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Handheld Raman Spectroscopy for the Distinction of Essential Oils Used in the Cosmetics Industry

Paul Vargas Jentzsch ^{1,2}, Luis A. Ramos ¹ and Valerian Ciobotă ^{3,*}

¹ Ecuadorian Agency for Quality Assurance in Agriculture, AGROCALIDAD, Av. Interoceánica km 14 ½, 170184 Tumbaco, Ecuador; E-Mails: paul_5151@yahoo.com (P.V.J.); luis.ramos@agrocalidad.gob.ec (L.A.R.)

² Faculty of Chemical Sciences, Central University of Ecuador, Ciudadela Universitaria, Av. América and Av. Universitaria, 170521 Quito, Ecuador

³ Rigaku Raman Technologies, Pasedagplatz 3-4, 13088 Berlin, Germany

* Author to whom correspondence should be addressed; E-Mail: valerian.ciobota@rigaku.com; Tel.: +49-179-6265-473.

Academic Editor: Amparo Salvador-Carreño

Received: 11 April 2015 / Accepted: 20 May 2015 / Published: 27 May 2015

Abstract: Essential oils are highly appreciated by the cosmetics industry because they have antimicrobial and antioxidant properties, among others. Since essential oils are natural products, their inclusion in cosmetic formulations is a common practice. Currently, low-quality and/or adulterated essential oils can be found on the market; therefore, analytical methods for control are required. Raman spectroscopy is a versatile technique that can be used for quality control tasks; the portability of modern devices expand the analytical possibilities also to *in situ* measurements. Fifteen essential oils of interest for the cosmetics industry were measured using a handheld Raman spectrometer, and the assignment of the main bands observed in their average spectra was proposed. In most cases, it is possible to distinguish the essential oils by a simple visual inspection of their characteristic Raman bands. However, for essential oils extracted from closely-related vegetable species and containing the same main component in a very high proportion, the visual inspection of the spectra may be not enough, and the application of chemometric methods is suggested. Characteristic Raman bands for each essential oil can be used to both identify the essential oils and detect adulterations.

Keywords: essential oils; cosmetics; Raman spectroscopy

1. Introduction

The cosmetics industry is, probably, one of the most profitable industries worldwide. Everyone can intuitively understand the meaning of the word “cosmetics”, but it may be difficult to formulate a universal definition of “cosmetics” and, in some cases, to classify certain products as cosmetics or medicinal products. A discussion about these aspects, including a short exposition of some legal definitions, was published by Zesch [1]. Independent of the definitions and the interpretations assumed by different countries concerning cosmetic products, there is a trend in the cosmetics industry to produce new products having also certain medicinal effects. For example, a skin cream can be formulated with the intention to improve the appearance and, at the same time, act against irritations and/or pathogenic organisms. Another common trend is the use of natural ingredients as raw materials instead of synthetic components. Among consumers, there is a marked preference for natural products, resulting in the present good market positioning of products containing natural ingredients.

Taking into account both their medicinal properties and the natural origin of the substances, essential oils are ideal for cosmetic formulations. In fact, the use of vegetable extracts for medicinal and cosmetic purposes is well known [2]. The specific properties of essential oils (bactericidal, fungicidal, antiparasitic, insecticidal, *etc.*) make them highly appreciated raw materials to be used in the cosmetic industry [3–5]. There are several scientific reports about the antibacterial activity of essential oils [6,7], and in many cases, applications for cosmetic products are evident. For example, Zu and coworkers [8] tested the antibacterial activity of ginger, lemon and thyme essential oils (among others) against *Propionibacterium acnes*, which is considered the key factor for the development of acne, suggesting their use as ingredients in anti-acne creams.

Another highly-appreciated property of essential oils is their antioxidant activity. The antioxidant activity of lemon, patchouli, bitter orange, nutmeg, sage and thyme essential oils (among many others) was reported in the past [9–12]. The incorporation of essential oils in cosmetic preparations may help to prevent oxidative skin damage. In addition, it was reported that the presence of certain essential oils (e.g., lemon essential oil) in anti-ageing formulations can enhance the skin penetration of other active ingredients, like vitamins, thus resulting in improvements in the action of such formulations [13]. Some essential oils can also be used for sunscreen formulations. Kaur and Saraf [14] have calculated the sun protection factor (SPF) of many essential oils and reported that peppermint essential oil has a remarkably good SPF value; therefore, it is a good ingredient for sunscreen formulations.

There are also scientific reports on the use of essential oils to repel insects. The mosquito repellent activity of 38 essential oils was tested by Trongtokit *et al.* [15]. These authors found that many essential oils prevent mosquito bites, and the essential oils with higher repellent effect were those of citronella, patchouli, clove and makaen. These kinds of effects on insects may also be desirable in some cosmetic formulations.

In general, in the cosmetics industry, the detection of adulterated raw materials is crucial, especially for important ingredients, such as the case of essential oils. Conventionally, the analysis of essential

oils is performed using chromatographic methods, providing good and reliable results. However, alternative techniques that allow not only fast, but also *in situ* measurements are required. In this context, spectroscopic techniques emerge as interesting options, and among them, Raman spectroscopy stands out due to its versatility. These techniques can provide valuable analytical information in shorter times than conventional techniques.

Raman spectroscopy is a remarkable analytical technique applicable to almost all disciplines of natural sciences, since it is a non-destructive technique and only requires minimal or no sample preparation [16]. It has several applications in chemistry, physics and biology, highlighting studies on the composition of materials in different areas, such as microbiology, arts, archaeology, geology and atmospheric sciences, among many others [17–24]. Since handheld Raman spectrometers with short acquisition times and reasonable spectral resolutions are available, the applicability of this technique in industry has remarkably increased.

Concerning the Raman spectroscopic evaluation of essential oils, we have distinguished some essential oils based on their Raman spectra in the past [25]. It was suggested that both the presence and relative intensity of certain bands, due to key components in essential oils, can be used to evaluate the quality of essential oils and even to detect adulterations.

In this work, we present the average Raman spectra of fifteen commercial essential oils, which were compared to each other in order to evaluate the capability of Raman spectroscopy for the distinction of essential oils. The strongest Raman bands were assigned, since they can be used to identify the individual essential oils (as well as to detect adulterations). The expected composition of each essential oil, as determined from a rigorous literature review of previous works, supported the evaluation of the spectroscopic data.

2. Materials and Methods

2.1. Essential Oils

The essential oils of anise (*Pimpinella anisum*), basil oil (*Ocimum basilicum*) linalool type, basil oil (*Ocimum basilicum*) methyl chavicol type, geranium oil (*Pelargonium graveolens*), ginger oil (*Zingiber officinale*), lavandin oil (*Lavandula hybrida*), lavender oil (*Lavandula angustifolia*), lemon oil (*Citrus limon*), nutmeg oil (*Myristica fragrans*), orange (bitter) oil (*Citrus auroantium amara*), orange (sweet) oil (*Citrus sinensis*), patchouli oil (*Pogostemon cablin*), peppermint oil (*Mentha piperita*), sage oil (*Salvia officinalis*) and thyme oil (*Thymus vulgaris*) were purchased from a local distributor in Germany. The distributor provided certificates that ensured the quality of the essential oils. The recorded Raman spectra of the essential oils were in good agreement with the expected composition for each case.

2.2. Raman Spectroscopy and Chemometrics

Raman measurements were performed using a handheld Raman spectrometer, ProgenyTM (Rigaku Raman Technologies, Wilmington, DE, USA), equipped with a 1064-nm Nd:YAG laser. The detection of the Raman scattered light was done by a Peltier cooled InGaAs detector. The total exposure time for a Raman spectrum was 5 s, and the laser power was 490 mW. The accuracy and precision of the

Raman bands of the acquired spectra were below 3 cm^{-1} and 1.5 cm^{-1} , respectively. Fifteen spectra were recorded for each sample, and each sample was measured three times on three different days in order to evaluate the reproducibility of the measurements. The average Raman spectra of each essential oil were used for comparisons and spectroscopic evaluation.

The chemometric analysis was performed using the “R” software [26]. Before data analysis, the already baseline-corrected Raman spectra were vector normalized. The analysis was carried out using principal component analysis (PCA).

3. Results and Discussion

Anise, basil (two types), geranium, ginger, lavandin, lavender, lemon, nutmeg, orange (bitter and sweet), patchouli, peppermint, sage and thyme essential oils are considered in this study. The composition of these essential oils was studied by various authors in the past, and a summary of their findings is given in Table S1 (Supplementary Materials). Essential oils contain a large variety of compounds (terpenes and non-terpene compounds) that are reflected by prominent differences in the Raman spectra. Therefore, characteristic Raman bands for each essential oil can be expected. Despite the differences in the Raman spectra, there are certain common features related to functional groups in compounds present in essential oils. At least one band in the vicinity of 1450 cm^{-1} attributed to bending modes of CH_3 and CH_2 groups [27] can be expected in the Raman spectra of all essential oils considered in this study. Bands in this region can be weaker or stronger, depending on the presence of CH_3 and CH_2 groups in the compounds of the essential oils and cannot easily be attributed to specific compounds, since most of the compounds have CH_3 and CH_2 groups in their structure. Figure 1 shows the average Raman spectra of all essential oils considered in this study. In addition, all Raman spectra, including the wavenumbers of the bands, can be found in the Supplementary Materials (Figures S1–S15). Comparing the spectra to each other, most often, the differences are easily visible by the naked eye, but in other cases (e.g., essential oils extracted from closely-related vegetable species), the differences are imperceptible by a fast inspection of the spectra. For the distinction of essential oils from each other, as well as for general quality control purposes, it is necessary to assign and understand the meaning of the most important bands. The assignment of the strongest bands occurring in the average Raman spectra of the essential oils considered in this study is summarized in Table 1 and discussed in the following.

The most important component of anise essential oil is anethole (sometimes more than 90%) [11,28]. In fact, the Raman spectrum of anise essential oil (Figure 1a) shows similar features compared to those reported in the past for anethole [29]. The stronger bands in the Raman spectra of anise appear at 1654 and 1608 cm^{-1} and are assigned to the $\text{C}=\text{C}$ and ring quadrant stretching modes, respectively. Since anethole has an ether group, the band at 1282 cm^{-1} in the Raman spectrum of anise essential oil was assigned to the $\text{Ar}-\text{O}$ stretching mode (where Ar = benzene ring). There are two Raman signals expected for aryl alkyl ethers: first, the $\text{Ar}-\text{O}$ stretching mode in the range $1310\text{--}1210\text{ cm}^{-1}$, and second, the $\text{C}-\text{O}$ stretching mode in the range $1050\text{--}1010\text{ cm}^{-1}$ [30]. The $\text{C}-\text{O}$ stretching mode appears as a weak band at 1039 cm^{-1} for the anise essential oil. The Raman band at 1173 cm^{-1} for anise essential oil was assigned to an in-plane $\text{C}-\text{H}$ bending mode of the ring.

There are many studies on the composition of basil essential oil reporting important variations in the proportion of its major components [31,32]. Many basil chemotypes are usually named by the main component in their essential oil [33]. In this work, two basil essential oils were considered: first, linalool type, and second, methyl chavicol (estragole) type. This means that the major components in these basil essential oils were linalool and estragole, respectively. The most important bands in the Raman spectrum of basil essential oil, linalool type, appear at 1672, 1640, 1452, 1378, 1293, 803 and 651 cm^{-1} , as shown in Figure 1b. These bands are similar to those observed in an earlier reported Raman spectrum of linalool [34]. The strong bands at 1672 and 1640 cm^{-1} are attributed to C=C stretching modes, and the band at 1452 cm^{-1} appears in the typical region for CH_3/CH_2 bending modes. There are two CH_3 groups directly attached to a C=C in the chemical structure of linalool; therefore, the band at 1378 cm^{-1} can be assigned to a CH_3 bending mode (more details concerning this assignment are provided in the discussion of the spectroscopic features of geranium essential oil). The band at 1293 cm^{-1} is assigned to the =CH rocking mode, and the band at 803 cm^{-1} seems to be related to the OH group of linalool. The band 651 cm^{-1} seems to indicate the presence of camphor in this essential oil, since a very strong band in this region is expected for the ring deformation of this compound [34].

Table 1. Assignment for the most intense Raman bands observed for the essential oils.

Essential Oil	Wavenumbers of Bands (cm^{-1})	Proposed Assignment	Essential Oil	Wavenumbers of Bands (cm^{-1})	Proposed Assignment
Anise	1654	C=C stretch	Lavender	1672	C=C stretch
	1608	Ring quadrant stretch		1640	C=C stretch
	1282	Ar-O stretch (ether)		1452	CH_3/CH_2 bend
	1173	In-plane C-H bend (ring)		1413	CH_3/CH_2 bend
Basil (linalool)	1672	C=C stretch	Lemon	1378	CH_3 bend (attached to a C=C)
	1640	C=C stretch		1298	=CH rock
	1452	CH_3/CH_2 bend		652	Ring deformation
	1378	CH_3 bend (attached to a C=C)		1676	C=C stretch
	1293	=CH rock		1644	C=C stretch
	803	C-O stretch (alcohol)		1432	CH_3/CH_2 bend
	651	Ring deformation		757	Ring deformation
Basil (methyl chavicol)	1640	C=C stretch	Nutmeg	1654	C=C stretch
	1608	Ring quadrant stretch		1640	C=C stretch
	1298	Ar-O stretch (ether)		1608	Ring quadrant stretch
	1179	In-plane C-H bend (ring)		1447	CH_3/CH_2 bend
	843	Aromatic 2 adjacent H		956	Ring deformation
		CH wag ¹		670	Ring deformation
	820	Aromatic 2 adjacent H		646	Ring deformation
		CH wag ¹		Sweet/Bitter	1676
640	Ring in-plane bend	Orange	1645	C=C stretch	
			1433	CH_3/CH_2 bend	
			763	Ring deformation	

Table 1. Cont.

Essential Oil	Wavenumbers of Bands (cm ⁻¹)	Proposed Assignment	Essential Oil	Wavenumbers of Bands (cm ⁻¹)	Proposed Assignment
Geranium	1671	C=C stretch	Patchouli	1640	C=C stretch
	1640	C=C stretch		1452	CH ₃ /CH ₂ bend
	1452	CH ₃ /CH ₂ bend		1438	CH ₃ /CH ₂ bend
	1378	CH ₃ bend (attached to a C=C)		604	Ring deformation
	803	C–O stretch (alcohol)		1457	CH ₃ /CH ₂ bend
Ginger	1671	C=O stretch	Peppermint	769	Ring deformation
	1635	C=C stretch		1447	CH ₃ /CH ₂ bend
	1590	C=C stretch	Sage	652	Ring deformation
	1447	CH ₃ /CH ₂ bend		1613	Ring quadrant stretch
	1438	CH ₃ /CH ₂ bend		1457	CH ₃ /CH ₂ bend
Lavandin	1378	CH ₃ bend (attached to a C=C)	1442	CH ₃ /CH ₂ bend	
	1672	C=C stretch	1378	CH ₃ bend (attached to an aromatic ring)	
	1640	C=C stretch	1210	In-plane C–H bend (ring)	
	1452	CH ₃ /CH ₂ bend	1061	In-plane C–H bend (ring)	
	1413	CH ₃ /CH ₂ bend	803	Ring deformation	
	1378	CH ₃ bend (attached to a C=C)	740	Ring quadrant in-plane bend	
	1298	=CH rock			
652	Ring deformation				

¹ The propenyl substituent of estragole has a mechanical interaction effect on the ring CH wagging vibrations, resulting in the enhancement of these Raman signals compared to analogous signals in anethole.

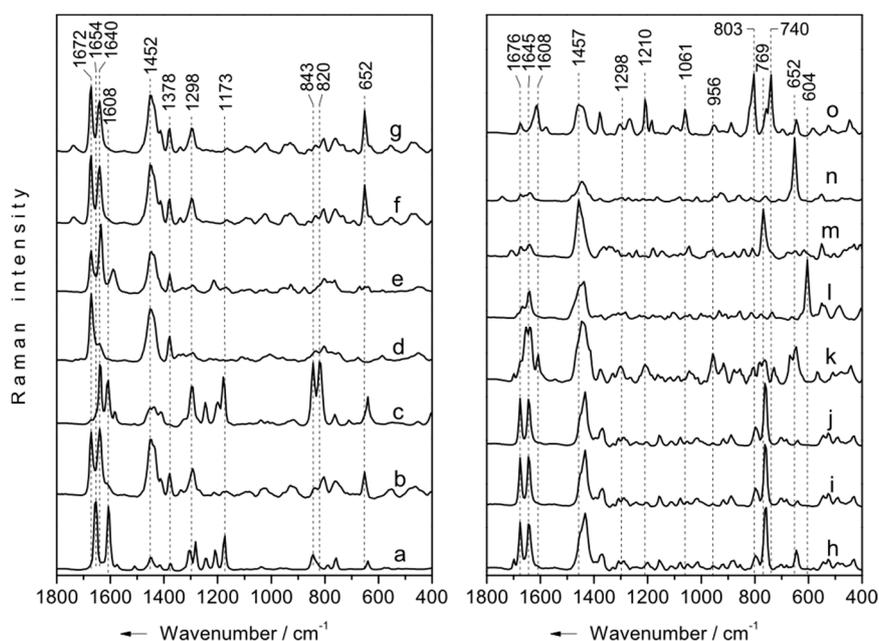


Figure 1. Raman spectra of essential oils: (a) anise, (b) basil (linalool), (c) basil (methyl chavicol), (d) geranium, (e) ginger, (f) lavender, (g) lavandin, (h) lemon, (i) bitter orange, (j) sweet orange, (k) nutmeg, (l) patchouli, (m) peppermint, (n) sage and (o) thyme.

The most important bands in the Raman spectrum of basil essential oil, methyl chavicol (also known as estragole) type, appear at 1640, 1608, 1298, 1179, 843, 820 and 640 cm^{-1} , as shown in Figure 1c. These bands are similar to those presented in an earlier Raman spectroscopic study of basil essential oil extracted from basil plants of estragole type [33]. The bands at 1640 and 1608 cm^{-1} are attributed to C=C stretching and ring quadrant stretching modes, respectively. The strong band at 1298 cm^{-1} seems to be related to the =CH rocking mode of estragole. The band at 1179 cm^{-1} is assigned to a ring in-plane C-H bending mode. In the region 650–630 cm^{-1} appears a characteristic band for *para*-disubstituted benzenes [30]; therefore, the band at 640 cm^{-1} is assigned to the ring in-plane bending mode of estragole. Comparing the Raman spectrum of anise essential oil with the corresponding one of basil (methyl chavicol type), the bands at 843 and 820 cm^{-1} are considerably stronger for the last one. The chemical structures of estragole and anethole differ only in the position of one double bond; therefore, minimal differences in the Raman spectra of basil (methyl chavicol type) and anise essential oils may be expected. However, there are important differences, especially in the intensity of a couple of bands. While for anise essential oil, a weak band and an almost imperceptible shoulder appear at 843 and ~ 825 cm^{-1} , respectively, for basil (methyl chavicol type) essential oil, two strong bands at 843 and 820 cm^{-1} occur. The same differences can be observed in the Raman spectra of anethole and estragole reported by Gudi *et al.* [29]. How can these changes in the intensity of bands be explained? In the region 880–795 cm^{-1} , ring C-H wagging vibrations for *para*-disubstituted benzenes are expected. These wagging vibrations are coupled to each other, but there is also a mechanical interaction effect of the substituents [35]. Apparently, the interaction effect of the two propenyl substituents is markedly different, and the prop-2-enyl substituent (in estragole) has an enhancement effect on the wagging signals compared to the prop-1-enyl substituent (in anethole). In addition, the response of certain Raman bands attributed to estragole (e.g., 1640 and 1608 cm^{-1}) may be lowered compared to those of anethole.

The main components of geranium essential oil are geraniol, citronellol and linalool [36,37]. As happens with other essential oils, the proportion can change depending on the region and other more specific factors. An interesting aspect is that these three compounds have some structural similarities; for example, all three compounds have a =C(CH₃)₂ group. A CH₃ bending mode appears in the vicinity of 1380 cm^{-1} as a weak Raman band, except when the CH₃ group is directly attached to C=C, C≡C, C=O or an aromatic ring. In these cases, the band appears medium to strong [35]. In fact, the Raman spectrum of geranium essential oil (Figure 1d) shows an intense band at 1378 cm^{-1} , which is assigned to a CH₃ bending mode. The bands at 1671 and 1640 cm^{-1} can unambiguously be assigned to C=C stretching modes, while the band at 1452 cm^{-1} is clearly related to CH₃/CH₂ bending modes. Geraniol, citronellol and linalool have in their chemical structure a OH group; therefore, the band at 803 cm^{-1} in the Raman spectrum of geranium essential oil has been assigned to the C–O stretching mode.

The main components of ginger essential oil are zingiberene, geranial and neral [38,39], and these compounds are expected to shape its Raman spectrum. Geranial (citral A) and neral (citral B) are geometric isomers [40]. Both geranial and neral are aliphatic unsaturated aldehydes, thus Raman signals related fundamentally to the bonds C=O, C=C, as well as the groups CH₂ and CH₃ are expected to appear. For zingiberene, vibrations related to bonds C=C and groups CH₂ and CH₃ are expected to be observed as bands. The most important bands in the Raman spectrum of ginger essential oil appear

at 1671, 1635, 1590, 1447, 1438 and 1378 cm^{-1} , as shown in Figure 1e. The first three bands can be assigned to C=C stretching modes. A wavenumber as low as 1590 cm^{-1} for the C=C stretching mode can be explained by conjugation [30]; in the chemical structure of geranial and neral, the double bond is conjugated with the aldehyde group. The bands at 1447 and 1438 cm^{-1} are assigned to CH_3/CH_2 bending modes, while the band at 1378 cm^{-1} is assigned to a CH_3 bending mode from CH_3 groups directly attached to a C=C.

The main components of lavender essential oil are linalool and linalyl acetate, but other components, like camphor, can also be present in lower proportions [41,42]. The essential oil extracted from lavandin, a hybrid of lavender, contains likewise linalool and linalyl acetate as major components, and the presence of camphor and 1,8-cineole was also reported [43]. It is not possible to distinguish between lavender and lavandin essential oils only by visual comparison of their Raman spectra. The Raman spectra of these two essential oils (Figure 1f,g) show the same main bands: 1672, 1640, 1452, 1413, 1378, 1298 and 652 cm^{-1} . Similar to the Raman spectrum of basil essential oil (linalool type), the bands at 1672 and 1640 cm^{-1} are attributed to C=C stretching modes, while the bands at 1452, 1413 and 1378 cm^{-1} are assigned to CH_3 and CH_2 bending modes. The band at 1298 cm^{-1} is assigned to the =CH rocking mode. The band at 652 cm^{-1} is characteristic of camphor. For camphor, Daferera *et al.* [34] have reported a very strong band at 651 cm^{-1} (ring deformation) and weaker bands at 1741 and 1448 cm^{-1} . In the Raman spectra of both lavender and lavandin essential oils, a weak band emerges at 1738 cm^{-1} , confirming the presence of camphor in these oils. The band at 1448 cm^{-1} for camphor cannot be observed, because it may be overlapped with the strong band of linalool at 1452 cm^{-1} . Considering that there are important structural similarities between linalool and linalyl acetate, the Raman bands of both compounds may occur in similar spectral regions. Linalyl acetate contains an ester group in its structure. The characteristic Raman signal for the ester group (C=O stretching mode) appears in the same region of ketones ($\sim 1740 \text{ cm}^{-1}$) [30]; therefore, the signal of linalyl acetate may be overlapped with the Raman band of camphor.

The major component of lemon and orange (bitter and sweet) essential oils is limonene. Limonene constitutes more than 90% of the essential oil extracted from orange peel, but this percentage decreases for lemon to values slightly below 70% [11,44]. Considering these high contents of limonene in the essential oils of lemon and orange, it is easy to deduce that the Raman spectra of these essential oils are dominated by Raman bands of limonene. The main features in the Raman spectrum of limonene appear at 1678, 1645, 1435 and 760 cm^{-1} [45,46], and similar bands are clearly identifiable in the Raman spectrum of lemon and orange essential oils, as shown in Figure 1h,i,j. The two strong bands in the region 1680–1640 cm^{-1} are attributed to C=C stretching modes. Two bands are expected for the C=C stretching, because limonene has two double bonds in its chemical structure. The strong and broad band centered at $\sim 1433 \text{ cm}^{-1}$ is assigned to the CH_3/CH_2 bending mode, while the intense signal at 763 and 757 cm^{-1} (for bitter/sweet orange and lemon essential oils, respectively) can be attributed to a ring deformation mode of limonene.

The main components of nutmeg essential oil are α -pinene, β -pinene, myristicin and sabinene [9,47]. As happens with other essential oils, the proportion of these components can change depending on the characteristics of the vegetable material and the extraction method. The most important Raman bands of nutmeg essential oil (see Figure 1k) appear at 1654, 1640, 1608, 1447, 956, 670 and 646 cm^{-1} . The bands at 1654 and 1640 cm^{-1} can be assigned to the C=C stretching modes of

α -pinene and β -pinene, respectively. According to an earlier report, both α -pinene and β -pinene show an intense band for the CH_2/CH_3 bending modes in the region $\sim 1440\text{ cm}^{-1}$ [46]. This band appears at 1447 cm^{-1} in the Raman spectrum of nutmeg essential oil. The bands at 670 and 646 cm^{-1} can be attributed to ring deformations of α -pinene and β -pinene, respectively. The band at 1608 cm^{-1} can be attributed to the ring quadrant stretching mode of myristicin. An earlier reported Raman spectrum of sabinene shows the strongest signals at 1653 , 1446 , 1415 , 957 , 915 and 652 cm^{-1} [48]. Although the strongest Raman bands of sabinene seem to be overlapped with the strong signals of α -pinene and β -pinene, there are some bands in the Raman spectrum of nutmeg essential oil that are attributable to sabinene. The bands at 957 and 915 cm^{-1} can be attributed to ring deformations of sabinene. In addition, a shoulder at 1414 cm^{-1} can be assigned to CH_3/CH_2 bending modes of sabinene.

The main components of patchouli essential oil are α -guaiene, δ -guaiene and patchoulol [10,49,50]. Considering the chemical structure of the sesquiterpenes α -guaiene and δ -guaiene, as well as the terpene patchoulol, it is possible to infer that the Raman spectrum of a mixture of these compounds, *i.e.*, the patchouli essential oil, should be dominated by bands related to $\text{C}=\text{C}$ and CH_2 and CH_3 groups. In addition, the Raman spectrum should also be influenced by the OH group of patchoulol. The most important bands in the Raman spectrum of patchouli essential oil appear at 1640 , 1452 , 1438 and 604 cm^{-1} , as shown in Figure 1l. The band at 1640 cm^{-1} is assigned to a $\text{C}=\text{C}$ stretching mode. The bands in the vicinity of $\sim 1450\text{ cm}^{-1}$ can be attributed to CH_2 and CH_3 bending modes. The band at 604 cm^{-1} appears in a spectral region in which ring deformations are expected; hence, this band is assigned to this kind of vibrations.

The main components of peppermint essential oil are menthone, menthol and 1,8-cineole [51,52]. The Raman spectrum of peppermint essential oil (Figure 1m) shows two strong bands that can be used for its identification: 1457 and 769 cm^{-1} . It seems that the peppermint essential oil used in this study contains a remarkably low proportion of menthone compared to menthol. One of the strongest bands of menthone, the one corresponding to the $\text{C}=\text{O}$ stretching mode, appears as a weak band at 1708 cm^{-1} . The strongest band should appear at 1459 cm^{-1} , but this band may be overlapped with the very strong band at 1457 cm^{-1} , which seems to be related to CH_3/CH_2 bending modes of menthol. Based on the Raman spectrum of a supersaturated solution of menthol in ethanol reported by Findlay and Bugay [53], the strong band at 769 cm^{-1} can be attributed to menthol. Taking into account the spectral region, the band at 769 cm^{-1} can be assigned to a ring deformation. For 1,8-cineole, the strongest Raman band appears at 652 cm^{-1} [54]. With the Raman spectrum of peppermint essential oil showing a weak band at this wavenumber, the presence of small amounts of 1,8-cineole cannot be ruled out. Since the weak bands at 1671 and 1640 cm^{-1} do not appear in the Raman spectra of menthone and menthol, they could be attributed to minor components. β -Caryophyllene and myrcene show intense bands for $\text{C}=\text{C}$ stretching modes at these wavenumbers [46].

As was reported by various authors, the main components of sage essential oil are camphor, α -thujone, β -thujone and 1,8-cineole [11,55]. Chalchat *et al.* [55] found that the proportion of the main components of sage essential oil depends on the origin of the plant; camphor or α -thujone appeared as the major compounds depending on the country from which the plants originated. The Raman spectrum of camphor shows a very strong band at 651 cm^{-1} (ring deformation) and characteristic bands for the $\text{C}=\text{O}$ stretching and CH_3/CH_2 bending modes at 1741 and 1448 cm^{-1} , respectively [34]. The Raman spectrum of sage essential oil (Figure 1n) shows a very strong band at 652 cm^{-1} .

In addition, a Raman band is found at 1447 cm^{-1} (medium intensity) and a weak band at 1743 cm^{-1} , confirming that camphor is present in sage essential oil in a high proportion. However, the bands at 1447 cm^{-1} and 1743 cm^{-1} can also be influenced by the bands of α -thujone and β -thujone, since vibrations related to CH_3 and CH_2 , as well as the ketone group are also expected for these compounds. Taking into account that the most important Raman bands of 1,8-cineole appear at 652 cm^{-1} and in the region $\sim 1440\text{ cm}^{-1}$ [54], its presence cannot be ruled out (bands of 1,8-cineole may be overlapped with the strong bands of camphor).

The main components of thyme essential oil are thymol, *p*-cymene, carvacrol and γ -terpinene [9,11,56,57]. The first three components have a benzene ring in their chemical structure, while the fourth has a ring of six carbons with two $\text{C}=\text{C}$. The Raman spectra of thymol, *p*-cymene, carvacrol and γ -terpinene were reported by Daferera *et al.* [34]. The Raman spectrum of thyme essential oil (Figure 1o) shows the most prominent bands at 1613, 1457, 1442, 1378, 1210, 1061, 803 and 740 cm^{-1} . The band at 1613 cm^{-1} is assigned to the ring quadrant stretching mode of *p*-cymene. The bands at 1457 and 1442 cm^{-1} are assigned to CH_3/CH_2 bending modes. The band at 1378 cm^{-1} is assigned to a CH_3 bending mode from CH_3 groups directly attached to an aromatic ring. Many of the bands observed for substituted benzenes in the range $1600\text{--}1000\text{ cm}^{-1}$ involve in-plane C–H bending vibrations interacting with various ring $\text{C}=\text{C}$ vibrations [30]. Therefore, the bands at 1210 and 1061 cm^{-1} are attributed to this type of vibration. The bands at 803 and 740 cm^{-1} are assigned to ring deformations of *p*-cymene and thymol, respectively.

In general, the essential oils considered in this study can be distinguished by the naked eye from each other based on their Raman spectra. The comparison of the Raman spectra (Figure 1) shows that there are characteristic (key) bands for each essential oil, which allow the identification of the respective essential oils by a simple visual inspection of the spectra. Nevertheless, in some cases, the identification based on the Raman spectra of the substances may not be that easy, requiring supplementary analyses. This is the case of orange (bitter and sweet) essential oils, which cannot be distinguished from each other by a simple inspection of their Raman spectra. Since they have a common main component in a very high proportion, these bands dominate the Raman spectra, and other minor bands tend to be masked or they are too weak to be appreciated in a fast visual inspection of the spectra. The same applies for the Raman spectra of lavandin and lavender essential oils. Therefore, the use of chemometric methods for these cases is necessary. The results of principal component analysis (PCA) performed on the Raman spectra of bitter and sweet orange oil are shown in Figure 2a. The Raman spectra of the investigated orange oils are clustered into two distinct groups, corresponding to their oil type. The groups are clearly separated, and therefore, discrimination between the two oil types is straightforward. Furthermore, the Raman spectra of lavandin and lavender oil can be discriminated by this unsupervised chemometric method, as shown in Figure 2b.

It is important to notice that in this study, we only compared one specimen of bitter orange oil with one specimen of sweet orange oil. The same happened when lavender and lavandin essential oils were compared. This is just a first approach, and in order to get more conclusive results, more specimens from different origins and extracted by different methods should be measured and their spectra evaluated. However, the outcome suggests that Raman spectroscopy can be used for analysis of essential oils originating even from closely-related species. The sensitivity shown by Raman spectroscopy can be therefore used not only to distinguish between oils with similar chemical

composition, but also for detection of adulterated essential oils. There is no question about the benefits of the use of handheld Raman spectrometers and chemometrics for the *in situ* quality control of essential oils, because the crucial stages in which the quality of materials must to be verified can be optimized.

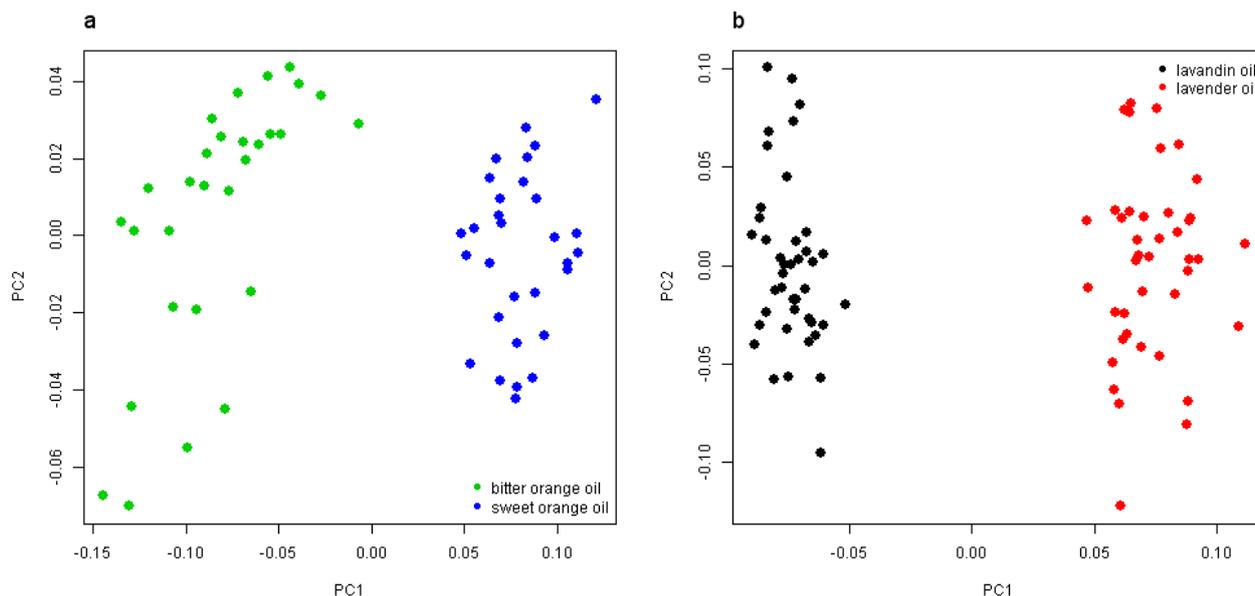


Figure 2. (a) Principal component analysis scores plot of bitter orange (green) and sweet orange (blue) oil. (b) Principal component analysis scores plot of lavandin (black) and lavender oil (red).

4. Conclusions

The Raman spectra of important essential oils used in cosmetics show characteristic bands that can be used for both the differentiation of essential oils and their quality control. For most of the essential oils considered in this study, the simple visual inspection of the Raman spectra is sufficient to distinguish the essential oils from each other. For some essential oils extracted from closely-related vegetable species (e.g., lavandin and lavender), the spectroscopic data require additional processing stages by chemometric methods for their identification. Key bands for the identification and discrimination of each essential oil were selected based on their relative intensity and the assignments proposed. The occurrence of foreign bands can be used to detect adulterations. Raman spectroscopy seems to be a strong technique for use in quality control, since modern equipment possesses handheld characteristics, providing spectra in a very short time with an acceptable spectral resolution.

Supplementary Materials

Supplementary materials can be accessed at: <http://www.mdpi.com/2079-9284/2/02/0162/s1>.

Acknowledgments

Paul Vargas Jentsch acknowledges the Prometeo Project of the Secretariat for Higher Education, Science, Technology and Innovation (SENESCYT) of the Republic of Ecuador for the support of the

investigation in Ecuador. Luis A. Ramos acknowledges the Ecuadorian Agency for Quality Assurance in Agriculture, AGROCALIDAD.

Author Contributions

Paul Vargas Jentsch, Luis A. Ramos and Valerian Ciobotă conceived of and designed the experiments. Valerian Ciobotă performed the measurements. Paul Vargas Jentsch, Luis A. Ramos and Valerian Ciobotă analyzed the data, and wrote the paper.

Conflicts of Interest

The authors declare no conflict of interest.

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