude for essential oil production for each variety cultivated in Arquà Petrarca. For each variety, the table indicates the number of plants harvested to obtain 1 kg of flowers and the essential oil yields obtained from this material, both in ml/kg and percentage over the flower quantity. By considering a fixed quantity of flowers (1 kg), it has been possible to relate the essential oil yields to the number of plants harvested for each distillation.

Among *L. angustifolia*, a large variability has been observed in essential oil yields, which assume all possible middle values within the expected range (0,5-1,5%), as reported in literature (Aprotosoaie et al., 2017). Important discrepancy is present also in relation to the number of plants harvested to obtain 1 kg of flowers, demonstrating that different varieties of *L. angustifolia* show a significant variation in development and size. Results have highlighted that *Maillette* gave the higher yield per kg of flowers but required 3,5 plants. It has been favourably observed that similar yields were obtained from *Miss Katerine*, by distilling only half plant. Satisfactory yields have been obtained also from varieties *Blue Mountain White* and *Melissa lilac*. Additionally, data have supported that drying biomass before distillation drastically reduces oil yields. The same essential oil yield has been obtained from 1,5 kg of fresh flowers, and 2,5 kg of dried flowers.

Variability in essential oil yields has been detected also among varieties of *L. x intermedia*, which show low values as compared to the expected 3% reported in prior literature (Aprotosoaie et al., 2017). The highest yield has been produced by the *Silver Dwarf* variety, but *Grosso* has also given satisfactory results.

	Plants (n.)	Flowers (kg)	EO yield (ml)	EO yield (%)
Maillette	3,5	1	15	1,50%
Blue mountain white	1,5	1	12,5	1,25%
Miss Katerine	0,5	1	14	1,40%
Elizabeth	1	1	7,5	0,75%
Royal purple	1	1	7,5	0,75%
Melissa lilac	1	1	10	1,00%
Imperial gem	1	1	5	0,50%
Hidcote Blue	2	1	8,5	0,85%
Contrast	1,5	1	5,5	0,55%
Loddon Blue	1,5	1	6,5	0,65%
Bleue velours Charles	1,5	1	7	0,70%
Bleue velours Paul	1,5	1	7,5	0,75%
Bleue velours Paul[1]	2,5	1	7,5	0,75%
Grosso	0,25	1	15	1,50%
Provence	0,5	1	7,5	0,75%
Hidcote Giant	1	1	7,5	0,75%
Silver Dwarf	0,25	1	20	2,00%

Table 4.1 Essential oil yields from *L. angustifolia* and *L. x intermedia* varieties cultivated in Arquà Petrarca.
[1]Cultivated in Asiago, distilled from dry material.

4.1.2 Chemical composition

By gas chromatographic analysis on essential oil samples, a total of 133 components have been identified and measured. Peak areas have been recorded and converted into percentages over the total area for each sample.

A first evaluation of the chemical composition of the samples has been based on the mean values of each molecule (See Appendix 2a) identified by the gas chromatographic analysis. On average, the monoterpenoid alcohol, linalool, and its ester, linalyl acetate, show the highest percentages, accounting for 34,04% and 27,38% respectively. When considered together, the sum of their values corresponds to 61,42%, thus representing almost two-thirds of the total oil composition. Although with considerably lower average values, 4-Terpineol, lavandulyl acetate, cis-β-ocimene and 1,8 cineole have a major role among terpenes, followed by trans-β-ocimene, camphor, β-caryophyllene, borneol, trans-β-farnesene, limonene, α-terpineol and β-myrcene, with mean percentages ranging from 1,87 to 0,84. Further observations can be derived by considering essential oils of *L. angustifolia* and *L. intermedia* separately (See Appendix 2b-c). Mean values of linalool and linalyl acetate are respectively 33,77% and 31,29% in *L. angustifolia* essential oils, but 36,76% and 23,51% for *L. intermedia* essential oils. When looking at the two main molecules altogether, their cumulative value reaches 65,06% for *L. angustifolia* and 60,26% for *L. intermedia*.

Among the total 133 components, 39 substances have not been identified and have been reported as "Unk", i.e. unknown. Although similar elution time and K index have been observed, spectral data have not supported a correct recognition of the molecule. These substances altogether account for 1,18% of the total composition, on average. However, they have been reported among results since they have been observed repeatedly in several samples. For instance, the molecule reported as "unk 57", showing a mean value of 0,2%, has been identified in 11 samples, and the molecule "unk 65", with a mean value of 0,16%, has been found in 15 samples.

Moreover, it has been evaluated that a limited number of molecules is present in all samples. Data show that 8 substances have been identified in all 34 samples. With the exclusion of water, substances shared among all samples are β-myrcene, limonene, linalool, α-terpineol, linalyl acetate, β-caryophyllene and trans-β-farnesene. When *L. angustifolia* and *L. intermedia* essential oils are considered separately, it has been highlighted that 9 molecules are present in all samples of *L. angustifolia* essential oils, while 17 molecules are found in all samples of *L. intermedia* essential oils.

In conclusion, data related to the samples' average composition have supported the role of linalool and linalyl acetate in characterising lavender essential oils and laid the foundations for explaining the different levels of variability in *L. angustifolia* and *L. intermedia* essential oils.

4.1.3 Quality standards

Different quality standards are available to evaluate the chemical composition of lavender essential oils. Among them, the quality standards ISO 3515:2002 for *Lavandula angustifolia* essential oil and ISO 8902:2009 for *Lavandula x intermedia* Grosso essential oil have been considered in the present experiment. The chemical composition of all samples has been compared with these quality standards and the results have been reported in table 4.2.

Data resulting from gas chromatography have not identified the presence of β-phellandrene, possibly due to the fact that β-phellandrene has been found coeluted with 1,8 cineole in many analyses (ISO 3515, 2002). In addition, hexyl butyrate has not been detected in any of the samples.

Among the 21 samples of L. angustifolia essential oils, only one variety, *L. angustifolia Royal Purple* completely satisfies ISO 3515:2002 quality standards. However, it might be accepted that the variety *Loddon Blue* also meets the quality requirements, since the content of linalyl acetate (47,76%) slightly exceeds the range of 25-27% established by the standard parameters. Additionally, attention should be drawn to composition of the essential oils from *L. angustifolia Buena Suerte*. As previously explained, this cultivar of *L. angustifolia* exhibits a branched inflorescence, which is a typical morphological characteristic of lavandin varieties. Although differing in both location and year of production, both essential oil samples of *L. angustifolia Buena Suerte* show similar characteristics in terms of chemical composition. In general, the chemical composition of these essential oils mostly satisfies the requirements established for *L. angustifolia*. However, higher levels of 1,8 cineole, camphor and borneol have been observed, as compared to all other essential oils from *L. angustifolia* varieties. In sample "A19PE Buena Suerte", levels of 1,8 cineole (3,86%), camphor (3,5%) and borneol (8,68%) exceed the standard parameters for *L. angustifolia* essential oils. If compared to *L. x intermedia* essential oil standards, only borneol content largely exceeds the fixed range. In sample "A21FR Buena Suerte", high levels of 1,8 cineole (3,15%) and borneol (4,79%) have been measured. Camphor (1,36%) remains within the standard range, even though its value largely exceeds the average camphor content for *L. angustifolia* essential oils (0,36%). This sample completely respects the threshold for lavandin essential oils.

With regards to the percentages of linalool and linalyl acetate, it has been observed that several samples do not fall within the expected parameters, both for *L. angustifolia* and *L. x intermedia* varieties. In general, high levels of linalool are mostly associated with low levels of linalyl acetate. But to a lesser extent, higher amounts of linalyl acetate correspond to lower amounts of linalool. However, linalool content is lower than the expected parameters only in the case of *L. angustifolia Munstead* and *Folgate* from Scotland, and *L. x intermedia Silver Dwarf* from Arquà Petrarca. The low percentage of linalool in "A21SC Munstead" (18,46%) and "A21SC Folgate" (19,39%) is associated with a percentage of linalyl acetate that exceeds the average values. Instead, "H21AP Silver Dwarf" is the only sample that does not meet the minimum requirements both for linalool and linalyl acetate. Lower contents of linalool along with stable content of linalyl acetate have been ascribed to particular environmental conditions during the flowering period, characterised by rainfall and subsequent decrease in temperature (Aprotosoaie et al., 2017).

Relatively high contents of linalool and linalyl acetate have been detected in *L. x intermedia Grosso*, in line with the trends expressed by prior literature (Aprotosoaie et al., 2017).

A high variability has been observed in the case of cis-β-ocimene and 4-Terpineol. Percentages of cis-β-ocimene range from 0 to 10,44 in *L. angustifolia*, and from 0 to 5,98 in *L. x intermedia* varieties, with the highest values in samples "A21PE Hidcote Superior" and "H21AP Hidcote Giant". Possible explanations related to the "Hidcote" group of lavenders have been overturned since no amounts of cis-β-ocimene have been detected in "A21AP Hidcote Blue". In relation to 4-Terpineol, values vary from 0 to 16,08% in *L. angustifolia*, and from 1 to 8,38% for *L. intermedia* varieties, with the highest percentages in samples "A21AP Miss Katherine" and "H21AP Silver Dwarf", both largely exceeding the maximum threshold.

L. x intermedia has been generally recognised for the higher presence of 1,8 cineole and camphor, as compared to *L. angustifolia* (Aprotosoaie et al., 2017). Nevertheless, samples of *L. x intermedia* essential oils analysed in the present experiment have shown low levels of both 1,8 cineole and camphor. With regards to camphor content, only two samples show values that lie within the standard range, while most values do not reach the minimum requirements, and no values exceed the maximum threshold. In relation to 1,8 cineole content, the *Grosso* variety is characterised by lower values, that mostly do not reach the minimum requirements, while other varieties show slightly higher values that remain within the standard range.

It has been reported by Aprotosoaie et al. (2017) that lavandulol and lavandulyl acetate assume intermediate values in the composition of lavender essential oil, with average values of 2.1 and 4.3% for *L. angustifolia*, and 4.80 and 5.48% for *L. x intermedia*, respectively. In this case, no amounts of lavandulol have been detected in most samples, except for a low value in "A21AR Blue Velours Paul", which corresponds to the one case of distillation from dry material.

	3-Octanone	B-phellandrene	Linalool	Linalyl acetate	1,8-Cineole	Limonene	cis-B-Ocimene	trans-B-Ocimene	Camphor	Lavandulol	Terpinen-4-ol	Lavandulyl acetate	alpha-Terpineol	Myrcene	Borneol	Hexyl butyrate
ISO 3515:2002	0-3	0-1	20-43	25-47	0-3	0-1	1-10	0,5-6	0-1,5	0-3	0-8	0-8	0-2	0,3-1	1,5-3,5	0,3-0,5
A21AP Maillette	0,04		40,63	41,73	0,15	0,06	0,38	0,10	0,00	0,00	0,09	3,57	0,97	0,28	0,47	
A21AP Blue Mountain White	0,05		27,47	38,22	0,00	0,13	1,01	0,66	0,00	0,00	9,95	8,52	1,71	0,31	0,00	
A21AP Miss Katherine	0,43		41,19	*17,08	0,18	0,18	0,78	0,15	0,00	0,00	16,08	5,26	0,62	0,43	0,10	
A21AP Elizabeth	0,13		37,08	41,54	0,25	0,28	1,20	0,40	0,15	0,00	2,87	2,55	1,20	0,43	0,77	
A21AP Royal Purple	0,20		39,30	31,72	0,57	0,29	2,91	0,54	0,28	0,00	6,58	2,18	1,08	0,56	0,47	
A21AP Melissa Lilac	0,72		21,87	32,68	0,55	0,28	0,10	0,00	0,00	0,00	14,75	6,68	0,90	0,30	0,41	
A21AP Imperial Gem	0,20		28,31	48,44	0,15	0,11	0,32	0,54	0,16	0,00	4,00	4,59	1,45	0,21	0,66	
A21AP Hidcote Blue	0,48		30,00	44,96	2,79	0,15	0,00	2,14	0,00	0,00	4,06	3,71	0,88	0,41	0,26	
A21AP Contrast	0,08		*53,67	*13,52	0,44	0,07	0,19	0,16	0,13	0,00	10,83	1,53	1,17	0,05	0,63	
A21AP Loddon Blue	0,18		21,60	47,76	0,49	0,53	3,20	1,31	0,11	0,00	2,14	4,90	1,59	0,57	0,94	
A21AP Bleue Velours Charles	0,49		*45,72	*19,98	0,35	0,18	0,05	0,06	0,08	0,00	13,02	2,34	1,31	0,14	0,35	
A21AP Bleue Velours Paul	0,47		39,49	*19,62	0,00	0,21	4,36	3,73	0,00	0,00	14,82	2,35	0,99	0,38	0,39	
A21AP Bleue Velours Paul	0,51		23,00	25,76	0,44	0,14	0,96	0,73	0,00	0,63	7,40	4,13	2,43	0,54	0,12	
A21SC Munstead	1,36		*18,46	44,98	0,29	0,94	8,80	2,85	0,44	0,00	0,96	3,89	1,16	1,23	0,38	
A21SC Little Lady	0,36		28,67	30,28	0,12	3,34	4,35	2,50	0,00	0,00	9,82	2,88	0,70	1,14	0,09	
A21SC Folgate	1,51		*19,39	38,58	0,00	1,59	9,39	2,61	0,16	0,00	4,00	3,46	1,14	1,09	0,37	
A19PE Buena Suerte	0,00		42,75	*6,23	3,86	7,78	6,75	1,63	3,50	0,00	2,45	1,24	0,63	1,74	6,68	
A21PE Hidcote Superior	0,47		22,70	31,48	0,41	1,70	10,44	3,91	0,11	0,00	2,07	4,78	0,55	0,79	0,72	
A20GO Maillette	1,18		32,13	*49,85	0,00	0,43	1,89	1,03	0,50	0,00	0,00	0,40	0,66	0,81	0,80	
A21GO Maillette	0,68		*46,79	29,77	0,00	0,41	1,44	1,02	0,66	0,00	0,00	0,42	0,82	0,82	1,55	
A21FR Buena Suerte	0,15		*48,98	*2,94	3,15	5,84	7,68	2,24	1,36	0,00	4,01	0,68	0,42	1,64	4,79	
ISO 8902:2009	0-3	0-1	24-37	25-38	4-8	0,5-1,5	0,5-1,5	0-1	6-8,5	0,2-1	1,5-5	1,5-3,5	0,3-1,3	0,3-1	1,5-3,5	0,3-0,5
H21AP Silver Dwarf	0,49		22,08	23,24	6,20	1,20	0,00	1,25	0,00	0,00	8,38	0,10	1,68	0,95	3,21	
H21AP Grosso	0,00		37,16	27,58	4,52	1,46	2,16	0,60	3,99	0,00	3,47	2,47	0,52	1,35	1,80	
H21AP Provence	0,67		*50,07	*3,30	5,96	0,83	0,58	0,00	2,66	0,00	8,15	0,77	1,75	0,34	9,14	
H21AP Hidcote Giant	0,34		*38,17	3,39*	6,05	0,67	5,98	11,19	7,96	0,00	4,77	0,00	0,61	0,52	1,90	
H20BO Grosso	0,00		28,66	37,40	2,13	0,95	1,24	0,47	3,97	0,00	3,06	2,87	0,35	0,83	1,52	
H21BO Grosso	0,00		26,78	36,76	3,03	1,00	0,79	0,35	4,41	0,00	2,50	3,98	0,50	0,84	1,43	
H21AR Grosso	0,44		*55,28	*14,50	2,86	1,09	1,02	0,82	6,00	0,00	3,67	0,00	0,40	0,70	1,61	
H21SC Grosso	0,00		35,25	37,14	2,01	0,93	2,73	0,47	3,49	0,00	1,00	2,59	0,99	0,93	1,80	
H20GO Grosso	0,00		37,35	28,24	2,72	1,42	1,64	0,66	3,75	0,00	2,59	2,77	0,77	1,38	2,49	

Table 4.2 Results of the comparison between the chemical composition of the essential oils' samples and the correspondent quality standards (ISO 3515:2002 for *L. angustifolia* essential oils and ISO 8902:2009 for *L. x intermedia* essential oils).

4.1.4 Multiple Variable Analysis for correlations

The Multiple Variable Analysis has been performed on the complete dataset, but correlations have been highlighted within a limited selection of data. Significant levels of correlation coefficients (r) have been evaluated with $p<0,05$. However, interpretation has been focused on those molecules included in the standards ISO 3515:2002 and ISO 8902:2009. These molecules have been detected in a large number of samples, ranging between 23 and 34, with except for lavandulyl acetate, which has been found in only 3 samples. Results have been reported in Table 4.3.

Based on what was illustrated in the introductory chapter, correlations between molecules might surmise the presence of relationships among biosynthetic pathways or enzymatic activities leading to the production of secondary metabolites.

The correlation analysis has highlighted significant relationships between linalool and linalyl acetate, and between camphor and borneol. In the first case, linalool and linalyl acetate show a negative correlation coefficient ($r=-0,5207$). This observation is in line with the interpretation of the samples' chemical composition. As a matter of fact, high levels of linalool correspond to low levels of linalyl acetate and, to a lesser extent, lower amounts of linalool are associated with higher amounts of linalyl acetate. This mechanism might be related to the conversion of linalool into linalyl acetate, by addition of an acetyl group (Woronuk et al., 2011) In the second case, camphor and borneol show a positive correlation coefficient ($r=0,4693$), that might be connected to the oxidization of borneol in camphor by a borneol dehydrogenase (Sarker et al., 2012).

Among monoterpenes, positive correlations have been shown among myrcene, cis-β-ocimene, trans-β-ocimene (acyclic) and limonene (monocyclic). In particular, associations have been observed between myrcene and limonene, between cis-β-ocimene and limonene, and between trans-β-ocimene and myrcene.

Furthermore, significant correlations have been highlighted within the entire set of molecules, thus suggesting that a cross-talk might occur between 2-C-methyl-D-erythritol-4-

phosphate (MEP) and mevalonate (MVA) biosynthetic pathways, mainly resulting in the production of monoterpenoids and sesquiterpenoids respectively.

	beta-Myrcene	Limonene	cis-beta-Ocimene	1,8-Cineole	trans-beta-Ocimene	Linalool	Camphor	Lavandulol	Borneol	4-Terpineol	alpha-Terpineol	Linalyl acetate	Lavandulyl acetate
3-Octanone	0,0050	-0,1350	0,2794	-0,0860	0,1514	-0,3130	-0,2841	0,2456	-0,1637	0,0492	0,1975	0,1272	0,0580
beta-Myrcene		0,4744	0,2791	0,5860	0,4563	-0,2685	0,2475	0,4708	0,2575	-0,3536	0,0048	-0,2563	-0,1197
Limonene			0,5309	0,1114	0,0344	0,1676	0,1394	-0,0914	0,5068	-0,1678	-0,3301	-0,3734	-0,2517
cis-beta-Ocimene				-0,1869	0,3197	-0,1875	0,0408	-0,2132	0,0697	-0,2314	-0,2549	-0,0403	-0,0683
1,8-Cineole					0,5031	-0,0230	0,5427	0,4798	0,5374	-0,1734	0,0993	-0,5776	-0,2918
trans-beta-Ocimene						-0,2227	0,1981	0,4903	-0,0595	-0,0883	0,0478	-0,3381	-0,0843
Linalool							0,2024	-0,2852	0,3812	0,0622	-0,4204	-0,5207	-0,5869
Camphor								-0,1865	0,4693	-0,3198	-0,3345	-0,4064	-0,4362
Lavandul									-0,1583	0,0282	0,4703	-0,1966	0,2876
Borneol										-0,1942	-0,0921	-0,5901	-0,5260
4-Terpineol											0,2537	-0,3007	0,3034
alpha-Terpineol												0,0634	0,3617
Linalyl acetate													0,4426

Table 4.3 Correlation table, with molecules included in the standards ISO 3515:2002 and ISO 8902:2009.

4.1.5 Principal Component Analysis

The Principal Component Analysis has been computed with the aim of underlining the main sources of variations in the dataset. As molecules have been arranged in descending order based on their contribution to the total variability, those molecules listed in the standards ISO 3515:2002 and ISO 8902:2009 have been identified among the first 28 molecules. Therefore, in order to plot the molecules of interest for further discussion, the first 28 molecules have been considered.

A Plot of the first two Component Weights has been reproduced in order to visualise a projection of all selected molecules on the Cartesian plane. The graph including the complete set of molecules (Fig. 4.1a) demonstrates a major contribution of linalool and linalyl acetate to the differentiation of the samples. This result has been supported by data related to the chemical composition, quality assessment and correlation analysis. It has been established that linalool and linalyl acetate are present in high percentages within the essential oils, thus strongly contributing to the differentiation of the samples. To observe and assess the role of the other molecules, a second graph has been reproduced by changing the scale (Fig. 4.1b). This plot demonstrates that the other molecules considered in the analysis give a lower contribution to the samples' differentiation. Among these molecules, borneol, camphor, 4-terpineol, limonene, 1,8-cineole and trans-β-ocimene, located in quadrant III, show the highest weights. In the same quadrant, trans-pinocarveol, β-myrcene, trans-β-farnesene, α-pinene, β-pinene, γ-terpinene, lavandulol and coumarin represent minor contributions to the total variability. Within quadrant I, the largest weight is associated to lavandulyl acetate, followed by cis-β-ocimene, trans-caryophyllene, geranyl acetate, octen-3-yl acetate, α-terpineol, germacrene and 3-octanone. Endo-fenchol and unk 57 are the only secondary molecules placed in the first and second quadrants respectively.

A Scatterplot (Fig. 4.2) has been constructed to obtain a visual representation of the samples' variability of the first two components of PCA. Sharp clusters have not been recognised within the plot, thus confirming a high similarity among samples. To evaluate the influence of the chemical composition on the samples' differentiation, essential oils have been highlighted with different colours based on the species. Considering *L. angustifolia* varieties, two groups have been identified. Essential oils of *L. angustifolia Buena Suerte* are closely located in quadrant II, thus proving similarities in their chemical composition. Similarly, essential oils of *L. angustifolia Maillette* are grouped in quadrant I. It has been favourably acknowledged that the sample of *L. angustifolia Elizabeth* is located close to all samples of *Maillette* variety. Among *L. x intermedia* essential oils, most *Grosso* samples are grouped in quadrant I. One single sample, "H21AR Grosso" is located distantly in quadrant II. It has been observed that these samples mainly differ for location and year of production. An additional clustering, including samples "A21SC Little Lady", "A21SC Folgate" and "A21SC Munstead" has been highlighted in quadrant I. This grouping involves all samples of *L. angustifolia* essential oils originating from Scotland.

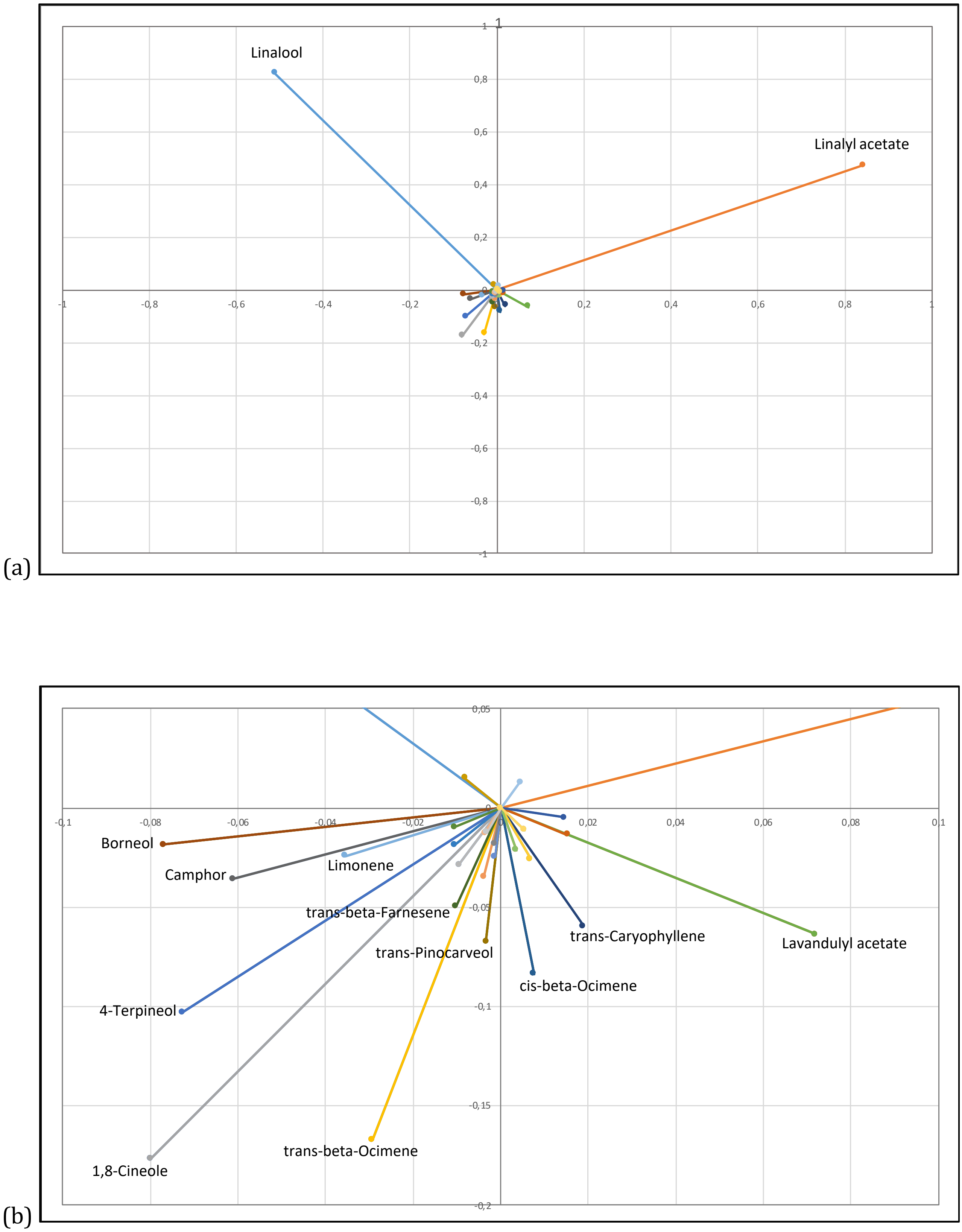

Figure 4.1 Plots of Component Weight for the complete set of variables, at smaller (a) and larger (b) scale.

Figure 4.2 Scatterplot representing variability among all samples, based on chemical composition.

4.1.6 Hierarchical Cluster Analysis

The Hierarchical Cluster Analysis has been performed in order to assess the level of similarity or dissimilarity among samples, thus identifying relationships between samples, based on their chemical composition. The analysis has been computed on the complete dataset. However, in order to evaluate the obtained results, two dendrograms have been constructed.

The first dendrogram (Fig. 4.3a) includes samples originating from the main location of Arquà Petrarca. A separate assessment of this first set of samples allows to evaluate the variability in terms of species and variety. Indeed, all essential oils have been obtained from the same site, produced in the same year and with the same distillation method, as thoroughly illustrated in the previous chapters. The species and variety represent the discriminant variable. The graph illustrates one major cluster including *L. angustifolia* varieties, characterised by large variability in terms of chemical composition. Although highly variable levels of similarity have been detected, similarities among samples have also been acknowledged, especially regarding cultivars *Maillette* and *Elizabeth*, but also *Imperial Gem* and *Hidcote Blue*. This result integrates and supports the observations that have been previously developed from the Principal Component Analysis. Furthermore, this analysis has distinguished *L. angustifolia* from *L. x intermedia* essential oils. As previously stated, samples of *L. angustifolia* have been grouped together on the left-side of the graph. Similarly, samples of *L. x intermedia* have been closely located in the dendrogram, but a high variability has been measured among them. In contrast to what was observed for *L. angustifolia* essential oils, a large distance has been detected among all samples of *L. x intermedia* essential oils. In particular, the sample "H21AP Grosso", located in a central position among essential oils of *L. angustifolia*, has shown a high similarity to the sample "A21AP Royal Purple".

The second dendrogram (Fig. 4.3b) represents the entire dataset. A significantly higher level of variability has been observed among samples, suggesting that the composition of essential oils might be strongly related to changes in location and year of production. In line with the expectations, *L. angustifolia Maillette* and *Elizabeth* have confirmed a high similarity. Similarly, associations between "A19PE Buena Suerte" and "A21FR Buena Suerte" have been further confirmed, despite differences in location and year of production. A strong relationship has been detected between *L. angustifoila Imperial Gem* and *Loddon Blue*, both cultivated in

Arquà Petrarca, and between *L. angustifolia Folgate* and *Munstead*, both cultivated in Scotland. On the contrary, "A21SC Little Lady" has shown high dissimilarity with respect to the other samples produced in Scotland. In relation to the year of production, contrasting results have been obtained. While a strong association has been identified between "H20BO Grosso" and "H21BO Grosso", a high level of dissimilarity has been observed between "A20GO Maillette" and "A21GO Maillette", thus suggesting that other sources of variations might be involved in the latter differentiation. As demonstrated in the experiment by Zagorcheva et al. (2020), a low level of genetic diversity is present between *L. angustifolia Munstead* and *Melissa Lilac*. Results of the Hierarchical Cluster Analysis support these findings, by verifying similarities based on biochemical information.

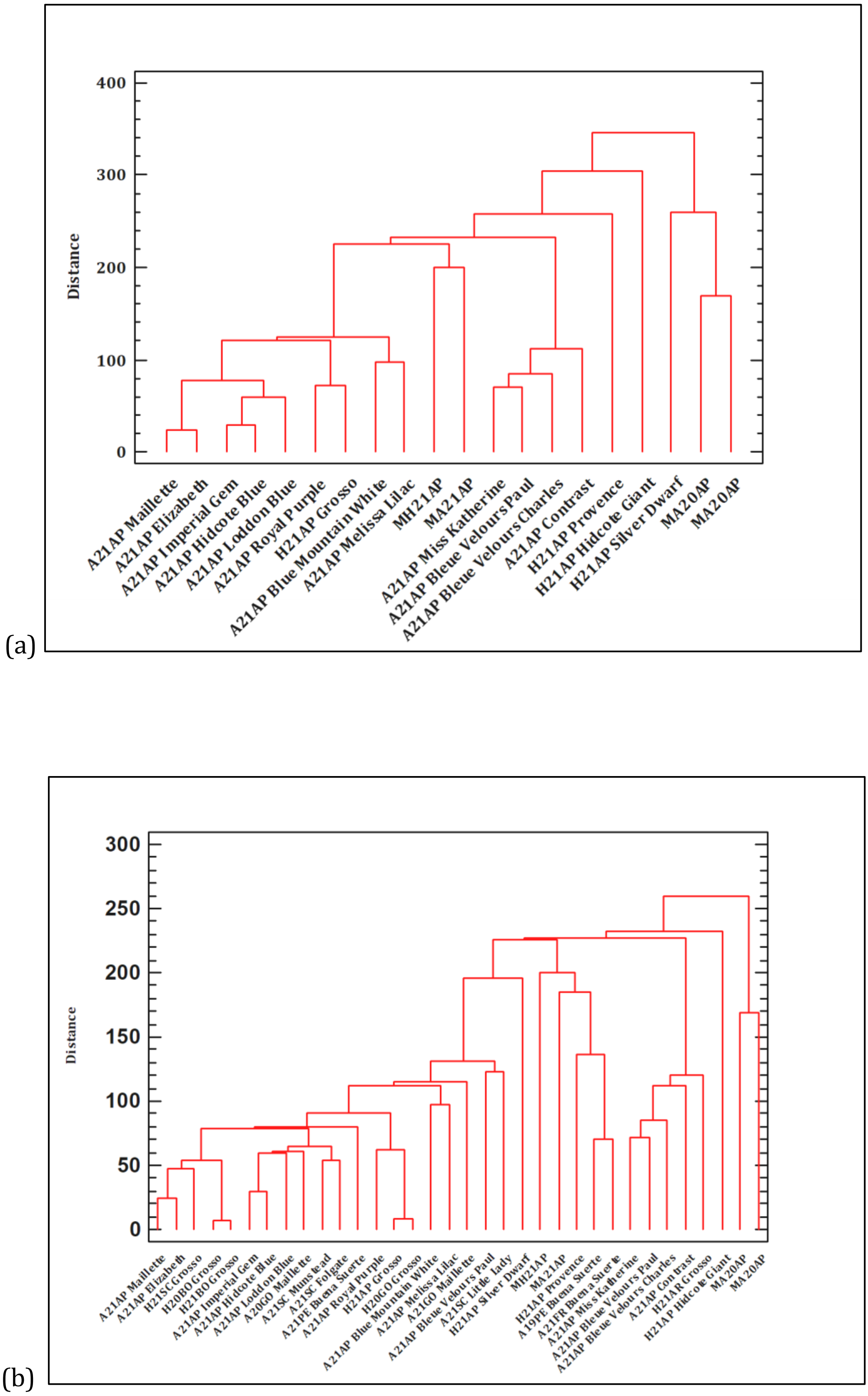

Figure 4.3 Dendrogram outlining results of the Hierarchical Cluster Analysis (HCA). Representation of samples from Arquà Petrarca (a). Representation of the complete set of samples (b).

4.1.7 Sensory analysis

The sensory analysis aimed to evaluate the consumers' perception of the chemical variability of essential oils, differing in location, year of production or variety. In particular, samples have been specifically identified and paired, in order to assess the perception of some significant differences and similarities detected in the chemometric analysis.

Samples of *L. angustifolia Maillette* from Gorizia and *L. x intermedia Grosso* from Bovolenta have been selected to test whether a perceivable difference is present among oils produced in two different years (2020 and 2021). In terms of chemical composition, essential oils of *L. angustifolia Maillette* from Gorizia have demonstrated a high variation, mainly supported by the Hierarchical Cluster Analysis. Contrarily, both the Principal Component Analysis and the cluster analysis have shown a strong association between samples of *L. x intermedia Grosso* from Bovolenta. Differences in the site of cultivation have been tested for olfactory perception by including samples of *L. x intermedia Grosso* from Bovolenta and Arezzo. These two samples have been produced from the same cultivar, in the same year and with the same distillation method. Significant differences have been confirmed by both the PCA and cluster analysis. Essential oils of *L. angustifolia Munstead* and *L. angustifolia Little Lady*, both produced in Scotland, have been included in the test to assess whether different varieties of *L. angustifolia* might produce perceivable differences in the essential oils. Variations in chemical composition have been established mainly by Hierarchical Cluster Analysis, while a low genetic variability between these varieties has been demonstrated by Van Oost et al. (2021).

Figure 4.4 shows the primary results of the experiment. Estimation of correct and wrong answers has been performed for each of the four categories of essential oils, and over the total number of answers collected. Results have highlighted that the majority of the panellists have not selected the correct answer in most of the series. In relation to the green series, a slightly higher percentage of correct answers (51%) has been observed, as compared to the percentage of wrong answers (49%). The substantial number of incorrect answers might be ascribed mainly to the difficulty of recognising the highly diluted samples of essential oils. Indeed, a very low percentage of essential oil was present in each sample, with a dilution ratio of 2 drops of oil per 10 ml of alcohol. Furthermore, the testing environment did not ensure the appropriate conditions of comfort and quiet, required to avoid any bias (Sinkinson, 2017).

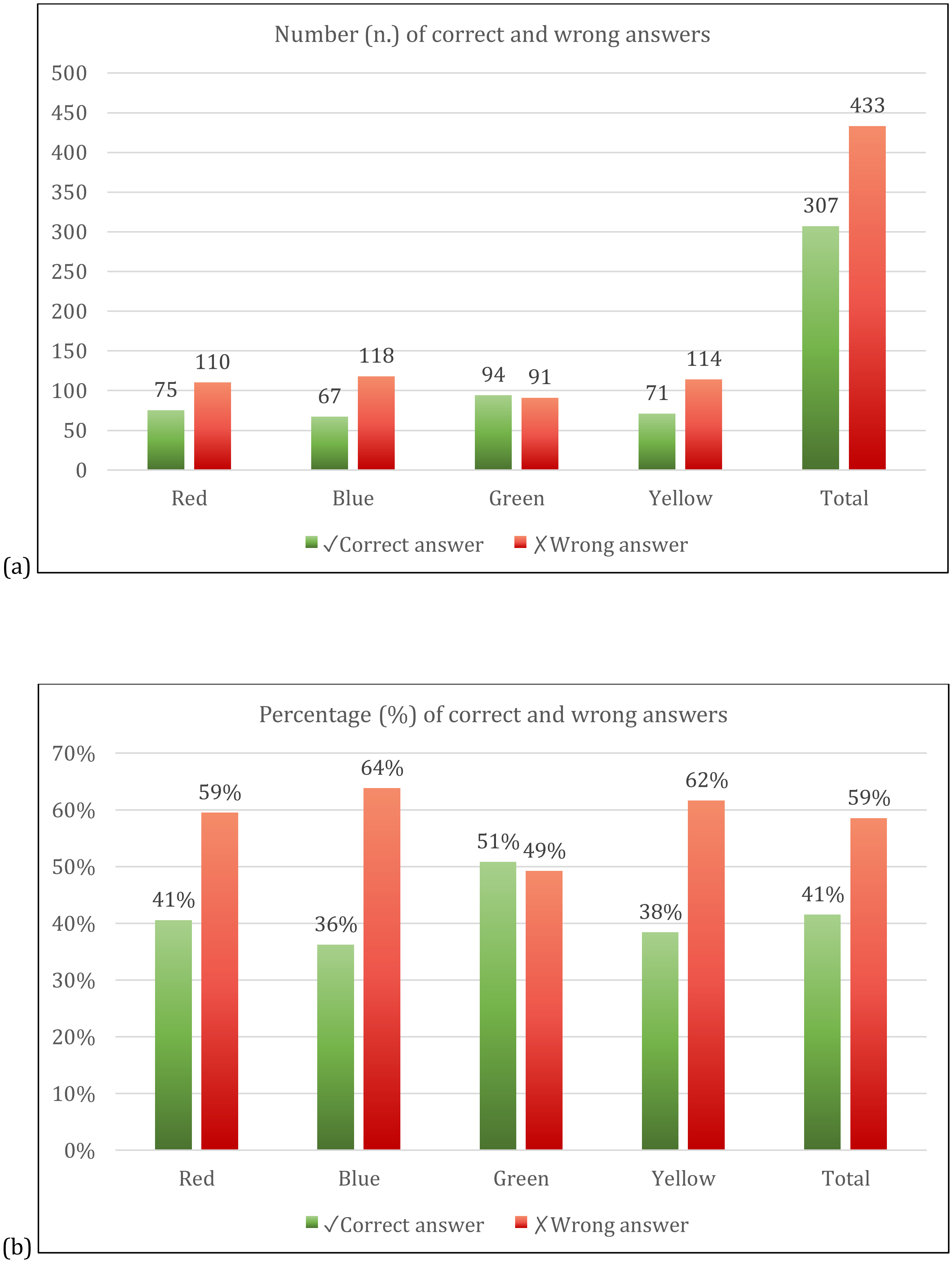

Figure 4.4 Estimation of correct and wrong answers as number (a) or percentage (b), calculated for each of the four series of essential oils, and over the total number of answers.

The statistical analysis has been performed on the entire dataset, replicating the test for the single series as a function of the single categories and subcategories chosen for the experiment. The general outcome of the analysis has evidenced that the panellists' ability in perceiving differences among samples is not correlated to any specific category. Results have been reported in Table 4.4 (a-g) which shows the number of correct answers in relation to each series and subcategory and highlights the level of significance for each computation. Statistically significant values have been identified with $p<0,05$, while high statistical significance has been established with $p<0,01$. Categories related to i) smoker/non-smoker, ii) expertise in using the sense of smell, iii) customary subjection to strong odours and iv) recent consumption of coffee have not provided a significant correlation with the ability to detect differences in scent. Significance has been observed in relation to the blue series within the gender category and depending on the education level for the green series. Data concerning age classes have highlighted significant results in relation to the blue series and highly significant results in relation to the red series.

(a)

Age				
	0-22	23-28	29-44	45-80
Red series				
✓	30	16	8	14
✗	23	23	29	27
				**
Blue series				
✓	18	20	17	8
✗	35	19	20	33
				*
Green series				
✓	25	21	22	20
✗	28	18	15	21
				NS
Yellow series				
✓	17	17	16	17
✗	36	22	21	24
				NS

(b)

Education level			
	High school	Middle school	University
Red series			
✓	0	40	35
✗	2	50	55
			NS
Blue series			
✓	0	35	30
✗	2	55	60
			NS
Green series			
✓	0	55	38
✗	2	35	52
			*
Yellow series			
✓	1	33	37
✗	1	57	90
			NS

(c)

Gender		
	Female	**Male**
Red series		
✓	40	34
✗	49	60
	NS	
Blue series		
✓	39	27
✗	50	67
	*	
Green series		
✓	46	47
✗	43	47
	NS	
Yellow series		
✓	37	34
✗	52	60
	NS	

(d)

Smoker/Non-smoker		
	Yes	**No**
Red series		
✓	56	18
✗	77	32
	NS	
Blue series		
✓	47	20
✗	86	30
	NS	
Green series		
✓	62	30
✗	71	20
	NS	
Yellow series		
✓	50	21
✗	83	29
	NS	

(e)

Olfactory expertise		
	Yes	**No**
Red series		
✓	27	48
✗	34	75
	NS	
Blue series		
✓	24	42
✗	37	81
	NS	
Green series		
✓	32	62
✗	29	61
	NS	
Yellow series		
✓	22	49
✗	39	74
	NS	

(f)

Subjection to strong odours		
	Yes	**No**
Red series		
✓	20	55
✗	28	81
	NS	
Blue series		
✓	19	48
✗	29	88
	NS	
Green series		
✓	27	66
✗	21	70
	NS	
Yellow series		
✓	17	54
✗	31	82
	NS	

(g)

Recent coffee consumption		
	Yes	**No**
Red series		
✓	10	65
✗	15	94
	NS	
Blue series		
✓	12	55
✗	13	104
	NS	
Green series		
✓	11	82
✗	14	77
	NS	
Yellow series		
✓	9	62
✗	97	16
	NS	

Table 4.4 Results of the chi-square for goodness of fit test, for each category and each series. Statistical significance has been highlighted for each series and subcategory, as “NS” not significant, “*” significant ($p<0,05$), “**” highly significant ($p<0,01$). Correct answers have been labelled ✓ and wrong answers have been labelled ✗.

4.2 Conclusion

The project aimed to evaluate the influence of genotypic and environmental factors on the chemical composition of lavender essential oils, by mainly assessing the effect of distinct locations, varieties and years of production.

Gas chromatographic analyses on samples of lavender essential oils detected 133 components. Estimations of the oils' average chemical composition supported the role of linalool and linalyl acetate in characterising lavender essential oils. On one side, the characterisation of *L. angustifolia* essential oils showed a higher percentage of linalool and linalyl acetate, but also a limited variation in chemical composition. On the other side, the composition of essential oils from *L. intermedia* presented a larger number of molecules. This may explain the more varied chemical composition.

The chemical composition of all samples was compared with the quality standards ISO 3515:2002 for *Lavandula angustifolia* essential oil and ISO 8902:2009 for *Lavandula x intermedia* Grosso essential oil. Several samples did not meet the quality requirements, with a large number of molecules exceeding the maximum threshold. Observations were further confirmed by the Multiple Variable Analysis for correlations. Significant correlations were identified within the entire set of molecules, thus suggesting a connection between biosynthetic pathways and enzymatic mechanisms responsible for the production of secondary metabolites. In comparison with the ISO standards, the content of linalool and linalyl acetate exceeded the ranges in several cases, both for *L. angustifolia* and *L. x intermedia* varieties. These molecules showed a negative correlation coefficient (r=-0,5207). As a matter of fact, too high levels of linalool were mostly associated with too low levels of linalyl acetate and, to a lesser extent, higher amounts of linalyl acetate corresponded to lower amounts of linalool. Since linalool and linalyl acetate were present in high percentages within the essential oils, their major contribution to the differentiation of the samples was clearly demonstrated by the Principal Component Analysis (PCA). Sharp clusters were not identified within the plot, but groups of samples have been highlighted in relation to common or recurring features. For instance, one group including all samples of *L. angustifolia* essential oils originating from Scotland was observed. In summary, the position of linalool and linalyl acetate within the first and second quadrants of the Cartesian plane strongly influenced the differentiation of the samples within the same quadrants.

Besides the Principal Component Analysis (PCA), the Hierarchical Cluster Analysis (HCA) outlined relationships between samples, based on similarities and dissimilarities in their chemical composition. Further evidence on the interspecific variability of essential oils was provided, in conjunction with additional proof on the involvement of the environment in the differentiation of essential oils.

A cross-sectional evaluation of the analysis estimates provided practical validation for the implementation of some *L. angustifolia* varieties for essential oil production. On one side, the marketed *L. angustifolia Buena Suerte*, morphologically similar to *L. x intermedia* varieties, preserved ambiguous features in terms of essential oil composition and quality assessment. However, strong similarities between samples of the same variety, cultivated in different locations, were supported both by the Principal Component Analysis (PCA) and the Hierarchical Cluster Analysis (HCA).

On the other sides, proximity between samples of *L. angustifolia Maillette* and *Elizabeth* was outlined both in the Principal Component Analysis (PCA) and Hierarchical Cluster Analysis (HCA). Although widely recommended as a first choice for distillation purposes, essential oil yields of *L. angustifolia Maillette* did not provide satisfactory results. In line with the farmer's opinion, *L. angustifolia Elizabeth* showed higher yields and higher quality as compared to *Maillette*. Additional trials might be useful to assess whether similarities in chemical composition and superior performance would be stable overtime.

The final stage of the project was focused on the consumers' perception of lavender essential oils variability. The sensory activity conducted within the Spices & Herbs Global Expo, at Macfrut 2022, evidenced the actual implications of the variability in the composition of lavender essential oils as a marketable product. Panellists of different age and expertise showed considerable interest in the experiment (Benelli, 2022). Professionals, farmers and enthusiasts of the medicinal plants field demonstrated great appreciation for this engaging activity, which perfectly intertwined with the major topics of the events, mainly related to the protection and enhancement of biodiversity.

Furthermore, the project adheres to the pillars of sustainability, beyond the environmental and ecological functions of lavender and its essential oil. This research reviewed and supported the association of genetic and biochemical studies, to assess the differentiation in essential oils' composition within and among species. This characterisation acquires a central role in breeding programs for essential oil quantity and quality, but also in the preservation of germplasm collections and natural biodiversity. Moreover, the identification of essential oil substances applies to several medical studies dealing with physical and mental health. In addition, the experiment encouraged connections among stakeholders, by increasing a multidirectional exchange of knowledge between researchers, farmers and professionals, in light of the recent update in the institutional framework of medicinal plants cultivation and transformation.

Based on the significant results obtained up to the present, this study ushers in the constitution of a global database collecting essential oils of *Lavandula* species and varieties.

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Appendix 1

Page 1

Anagrafica

Età	
Genere	☐ M ☐ F ☐ Altro
Fumatore	☐ Sì ☐ No
Titolo di studio	☐ Licenza media ☐ Scuola superiore ☐ Laurea
Nel lavoro adopera l'olfatto	☐ Sì ☐ No
È sottoposto a odori forti	☐ Sì ☐ No
Ha bevuto caffè nell'ultima mezz'ora	☐ Sì ☐ No

Test 1: riconoscere l'olio essenziale diverso.

Per ogni serie, inserire il codice corrispondente all'olio essenziale dal profumo diverso.

Serie rossa	Serie blu	Serie verde	Serie gialla

Test 2: riconoscere l'olio essenziale.

Nome scientifico	Nome comune	Codice
Lavandula officinalis	Lavanda	
Laurus nobilis	Alloro	
Lavandula x intermedia	Lavandino	
Salvia officinalis	Salvia	
Hissopus officinalis	Issopo officinale	
Mentha x piperita	Menta	
Thymus vulgaris	Timo	
Arnica montana	Arnica montana	
Artemisia dracunculus	Dragoncello	
Lippia citriodora Kunth.	Verbena odorosa	
Anthemis nobilis	Camomilla romana	
Rosmarinus officinalis	Rosmarino	

Page 2

Test 3: riconoscere la droga essiccata.

Nome scientifico	Nome comune	Codice
Cartaegus oxyacantha L.	Biancospino foglie e fiori	
Peumus boldus Molina	Boldo foglie	
Matricaria chamomilla L. / Chamomilla recutita Rausch.	Camomilla fiori	
Hibiscus sabdariffa L.	Karkadè fiori	
Malva sylvestris L.	Malva foglie e fiori	
Linum usitatissimum L.	Lino semi	
Orthosiphon stamineus Benth.	Giava foglie	
Passiflora incarnata L.	Passiflora sommità	
Plantago ovata Forsk.	Psillio semi	
Rheum officinale Baill.	Rabarbaro cinese rizoma	
Sambucus nigra L.	Sambuco fiori interi	
Cassia angustifolia Vahl.	Senna follicoli interi	
Arctostaphylos uva-ursi Spreng.	Uva orsina foglie	
Taraxacum officinale Weber	Radice di tarassaco	
Lippia citriodora Kunth.	Verbena odorosa foglie	
Echinacea angustifolia	Echinacea angustifolia radice	
Cardiospermum halicacabum	Cardiospermo parti aeree	

Appendix 2

2a) Average composition of all essential oils' samples.

N.	Component	%
1	Linalool	34,04
2	Linalyl acetate	27,38
3	4-Terpineol	5,34
4	Lavandulyl acetate	2,84
5	cis-β-Ocimene	2,52
6	1,8-Cineole	2,41
7	trans-β-Ocimene	1,87
8	Camphor	1,70
9	β-Caryophyllene	1,65
10	Borneol	1,65
11	trans-β-Farnesene	1,61
12	R(+)-Limonene	1,06
13	α-Terpineol	1,03
14	β-Myrcene	0,84
15	Water	0,66
16	Caryophyllene oxide	0,62
17	Geranyl acetate	0,61
18	1-Methyl-4-isopropylbenzene	0,51
19	trans-Pinocarveol	0,47
20	α-Pinene	0,42
21	3-Octanone	0,39
22	β-pinene	0,37
23	Neryl acetate	0,35
24	cis-5,8,11,14-Eicosatetraenoic acid methyl ester	0,31
25	Octen-3-ol	0,31
26	cis-Linalool oxide	0,31
27	octen-3-yl-acetate	0,31
28	Germacrene	0,29
29	Camphene	0,25
30	Endo-fenchol	0,25
31	Neoisomenthol	0,24
32	δ-3-Carene	0,22
33	unk 57	0,20
34	trans-Linalol oxide	0,20
35	alpha-Santalene	0,20
36	α-Bisabolol	0,18
37	α-Terpinolene	0,18
38	α-Bergamotene	0,17
39	unk 68	0,17
40	Coumarin	0,16
41	unk 65	0,16
42	γ-Terpinene	0,14
43	Valencene	0,13
44	Nerol	0,13

45	Sabinene	0,13
46	Lavandulol	0,11
47	Di-isooctylfumarate	0,11
48	unk 77	0,10
49	Hexyl acetate	0,09
50	Acetone	0,08
51	α-Thujene	0,07
52	unk 130	0,07
53	δ-Cadinene	0,06
54	unk 121	0,06
55	unk 134	0,06
56	trans-Citral	0,06
57	Hexyl tiglate	0,05
58	3-Octanol	0,05
59	α-Humulene	0,05
60	2-heptanol	0,05
61	Geraniol	0,04
62	α-Santalol(Z)	0,04
63	(R)-( )-Pulegone	0,04
64	1-Methyl-3-isopropylbenzene	0,04
65	unk 110	0,04
66	unk 52	0,04
67	Verbanol acetate	0,03
68	methyl docosatrienoate	0,03
69	cis-Nerolidol	0,03
70	Neo-Isopulegol	0,03
71	Bornyl acetate 76-49-3	0,03
72	β-Bourbonene	0,03
73	unk 100	0,03
74	α-Terpinene	0,03
75	Cumin aldehyde	0,03
76	β-Vetivene	0,03
77	Methyl cis-9,10-methyleneoctadecanoate	0,03
78	α-Phellandrene	0,03
79	unk 115	0,02
80	Isobornyl acetate	0,02
81	unk 124	0,02
82	α-Gurjunene	0,02
83	t,t-alpha-Farnesene	0,02
84	Acetic acid	0,02
85	Heptyl alcohol	0,02
86	unk 108	0,01
87	unk 78	0,01
88	unk 128	0,01
89	unk 96	0,01
90	unk 97	0,01
91	α-Bulnesene	0,01
92	Carvacrol	0,01
93	unk 15	0,01
94	unk 85	0,01

95	unk 61	0,01
96	unk 113	0,01
97	unk 133	0,01
98	unk 71	0,01
99	(1R)-endo-(+)-Fenchyl alcohol	0,01
100	unk 43	0,01
101	unk 62	0,01
102	Citronellol	0,01
103	unk 54	0,01
104	Hexane	0,01
105	gamma-Curcumene	0,01
106	unk 111	0,01
107	Allyl alcohol	0,01
108	unk 98	0,01
109	unk 17	0,01
110	unk 14	0,01
111	Toluene	0,01
112	unk 89	0,01
113	Octyl acetate	0,00
114	unk 49	0,00
115	n-Butyl alcohol	0,00
116	Ethyl alcohol	0,00
117	unk 66	0,00
118	Heptane	0,00
119	unk 103	0,00
120	unk 53	0,00
121	Piperitone	0,00
122	Eugenol	0,00
123	unk 101	0,00
124	trans-Nerolidol	0,00
125	unk 123	0,00
126	unk 33	0,00
127	isovaleraldehyde	0,00
128	trans-1,4-Hexadiene	0,00
129	Acetaldehyde	0,00
130	cis,cis-2,4-Hexadiene?	0,00
131	unk 92	0,00
132	Dimethyl sulfone	0,00
133	Isobutyraldehyde	0,00

2b) Average composition of *L. angustifolia* essential oils' samples

N.	Component	%
1	Linalool	33,77
2	Linalyl acetate	31,29
3	4-Terpineol	6,19
4	Lavandulyl acetate	3,34
5	cis-β-Ocimene	3,15
6	β-Caryophyllene	1,69
7	trans-β-Farnesene	1,55
8	trans-β-Ocimene	1,35
9	R(+)-Limonene	1,17
10	α-Terpineol	1,07
11	Borneol	1,00
12	Caryophyllene oxide	0,85
13	Geranyl acetate	0,69
14	1,8-Cineole	0,68
15	β-Myrcene	0,66
16	Water	0,62
17	1-Methyl-4-isopropylbenzene	0,55
18	3-Octanone	0,46
19	cis-5,8,11,14-Eicosatetraenoic acid methyl ester	0,41
20	Neryl acetate	0,40
21	octen-3-yl-acetate	0,40
22	Camphor	0,36
23	Endo-fenchol	0,34
24	Neoisomenthol	0,33
25	unk 57	0,29
26	Octen-3-ol	0,28
27	alpha-Santalene	0,27
28	cis-Linalool oxide	0,23
29	α-Pinene	0,20
30	Germacrene	0,20
31	δ-3-Carene	0,19
32	unk 65	0,19
33	α-Bergamotene	0,18
34	Nerol	0,15
35	Camphene	0,14
36	Hexyl acetate	0,11
37	trans-Linalol oxide	0,10
38	unk 77	0,10
39	Acetone	0,10
40	γ-Terpinene	0,10
41	unk 134	0,09
42	unk 68	0,08
43	unk 130	0,08
44	Coumarin	0,08
45	α-Terpinolene	0,08
46	δ-Cadinene	0,08

47	β-pinene	0,07
48	3-Octanol	0,07
49	Hexyl tiglate	0,06
50	α-Humulene	0,06
51	Sabinene	0,05
52	Geraniol	0,05
53	Valencene	0,05
54	1-Methyl-3-isopropylbenzene	0,05
55	Neo-Isopulegol	0,05
56	Bornyl acetate 76-49-3	0,05
57	trans-Citral	0,05
58	Verbanol acetate	0,05
59	Cumin aldehyde	0,05
60	α-Thujene	0,05
61	unk 121	0,04
62	unk 110	0,04
63	α-Bisabolol	0,04
64	α-Santalol(Z)	0,04
65	unk 100	0,03
66	unk 115	0,03
67	2-heptanol	0,03
68	Lavandulol	0,03
69	β-Bourbonene	0,03
70	trans-Pinocarveol	0,03
71	Acetic acid	0,03
72	Isobornyl acetate	0,02
73	Di-isooctylfumarate	0,02
74	α-Terpinene	0,02
75	(R)-( )-Pulegone	0,02
76	Carvacrol	0,02
77	unk 124	0,02
78	unk 133	0,02
79	unk 71	0,02
80	methyl docosatrienoate	0,02
81	unk 52	0,02
82	Citronellol	0,02
83	Hexane	0,01
84	α-Phellandrene	0,01
85	unk 113	0,01
86	α-Gurjunene	0,01
87	unk 108	0,01
88	unk 85	0,01
89	unk 17	0,01
90	unk 96	0,01
91	unk 97	0,01
92	unk 54	0,01
93	(1R)-endo-(+)-Fenchyl alcohol	0,01
94	unk 49	0,01
95	unk 111	0,01
96	Toluene	0,01

97	Octyl acetate	0,01
98	unk 14	0,01
99	unk 103	0,00
100	Allyl alcohol	0,00
101	n-Butyl alcohol	0,00
102	unk 53	0,00
103	unk 101	0,00
104	unk 33	0,00
105	Piperitone	0,00
106	Acetaldehyde	0,00
107	unk 92	0,00
108	trans-Nerolidol	0,00
109	Heptyl alcohol	0,00
110	t,t-alpha-Farnesene	0,00
111	unk 89	0,00
112	trans-1,4-Hexadiene	0,00
113	unk 78	0,00
114	unk 61	0,00
115	unk 62	0,00
116	cis-Nerolidol	0,00
117	Ethyl alcohol	0,00
118	α-Bulnesene	0,00
119	unk 43	0,00
120	unk 66	0,00
121	unk 128	0,00
122	Methyl cis-9,10-methyleneoctadecanoate	0,00
123	β-Vetivene	0,00
124	Isobutyraldehyde	0,00
125	Heptane	0,00
126	gamma-Curcumene	0,00
127	unk 15	0,00
128	unk 123	0,00
129	Dimethyl sulfone	0,00
130	cis,cis-2,4-Hexadiene?	0,00
131	Eugenol	0,00
132	isovaleraldehyde	0,00
133	unk 98	0,00

2c) Average composition of *L. x intermedia* essential oils' samples

N.	Component	%
1	Linalool	36,76
2	Linalyl acetate	23,51
3	4-Terpineol	4,18
4	Camphor	4,03
5	1,8-Cineole	3,94
6	Borneol	2,77
7	cis-β-Ocimene	1,79
8	trans-β-Ocimene	1,76
9	Lavandulyl acetate	1,73
10	trans-β-Farnesene	1,66
11	β-Caryophyllene	1,30
12	trans-Pinocarveol	1,18
13	R(+)-Limonene	1,06
14	β-Myrcene	0,87
15	α-Terpineol	0,84
16	Water	0,63
17	α-Pinene	0,56
18	α-Bisabolol	0,51
19	β-pinene	0,50
20	Germacrene	0,45
21	1-Methyl-4-isopropylbenzene	0,45
22	unk 68	0,40
23	Octen-3-ol	0,40
24	Geranyl acetate	0,36
25	Camphene	0,34
26	Valencene	0,34
27	Coumarin	0,27
28	α-Terpinolene	0,26
29	Di-isooctylfumarate	0,23
30	δ-3-Carene	0,22
31	3-Octanone	0,22
32	Neryl acetate	0,21
33	octen-3-yl-acetate	0,21
34	Caryophyllene oxide	0,20
35	Sabinene	0,16
36	cis-5,8,11,14-Eicosatetraenoic acid methyl ester	0,16
37	α-Bergamotene	0,15
38	γ-Terpinene	0,14
39	trans-Linalol oxide	0,13
40	unk 121	0,13
41	unk 77	0,12
42	β-Vetivene	0,09
43	cis-Nerolidol	0,09
44	unk 57	0,08
45	2-heptanol	0,08
46	unk 65	0,08

47	α-Thujene	0,07
48	α-Santalol(Z)	0,07
49	Methyl cis-9,10-methyleneoctadecanoate	0,06
50	cis-Linalool oxide	0,06
51	unk 78	0,05
52	(R)-( )-Pulegone	0,05
53	Hexyl acetate	0,05
54	alpha-Santalene	0,05
55	unk 130	0,05
56	Acetone	0,04
57	unk 43	0,04
58	Geraniol	0,04
59	Hexyl tiglate	0,03
60	Nerol	0,03
61	t,t-alpha-Farnesene	0,03
62	α-Gurjunene	0,03
63	unk 61	0,03
64	3-Octanol	0,02
65	δ-Cadinene	0,02
66	gamma-Curcumene	0,02
67	Endo-fenchol	0,02
68	α-Terpinene	0,02
69	unk 96	0,02
70	unk 98	0,02
71	α-Bulnesene	0,01
72	α-Humulene	0,01
73	β-Bourbonene	0,01
74	unk 110	0,01
75	unk 124	0,01
76	methyl docosatrienoate	0,01
77	α-Phellandrene	0,01
78	unk 113	0,01
79	Heptyl alcohol	0,01
80	Heptane	0,01
81	trans-Citral	0,01
82	unk 108	0,01
83	unk 111	0,01
84	Eugenol	0,00
85	Toluene	0,00
86	Isobornyl acetate	0,00
87	Verbanol acetate	0,00
88	unk 71	0,00
89	unk 15	0,00
90	cis,cis-2,4-Hexadiene?	0,00
91	Ethyl alcohol	0,00
92	Dimethyl sulfone	0,00
93	unk 101	0,00
94	unk 17	0,00
95	unk 97	0,00
96	unk 92	0,00

97	unk 128	0,00
98	Neoisomenthol	0,00
99	(1R)-endo-(+)-Fenchyl alcohol	0,00
100	Lavandulol	0,00
101	Allyl alcohol	0,00
102	unk 103	0,00
103	unk 49	0,00
104	unk 52	0,00
105	Isobutyraldehyde	0,00
106	Bornyl acetate 76-49-3	0,00
107	trans-1,4-Hexadiene	0,00
108	unk 54	0,00
109	unk 123	0,00
110	unk 66	0,00
111	1-Methyl-3-isopropylbenzene	0,00
112	unk 89	0,00
113	n-Butyl alcohol	0,00
114	Acetaldehyde	0,00
115	unk 33	0,00
116	isovaleraldehyde	0,00
117	unk 14	0,00
118	unk 115	0,00
119	Carvacrol	0,00
120	Octyl acetate	0,00
121	unk 85	0,00
122	trans-Nerolidol	0,00
123	Piperitone	0,00
124	Neo-Isopulegol	0,00
125	unk 134	0,00
126	unk 53	0,00
127	Acetic acid	0,00
128	Citronellol	0,00
129	unk 133	0,00
130	unk 62	0,00
131	Hexane	0,00
132	Cumin aldehyde	0,00
133	unk 100	0,00