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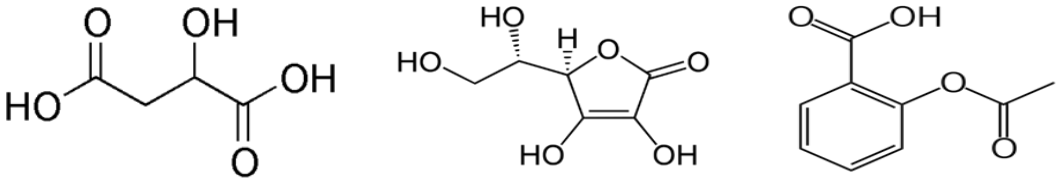

Effects of irrigation dose, plant density, application of organic acids, and harvest date on the quality and yield of commercial aromatic plants

Hussein Ali Sulayman El Zaeddi

Tesis Doctoral, 2018

Effects of irrigation dose, plant density, application of organic acids, and harvest date on the quality and yield of commercial aromatic plants

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Dr. D. **Ángel Antonio Carbonell Barrachina**, Catedrático de Universidad y Coordinador del Programa de Doctorado Recursos y Tecnologías Agrarias, Agroambientales y Alimentarias (ReTos-AAA) de la Universidad Miguel Hernández de Elche.

CERTIFICA:

Que la Tesis Doctoral titulada "Effects of irrigation dose, plant density, application of organic acids, and harvest date on the quality and yield of commercial aromatic plants", de la que es autor el Licenciado en Química Hussein Ali Sulayman El Zaeddi, ha sido realizada bajo la dirección de los doctores D. Francisco Miguel Burló Carbonell y D. Ángel Calín Sánchez, actuando como tutor el Dr. D. Ángel Antonio Carbonell Barrachina (UMH). Considero que la tesis es conforme en cuanto a forma y contenido a los requerimientos del Programa de Doctorado ReTos-AAA por tanto, es apta para su exposición y defensa pública.

Y para que conste a los efectos oportunos firmo el presente certificado en Orihuela a seis de diciembre de dos mil diecisiete.

Orihuela,___de_____de 2018

Esta Tesis Doctoral ha sido dirigida por el **Dr. Francisco Miguel Burló Carbonell,** Catedrático de Universidad del Departamento de Tecnología Agroalimentaria, de la Universidad Miguel Hernández de Elche, y del **Dr. Ángel Calín Sánchez,** investigador del grupo de investigación de la Universidad Miguel Hernández (CSA)**.**

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Catedrático de Tecnología de Alimentos. — Investigador CSA

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ESTRUCTURA DE LA TESIS

Para la realización de la presente Tesis Doctoral se ha seguido una metodología basada en la publicación de artículos de investigación. Con esta Tesis Doctoral se pretende obtener el título de Doctor con Mención Internacional, para ello en la redacción de la misma, se ha seguido la normativa vigente de la Universidad Miguel Hernández, concretamente el artículo 15 del Real Decreto 99/2011, donde se indica: "*Que parte de la tesis doctoral, al menos el resumen y las conclusiones, se haya redactado y presentado en una de las lenguas oficiales de la Unión Europea, distinta a alguna de las lenguas oficiales en España*". En este caso el idioma seleccionado es el inglés; y este idioma será empleado tanto para la redacción como para la exposición de parte de la Tesis.

Es importante mencionar, que las publicaciones presentes en esta Tesis Doctoral forman parte de un Contrato para Actividades de Asesoramiento y Asistencia Técnica entre el Grupo de Calidad y Seguridad Alimentaria y la empresa Rambla de los Molinos S.A. y la Cooperativa Agrícola Católica de Orihuela. Los experimentos se han llevado a cabo en las fincas propiedad de la empresa Rambla de los Molinos S.A., y, por lo tanto, los resultados obtenidos son de gran importancia y relevancia práctica en el sector de la producción agrícola, más concretamente el de las hierbas aromáticas.

La estructura de esta Tesis Doctoral consta de una breve Introducción en la que se incluye una revisión bibliográfica sobre hierbas aromáticas, sus usos, composición, propiedades funcionales y las prácticas agronómicas. Además, se incluye una descripción del efecto que provocan las prácticas agronómicas y culturales sobre algunos parámetros de las hierbas aromáticas, tales como la producción y la composición.

En el siguiente capítulo se describen los Objetivos planteados. A continuación, se recogen las Publicaciones Científicas que componen el núcleo de la presente Tesis Doctoral, y que se resumen a continuación:

- La primera publicación se titula "*Volatile composition of essential oils from different aromatic herbs grown in Mediterranean regions of Spain".* En este artículo, publicado en la revista *Foods*, se evalúa el contenido en aceites esenciales y su composición en distintas hierbas aromáticas en los distintos estados vegetativos en los que las empresas actuales del sector comercializan el material vegetal bajo estudio. También se describe el perfil sensorial de las hierbas aromáticas en el estado vegetativo en el que se detectaron los niveles más elevados de aceites esenciales.
- La segunda y tercera de las publicaciones, muestran los cambios en la concentración de los aceites esenciales y el perfil sensorial descriptivo de perejil y eneldo, como consecuencia de dos prácticas agronómicas: (i) la dosis de riego y (ii) la densidad de plantación. Los títulos de las publicaciones son "*Irrigation dose and plant density affect the essential oil content and sensory quality of parsley* (*Petroselinum sativum*)" and "I*rrigation dose and plant density affect the essential oil content and sensory quality of dill* (*Anethum graveolens* L.)", y se publicaron en las revistas *Scientia Horticulturae* y *Journal of the Science of Food and Agriculture*, respectivamente
- La cuarta y última publicación, publicada en la revista *Food Chemistry*, se titula "*Preharvest treatments with malic, oxalic and acetylsalicylic acids affect the phenolic composition and antioxidant capacity of coriander, dill and parsley*" y describe como una práctica agrónomica como los tratamientos pre-cosecha con compuestos de origen natural, modifican y en

algunos casos mejoran de manera notable las características funcionales de las hierbas aromáticas.

El cuarto capítulo recoge los Materiales y Métodos empleados para poder entender las distintas prácticas agronómicas utilizadas y las determinaciones físicas y químicas realizadas, así como, los Resultados y Discusión; donde se presenta un resumen global de los resultados más relevantes obtenidos en los diferentes estudios realizados.

Finalmente, en el quinto capítulo se recogen las Conclusiones generales de todos los estudios que forman parte de la presente Tesis Doctoral, mientras que el sexto y último capítulo corresponde a la Bibliografía consultada.

ABSTRACT - RESUMEN

Abstract

Nowadays, optimizing agronomical factors becomes very important to produce crops with both high production and quality. The optimization of several agricultural practices was the main purpose of this PhD dissertation. To reach this aim, the analysis of the volatile composition of different herbs was carried out to investigate the influence of (i) harvest date, (ii) irrigation dose, and (iii) plant density on the commercial yield and plant quality. Four herbs were studied: (i) **dill** (*Anethum graveolens*), (ii) **parsley** (*Petroselinum crispum*), (iii) **coriander** (*Coriandrum sativum*), (iv) and **mint** (*Mentha piperita*). Hydrodistllation, using a Deryng apparatus, to extract the herbs essential oils or volatile fractions, while gas chromatography with a mass spectrometry detector (GC-MS) was used to isolate and identify the volatile compounds; finally, the quantification was done by using gas chromatography with a flame ionization detector (GC-FID). Results showed that the predominant compounds of the volatile fractions of the aerial parts of: (i) dill were *α*-phellandrene, dill ether, and *β*-phellandrene, (ii) parsley were 1,3,8-*p*-menthatriene and *β*-phellandrene, (iii) coriander were *E*-2-dodecenal, dodecanal, and octane, and (iv) mint were carvone and limonene. All data generated in the current dissertation (e.g. total yield, volatile composition, and descriptive sensory profile) were used to provide a final recommendation about the optimal harvest date, irrigation dose, and plant density for each of the above mentioned aromatic herbs. The commercial production (total yield), composition of the volatile fraction, and sensory quality of dill, parsley, coriander, and mint were significantly affected by harvest date, irrigation dose, and plant density. Data obtained in the current study showed that the *optimal* harvest date were: for *dill* the second commercial harvest (approximately the 4th week of February), for *parsley* the first commercial harvest (approximately the 3rd week of November), for *coriander*

the second commercial harvest (approximately the 1st week of February), and for *mint* the first commercial harvest (approximately the 2nd week of December). The application of the irrigation dose 2180 m^3/ha and the plant density 7.41 plant/m^2 led to the highest aromatic and sensory quality of the dill plants; while, to produce dill plants with the highest yield, 1585 m^3/ha and 5.56 plant/m^2 must be applied. In a similar way, the use of 861 m^3/ha and 5.56 plant/m^2 led to the highest yield and quality of parsley plants. On the other hand, the effect of a preharvest treatment with malic acid, oxalic acid, and acetylsalicylic acid at 3 concentrations (1, 2, and 3 mM) on phenolic composition and antioxidant capacity of dill, parsley and coriander was also investigated. The results showed the presence of 30 phenolic compounds in dill, parsley, and coriander extracts. The major phenolic compounds found were (i) *dill*: neochlorogenic acid and quercetin glucuronide, (ii) *parsley*: apigenin-7-apiosylglucoside (apiin) and isorhamnetin-3-*O*-hexoside, and (iii) *coriander*: dimethoxycinnamoyl hexoside and quercetin-3-*O*-rutinoside. In fact, the preharvest treatment of both dill and parsley with organic acids did not significantly improve the quality of these herbs; however, experimental results proved that coriander shoots treated by a preharvest treatment with these organic acids had a significant enhancement of both the concentration of phenolic compounds and the antioxidant capacity.

Resumen

Actualmente, la optimización de las prácticas agronómicas, es de gran importancia para la obtención de cultivos de elevada producción y calidad. Esta optimización de diversas prácticas culturales, es el principal propósito de esta Tesis Doctoral. Para alcanzar este propósito, se ha realizado el análisis de los compuestos volátiles y la producción con el objetivo de investigar la influencia de la (i) fecha de recolección, (ii) la dosis de riego y (iii) la densidad de plantación sobre la producción calidad de **eneldo** (*Anethum graveolens*), **perejil** (*Petroselinum crispum*), **cilantro** (*Coriandrum sativum*) y **menta** (*Mentha piperita*). La hidrodestilación, con el aparato *Deryng,* se empleó para la extracción de los aceites esenciales o las fracciones volátiles, mientras que, para la separación e identificación de los compuestos volátiles, se empleó la cromatografía de gases con detector de espectrometría de masas; finalmente, la cuantificación se realizó mediante cromatografía de gases con detector de ionización de llama. Los resultados mostraron que los principales compuestos volátiles fueron (i) en eneldo *α*-felandreno, éter de eneldo y *β*-felandreno, (ii) en perejil, 1,3,8-*p*-mentatrieno y *β*-felandreno, (iii) en cilantro, *E*-2-dodecenal, dodecanal y octano, y (iv) en menta, carvona y limoneno. Los datos generados en esta Tesis Doctoral (por ejemplo, producción, concentración de compuestos volátiles y análisis sensorial descriptivo), se emplearon para alcanzar la decisión sobre la estimación de la fecha óptima de recolección, dosis de riego y la densidad de plantación. Todos los parámetros estudiados, se vieron afectados por la fecha de recolección, la dosis de riego y la densidad de plantación. Los datos obtenidos, revelaron que la fecha óptima de recolección de *eneldo* fue en el segundo corte (última semana de febrero), para *perejil*, en el primer corte (tercera semana de noviembre), para *cilantro* fue el segundo corte (primera semana de febrero) y para *menta*, el primer corte (segunda semana de diciembre). La aplicación de una dosis de riego de 2180 m^3/ha y densidad de plantación 7.41 plantas/m^2, condujo a la obtención de

eneldo de elevada calidad aromática y sensorial; mientras que para obtener una elevada producción se requirieron 1585 m^3/ha y 5.56 plantas/m^2. En lo que respecta al perejil, una dosis de riego de 861 m^3/ha y densidad de plantación 5.56 plantas/m^2 condujeron a una gran producción y elevada calidad. Además, se investigó el efecto de un tratamiento pre-cosecha con ácido málico, oxálico y acetil salicílico a 3 concentraciones (1, 2, y 3 mM) sobre la concentración de compuestos fenólicos y la capacidad antioxidante de eneldo, perejil y cilantro. Los resultados, indicaron la presencia de 30 compuestos fenólicos, siendo los más importantes en (i) *eneldo*: ácido neoclorgénico y quercetina glucorónido, (ii) en *perejil*: 7-apiosilglucósidos de apigenina y isoramnetina-3-*O*-hexosido, y (iii) en *cilantro*: quercetina-3-*O*-rutinósido. De hecho, el tratamiento pre-cosecha de eneldo y perejil, no mejoró de manera significativa la calidad de dichas hierbas aromáticas; mientras que, los resultados, mostraron que el cilantro tratado con estos ácidos orgánicos, tuvo una mejora significativa tanto en la concentración de compuestos fenólicos, como en la capacidad antioxidante.

Chapter 1.- Introduction

1. INTRODUCTION

1.1. Aromatic herbs

Aromatic herbs are important in different fields; they can be used in many areas such as flavoring, drinks, dyeing, repellents, fragrances, cosmetics, and medicine (Djeridane *et al.,* 2006; Wojdyło *et al*., 2007).

Apiaceae or ***Umbelliferae*** is one of the most popular family of aromatic plants, and includes well-known plants, such as anise, caraway, carrot, celery, coriander, dill, and parsley. These types of herbs have an importance usage in food and medicine. For instance, **dill** (*Anethum graveolens* L.) seeds and leaves are used as flavoring in sauces, vinegars, pastries, and soups, as well as in medicine as diuretic, stimulant, and carminative agents (Shahmohammadi *et al*., 2014). In addition, dill can be applied to treat many diseases, such as digestive disorders, bad breath, and as a hypolipidemic agent. Other studies reported that dill has anticancer, antimicrobial, antigastric irritation, anti-inflammatory and antioxidant activities (Jana & Shekhawat, 2010; Oshaghi *et al*., 2015). Zhang *et al*. (2006) reported that fresh, dried, and dehydrated leaves of **parsley** (*Petroselinum crispum*) can be used as condiment, garnish, and flavoring material. Leaves of **coriander** (*Coriandrum sativum* L.) plant are widely used in the cuisines of South and Central America, China, India and Southeast Asia. For instance, it is a common ingredient in sauces and in restaurant menus based on chicken and seafood (Potter, 1996). **Anise** (*Pimpinella anisum* L.) is an important medicinal plant. Its active substances are used in different pharmaceutical products and in the food industry (Nabizadeh *et al.*, 2012; Faravani *et al*., 2013).

Moreover, other herbs that belong to the ***Lamiaceae*** or ***Labiatae*** family, such as basil, mint, rosemary, marjoram, oregano, and thyme, also play a major role in the fields of food and medicine. **Mint** (*Mentha spp.*) have antimicrobial and antioxidant properties, and for this reason, it is used in traditional medicine (Tsai *et*

al., 2013). Rosemary (*Rosmarinus officinalis* L.), sweet basil (*Ocimun basilicum* L.) and thyme (*Thymus vulgaris* L.) have been applied for different purposes. Dried **rosemary** leaves are used in spices, salads, fried chicken, soaps, and perfumes. **Sweet basil** has extensively usage to add a distinctive aroma and flavor to food (e.g. salads, pizzas, meats, and soups) (Viuda-Martos *et al*., 2011; Calín-Sánchez *et al*., 2011). **Thyme** is also effective as antiseptic, carminative, antimicrobial and has antioxidative properties (Shati & Elsaid, 2009; Calín-Sánchez *et al*., 2013).

1.2. Essential oils

1.2.1. Definition and production

According to the European Pharmacopoeia, an **essential oil** (EO) is defined as an odorous product with a complex composition, obtained from a botanically defined plant raw material by steam distillation, dry distillation, or a suitable mechanical process without heating (European Pharmacopoeia, 2008; Bicchi *et al*., 2008). Therefore, an essential oil can be defined as ***the volatile fraction of a plant matrix***, which covers a number of other sampling approaches and/or techniques producing samples whose composition has the properties of a plant matrix (Bicchi *et al.*, 2008).

Essential oils are liquid, volatile, limpid, and rarely colored. Their density is less than 1.0 g mL^{-1}, and they are soluble in organic solvents and lipids. They can be extracted from different parts of plant, e.g. leaves, stems, flowers, seeds, buds, fruits, roots, and peel (Bakkali *et al*., 2008).

Brazil, India, United states of America (USA), and China are the main producers of essential oils (Schmidt, 2010; Zuzarte & Salgueiro, 2015), being the main trading markets USA, Germany, United Kingdom, Japan, and France (Bovill, 2010; Zuzarte & Salgueiro, 2015). Overall, 118 species are used for production of essential oil in the world. Fifteen of these species, namely citronella Ceylon (*Cymbopogon nardus*), Citronella java (*C. winterianus*), clove buds (*Syzygium aromaticum*), cornmint *(Mentha arvensis*), eucalyptus (*Eucalyptus globulus*), lemon-scented eucalyptus (*E. citriodora*), lavandin (*Lavandula x intermedia*), lemon (*Citrus limon*), lime distilled

(*C. aurantifolia*), sweet orange (*C. sinensis*), patchouli (*Pogostemon cablin*), peppermint (*M. x piperita*), sassafras Brazilian (*Ocotea odorifera*), sassafras Chinese (*Sassafras albidum*), and spearmint Scotch (*M. gracilis*), are the most relevant with over 1000 t year^{-1} of essential oils being produced (Franz & Novak, 2010; Zuzarte & Salgueiro, 2015).

1.2.2. Uses and chemical composition of essential oils

Essential oils are very important in nature to protect plants; they work as antibacterial, antiviral, antifungal, insecticide agents, and also against herbivores. They attract some insects to favor dispersion of pollens and seeds, or repel the undesirable ones (Bakkali *et al*., 2008). The essential oils of aroma yielding-plants can be used in different human activities, as active flavor and fragrant ingredients, mainly in perfumery but also nowadays in the food industry. These EOs are widely used in the formulation of hygiene and health care products. For instance, oils from thyme, mint, and basil are used in toothpastes and mouthwashes. Also, another commercial importance of essential oils is that some specific oil constituents are useful as chiral auxiliaries in synthetic organic chemistry (Sangwan *et al*., 2001).

The chemical composition of essential oils shows that terpenoids are the main constituents, but other chemicals such as phenylpropanoids are also present in high contents. In fact, other chemical families (e.g. monoterpenoids, sesquiterpenoids, etc.) are present in the EO composition (Sangwan *et al*., 2001).

In general, there are about 20 to 60 compounds present in the EOs at different concentrations. Mostly, two or three of these compounds represent a big percentage of the total volatile compounds content (20-70 %). For instance, the *Origanum compactum* EO contains carvacrol (30 %) and thymol (27 %) as the major compounds, while in the *Coriandrum sativum* EO, linalool (68 %) predominated. In a similar way, *α*-, *β*-thujone (57 %) and camphor (24 %) are abundant in *Artemisia herba-alba* oil, 1,8-cineole (50 %) in *Cinnamomum camphora* oil, *α*-phellandrene (36 %) and limonene (31 %) in the leaves and carvone (58 %) and limonene (37 %) in

the seeds of *Anethum graveolens* oil. Finally, menthol (59 %) and menthone (19 %) are the main compounds of the *Mentha piperita* oil (Bakkali *et al*., 2008).

The chemical composition of essential oils in many different types of herbs has received wide attention in the last decades. For example, the EO of *Thymus carmanicus* contains 21 components and the main essential oil components were carvacrol (60.1-70.4 %), thymol (6.5-13.1 %), *γ*-terpinene (1.4-8.6 %), borneol (4.2-8.5 %) and *p*-cymene (3.6-5.0 %) (Bahreininejad *et al*., 2014). Vokk *et al*. (2011) reported that *α*-phellandrene (47.7–62.5 %), myristicin (1.7–28.2 %), dill ether (0.9–14.8 %), *β*-phellandrene (7.4–7.5 %), and limonene (3.7–3.8 %) were the main compounds in the EO of dill leaves. In sweet basil (*Ocimum basilicum* L.) EO, 42 compounds were detected by Calín-Sánchez *et al*. (2012), with methyleugenol, eugenol, eucalyptol, and linalool being the major components.

Furthermore, Petropoulos *et al*. (2004) obtained essential oils from leaves, petioles and roots of 3 types of parsley (turnip-rooted, plain leaf and curly leaf). The main compounds detected were *β*-phellandrene, 1,3,8-*p*-menthatriene, *α*-, *p*-dimethylstyrene, myristicin, *β*–myrcene, and apiole, and in some cases *α*- and *β*-pinene were found; whereas, *β*-elemene was detected only in curly leaf type. The EO of coriander leaves consisted mainly of (*E*)-2-decenal (46.6 %) and other principal constituents were (*E*)-2-dodecanal, decanal, (*E*)-2-undecanal, (*E*)-2-tetradecanal, 1-decanol, and 2-decen-1-ol (Potter, 1996). In this way, it can be stated that the coriander EO is composed mainly from aliphatic aldehydes (Nurzyńska-Wierdak, 2013). Peppermint (*M. piperita* L.) and chocolate mint (*M. piperita* L.) oils were analyzed and the major components of the peppermint EO were menthol (30.4 %), menthone (21.1 %), and *trans*-carane (11.0 %), while the major components of chocolate mint oil were menthol (28.2 %), menthone (15.5 %) and 1,8-cineole (11.9 %) (Tsai *et al*., 2013). The monoterpenes limonene, isomenthone, menthol, menthofuran, D-neoisomenthol, 1,8-cineole (eucalyptol), D-carvone, linalool, linalyl acetate, piperitenone oxide, and pulegone were the predominant compounds in the EO from 11 plants of genus *Mentha*l (De souse barros *et al*., 2015).

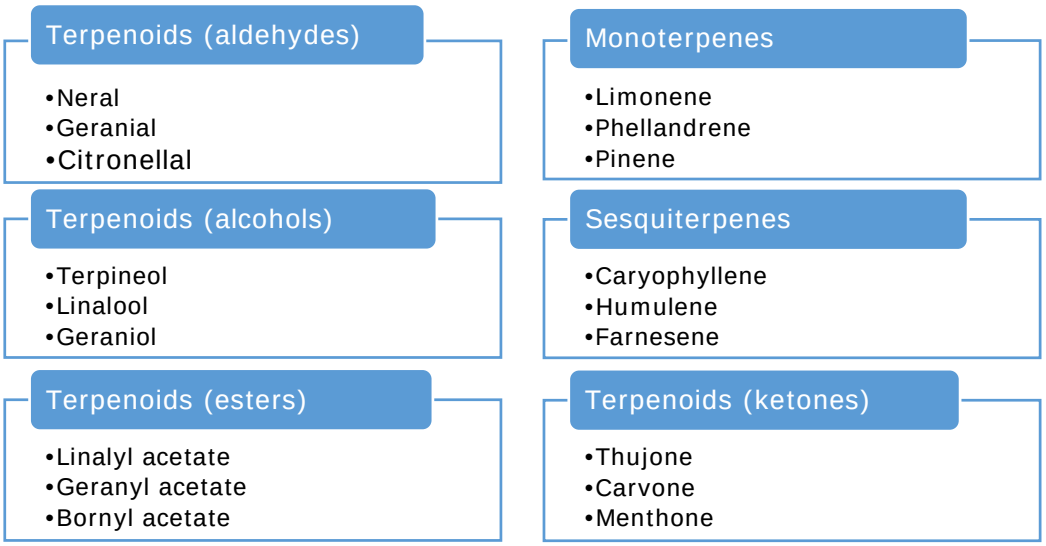


Figure 1. Main chemical groups in essential oils (Fahlbusch *et al.* (2000))

1.2.3. Extraction methods

There are several techniques that can be applied for the extraction of EOs from aromatic plants. Among these extraction techniques are supercritical carbon dioxide or microwave but, low or high pressure distillation is still considered as the main technique for extraction of EOs (Bakkali *et al*., 2008). Previous studies have used different methods for extracting essential oils. For instance, Vokk *et al*. (2011) isolated EOs of dill and parsley leaves using the **Clevenger** distillation apparatus. Petropoulos *et al*. (2004) obtained EOs from leaves, petioles and roots of three types of parsley using **simultaneous distillation–extraction** (**SDE**). Fan & Sokorai (2002) used **solid-phase microextraction** (**SPME**) technique to extract volatile compounds of coriander leaves. Hydrodistillation with **Deryng** apparatus (**Figure 2**) was used by Calín-Sánchez *et al.* (2015) to extract *Origanum majorana* L. EO. Moreover, **microwave** extraction process was applied by Costa *et al*. (2014) to obtain mint EO.

The isolation technique of volatile compounds from aromatic plants may affect the composition of the extracted volatile compounds (Melgarejo *et al*., 2013). For instance, 31 compounds were isolated from the volatile fraction of *Prunus domestica*, cv. Nancy, by using simultaneous **enzyme catalysis** extraction (Krammer *et al*.,

1991; Melgarejo *et al*., 2013), while only 6 compounds were extracted from cultivar D'Agen using SPME (Sabarez *et al*., 2000; Melgarejo *et al*., 2013). Five different isolation techniques: **dynamic headspace** (**DHS**), solid-phase microextraction (SPME), **headspace sorptive extraction** (**HSSE**), **solvent extraction** (SE) and simultaneous distillation–extraction (SDE) were applied to isolate the volatile compounds of buckwheat. The results indicated that SDE was the most suitable technique to obtain the aroma compounds of cooked buckwheat (Prosen *et al*., 2010).

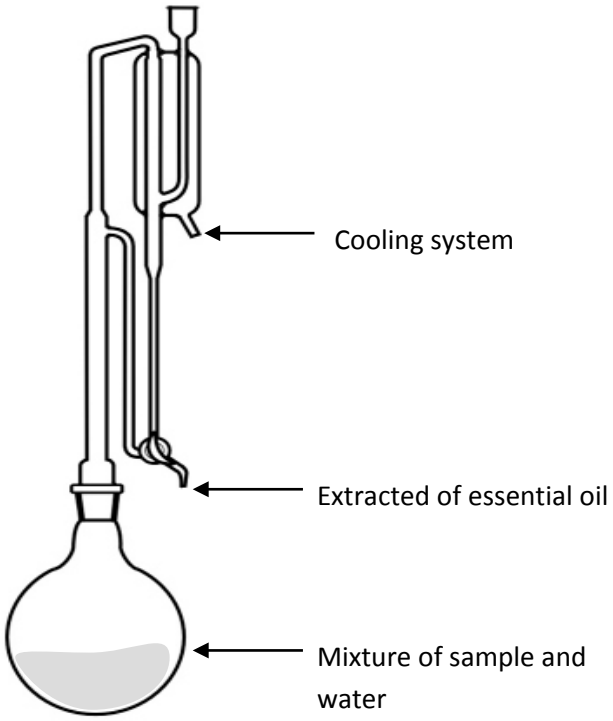


Figure 2. *Deryng apparatus for hydrodestillation*

1.2.4. Analysis methods

Many studies confirm that the gas chromatography (GC) technique is the most suitable technique that can be applied for the analysis of the EO chemical composition. Identification and quantification of components of an EO can be done using this technique, because of its simplicity, speed, and efficiency (Chamorro *et al*., 2012).

Employment of GC technique to determine unknown compounds in samples need two independent identification forms, retention time and mass spectral match

(Goodner, 2008). Retention times vary depending on GC temperature programming, and for this reason Kovat introduced a relative retention index scheme. A series of standards (n-alkanes) is used to calculate this relative retention index with other compounds referenced against standards using Equation 1 (Goodner, 2008):

$$I = 100\left[n + (N-n)\frac{\log t'_r(unknown) - \log t'_r(n)}{log\ t'_r(N) - \log t'_r(n)}\right] \quad \text{Eq. 1}$$

where,

n = number of carbon atoms in smaller alkane.

N = number of carbon atoms in larger alkane.

$t'_r(n)$ = adjust retention time of smaller alkane.

$t'_r(N)$ = adjusted retention time of larger alkane.

The internal standardization method is used for quantification of volatile compounds in GC, and includes the addition of a known concentration of an internal standard is added to the sample (Tissot *et al*., 2012). Semi-quantification is the most frequent way to estimate the contents of each compound in the volatile fraction or EO by adding an internal standard to the mixture and calculating the compounds contents by assuming that all response factors relative to this internal standard are equal to 1 (Bakkali *et al*., 2008). Many researchers have carried out the application of GC as the technique for the EO analysis. For instance, the composition of Polish fresh rosemary plant (*Rosmarinus officinalis* L.) EO was investigated by Calín-Sánchez *et al*. (2011) and 31 compounds were identified by using 3 different analytical methods: (i) Kovats indexes (KI), (ii) GC–MS retention times (authentic chemicals) and (iii) mass spectra (authentic chemicals and NIST05 spectral library collection; NIST, 2010) and for quantification analysis they used gas chromatography with flame ionization detector (GC-FID). Components of sweet basil (*Ocimum basilicum* L.) oil were identified by gas chromatography coupled with mass spectrometry (GC-MS) and the analysis was based on comparison of retention time

of components with those of authentic standards and by comparison of their mass spectral fragmentation patterns (WILEY and NIST databases, ChemStation data system) (Telci *et al*., 2006). GC-MS was applied for the analysis of the EOs of dried leaves, flowers and fruits of dill (*Anethum graveolens* L.) (Radulescu *et al*., 2010). GC–FID and GC–MS were used by Msaada *et al*. (2007) to analyze the composition of coriander (*Coriandrum sativum* L.) fruits EOs at three stages of maturity. Derwich *et al*. (2010) analyzed the EOs of the leaves of *Mentha pulegium* using GC-FID and GC-MS to determine the chemical composition of volatile fraction and identify their chemotypes.

1.2.5. Sensory analysis

The intensities of the main sensory properties and attributes of food can be quantitatively analyzed by using descriptive sensory analysis (DSA). This analysis requires the use of a trained panel and between 7 to 12 highly trained panelists (Meilgaard *et al*., 1999; AENOR, 1997). Previous studies have reported the application of DSA in the description of the main attributes of fruits, vegetables and especially aromatic herbs. For instance, sensory evaluation with a trained panel was used to distinguish the intensities of main aromatic characteristics of thyme, rosemary, oregano, and sweet basil (Calín-Sánchez *et al*., 2011, 2012, 2013 & 2015) Factors such as irrigation dose, plant density, and harvest date can affect the sensory properties of aromatic herbs. For instance, to have a dill EO with herbaceous characteristics, the plants should be harvested at early fruit formation or high plant density must be used (Callan *et al*., 2007). Increasing the irrigation level can improve the quality of the coriander essential oil (Hassan & Ali, 2014). The quality of spearmint (*Mentha spicata* L. var. *spicata*) could also be modified by harvesting at full flowering stage (Kizil & Tonçer, 2006).

1.3. Factors affecting yield and content of essential oil in herbs

Yield and composition of essential oil depend on genetic factor, climatic conditions during stages of fruit formation, and ripening (Sedlakova *et al*., 2003; Aćimović, 2015). Other factors, related to agronomic factors, can affect the yield and composition of essential oil, such as sowing date, irrigation doses, fertilization, and harvest time (Mohamed & Abdu, 2004; Rożek *et al*., 2013; Nurzyńska-Wierdak, 2013; Aćimović, 2015). The main parameters will be briefly explained here.

1.3.1. Irrigation dose

Water is an important resource for the growth and production of plants. An efficient use of water is important to ensure acceptable production, especially in dry or semi-dry areas, such as southeastern Spain, where the water is not enough to fulfil crops requirements (Boogaard, 1995; Zehtab-Salmasi *et al*., 2006). To produce high quality of dill seeds, Zehtab-Salmasi *et al*. (2006) reported that it is important to provide enough water to the dill plants during the stage "filling of seeds", especially for late sowings. For maize plants, an increase in irrigation water from 0 to 480 mm increased dry matter (dm) yield from 9.3 to 23.8 t ha^{-1}, and also increased grain from 92 to 315 g kg^{-1} dm, but decreased stover (leaves and stalks) of plants from 907 to 685 g kg^{-1} dm (Islam *et al.*, 2012). Impact of water deficit stress on growth, yield and composition of essential oils of 3 parsley cultivars (plain-leafed, curly-leafed and turnip-rooted) in Greece was investigated by Petropoulos *et al*. (2008), and results showed that plant growth (foliage and root weight, leaf number) was significantly reduced by water stress. The control treatment (no significant water stress, 0-10 %) for example gave 62.3 g of foliage of plain-leafed, where water stressed (45-60 %) produced only 29.6 g. However, the oil yield of both plain-leafed and curly-leafed cultivars increased from 0.04 mL 100 g^{-1} to 0.07 mL 100 g^{-1} and 0.05 mL 100 g^{-1} to 0.11 mL 100 g^{-1}, respectively, under stress conditions. In fact, these results did not agree with those of Zehtab-Salmasi *et al*. (2001), who reported that water stress reduced oil yields of rosemary and with Singh & Ramesh (2000)

who reported that water deficit stress reduced oil yield of rosemary, but oil yield on a plant fresh weight was not affected (Petropoulos *et al*., 2008). The effect of five irrigation levels (40 %, 60 %, 80 %, 100 % and 120 % of evapotranspiration potential) on growth, yield and chemical composition of coriander plants was investigated by Hassan & Ali (2014). Results showed that vegetative growth parameters were improved with applying higher irrigation levels, and volatile oil percentage was increased with increasing irrigation rate from 40 % to 120 %. Moreover, Laribi *et al*., (2009) studied the effect of three different water levels: [100 % (control), 50 % (moderate water deficit) and 25 % (severe water deficit) of crop evapotranspiration] on growth and essential oil composition of caraway (*Carum carvi* L.) and reported that plant growth (height, fresh and dry matter weight) was reduced by severe water deficit, while the essential oil yield (expressed as g 100 g^{-1}) was increased by applied moderate water deficit.

1.3.2. Plant density

It is necessary to optimize the plant density for maximum production of crop, especially when water is a limiting factor (Khazaie *et al*., 2008). Many researchers were interested in studying this factor. For instance, Khazaie *et al*. (2008) used 6.6, 8, and 10 (plants m^{-2}) to study the effect of plant density on herbage biomass and oil production of thyme (*Thymus vulgaris*), and the results showed that the highest herbage biomass and oil production were obtained at the lowest plan density (6.6 plants m^{-2}). The highest production of herbage biomass in years 2003 and 2004 were 1110 g m^{-2} and 1217 g m^{-2}, respectively, where the highest yield of essential oil was 20.9 and 20.6 g m^{-2} on 2003 and 2004, respectively. Also, for sweet basil (*O. basilicum*) the lowest planting distance between plants (15 cm) compared to (45 cm) gave higher biomass and higher yield of essential oil (El-Gendy *et al*., 2001; Khazaie *et al*., 2008). Another study about dill plant was carried out by Callan *et al.* (2007), and these authors reported that there was no effect of plant density on total biomass production and oil yield, but plant density influenced oil composition, where

application of low plant density led to high contents of carvone, but low contents of phellandrene, *α*-pinene and dill ether (3,9-epoxy-1-*p*-menthene) were lower. Three plant spacing (30×30, 40×40, 50×50 cm) were applied to study the effect of plant spacing on hyssop (*Hyssopus officinalis* L.) herb yield and quality, and results recorded that no significant effect was found on the contents of dry matter, such as essential oil content, while the treatment 40 × 40 cm plant spacing gave the highest fresh herb yield (on average 1.47 kg m^{-2}) (Zawislak, 2011). The effect of plant density on yield and quality of anise (*Pimpinella Anisum* L) was studied by Faravani *et al*. (2013). These authors applied three levels 17, 25, and 50 plants m^{-2}, and the results showed that the grain yield, biological yield, number of lateral branches, essential oil percentage, and yield of essential oil were affected by plant density. Seed and essential oil yield were the highest with plant densities of 50 and 25 plants m^{-2}, respectively. Moreover, the effect of plant density on yield and essential oil components of fennel (*Foeniculum vulgare* Mill, cultivar Soroksary) was investigated (Khorshidi *et al*., 2009); five plants spaces 10, 15, 20, 25, and 30 cm were applied. The highest essential oil percentage (3.53 %) was obtained with the lowest plant density. The higher percentage of anethole (83.07 %), estragol (3.47 %), fenchone (8.04 %), *p*-cymene (4.45 %), *α*-terpinene (0.54 %), sabinene (0.51 %) and *α*-pinene (0.48 %) were obtained with space between plants 25, 10, 20, 20, 15, 20, and 25 cm, respectively (Khorshidi *et al*., 2009).

1.3.3. Harvest date and plant development

The influence of different harvest stages on the yield and oil composition of spearmint (*Mentha spicata* L., cultivar *spicata*) was investigated. The highest yield of fresh herbage (20.66 t ha^{-1}), dry herbage (6.13 t ha^{-1}), dry leaf (4.16 t ha^{-1}), and plant height (57.4 cm) was obtained at the full flowering stage. Also, the highest essential oil content (2.63 %) and yield of essential oil (70.7 L ha^{-1}) were obtained at full flowering stage (Kizil & Tonçer, 2006). In a similar way, the flowering parts of *Salvia officinalis* had higher oil contents (1.6 versus 1.1 %) and *β*-pinene levels (27

versus 10 %) than leaves but lower thujone levels (16 versus 31 %) (Perry *et al.*, 1999).

Other previous studies stated that harvest of crops of herbs at different stages can affect yield and quality of the herb essential oil. For instance, anise plant essential oil was analyzed at 10 different plant maturation stages, and the results indicated that the highest yield of essential oil was obtained at the fourth harvest stage, where the first and second harvest stages gave the best quality of essential oil with a high (*E*)-anethole content (Özel, 2009; Olle & Bender, 2010). Volatiles of sweet fennel seeds were analyzed at three harvest stages (full bloom, waxy seed and seed ripening), and the highest yield of the volatile compounds was obtained at the waxy seed maturity stage (Marotti *et al.*, 1993; Olle & Bender, 2010). Also, thyme plant was harvested at 3 different stages (beginning of blooming, full blooming and fruit set), and the maximum yield of dry and fresh herbage, yield and content of oil and thymol yield were obtained at the beginning of blooming stage (Badi *et al*., 2004). In addition, Msaada *et al*. (2007) compared the variation of coriander fruit essential oil composition during maturation stages, and found great differences in oil composition. These authors reported that geranyl acetate (46.3 %), linalool (11.0 %), nerol (1.5 %) and neral (1.4 %) were the main compounds at first stage of maturity (immature fruits); while, at middle stage, linalool (76.3 %), *cis*-dihydrocarvone (3.2 %) and geranyl acetate (2.9 %) were the major constituents. At the final stage of maturity (mature fruits) consisted mainly of linalool (87.54 %) and *cis*-dihydrocarvone (2.36 %).

1.3.4. Date of sowing

Three different sowing dates (10 May, 20 May, and 30 May) were used for fennel plant (*Foeniculum vulgare* Mill.) to determine the effect of sowing date on the yield, yield components, and essential oil content. The highest number of plant height, number of umbel *per* plant, number of seed in umbel, 1000 seed weight, seed yield, and biomass were obtained at the second sowing date (20 May), and the

essential oil percentage also was the highest at the second sowing date, 2.73 % (Ghanbari-odivi *et al*., 2013). To get best results for seed production, dill must be sown in early Spring (3 to 18 April) (Zehtab-Salmasi *et al.*, 2006) and also to obtain the highest essential oil production, anise must be sown early in Spring (Zehtab-salmasi *et al*., 2001; Olle & Bender, 2010). Sowing dates (20 April, 5 May, 10 May, and 15 May) were applied to study the effects of sowing date on quantity and quality features in thyme (*Thymus vulgaris* L.). Sowing of thyme on 20 April gave the highest total biomass (914 kg ha^{-1}) and the highest amount of the main constituents of essential oil with 53.1 % of thymol, 4.8 % linalool, 2.7 % terpineol, and 1.3 % carvacrol (Moaveni *et al*., 2011).

1.3.5. Fertilization

Several studies pointed to the influence of several types of fertilizers on the quantity and quality of herbs. For instance, inorganic and organic fertilizers were utilized to evaluate their effect on yield and oil composition of rosemary (*Rosmarinus officinalis* L.). Herbage yield increased by 66.1 % and oil yield by 54.9 % with the application of organic fertilizer (vermicompost at a rate of 10 t ha^{-1}) + inorganic fertilizer (N:P:K at a rate of 100:25:25 kg ha^{-1}) compared control (no fertilizer) and other treatments (Singh & Guleria, 2013). Analysis of anise (*Pimpinella anisum* L.) plant also showed clearly influence by fertilizer. Four treatments of fertilizer: (i) the control, (ii) vermicompost 5 t ha^{-1}, (iii) cow manure 25 t ha^{-1}, and (iv) mineral fertilizer (NPK) 60 kg ha^{-1} (the same rate of each nutrient) were applied. The application of vermicompost and control gave the highest essential oil yields (14.93 kg ha^{-1}), while the highest biomass (1913 kg ha^{-1}) was obtained after the application of vermicompost (Faravani *et al*., 2013). Chemical N fertilizer (urea, 46 % N), two organic fertilizers (compost and vermicompost), two biological fertilizers (*Pseudomonas putida* and *Azotobacter chroococcum*), combinations of organic and biological fertilizers (*Ps. putida* + compost; *Ps. putida* + vermicompost; *A. chroococcum* + compost; *A. chroococcum* + vermicompost) and control (no

fertilized) were applied to evaluate their effects on quantity and quality of dill (*Anethum graveolens* L.) essential oil. The highest essential oil yield (53.2 L ha^{-1}) was obtained in the vermicompost + *Pseudomonas* treatment. While, the highest seed yield was obtained after the application of chemical fertilizer and vermicompost plus *Pseudomonas* treatments (Tajpoor *et al.*, 2013). Moreover, application of phosphorus fertilization led to change in content and yield of essential oil of cumin (*Cuminum cyminum*) plant; the highest oil content and essential oil yield were obtained after application of 40 kg ha^{-1} P_2O_5 treatment (Tunctürk & Tunctürk, 2006; Olle & Bender, 2010). Addition of fertilization with different concentrations of nickel (0, 25, 50, or 100 mg kg^{-1}) have affected both yield and quality of parsley leaves; the application of the low level of fertilization with nickel (50 mg kg^{-1}) strongly improved parsley oil yield (Atta-Aly, 1999; Olle & Bender, 2010).

1.4. Polyphenols in herbs

1.4.1. Classification and Composition

According to the number of phenol rings and structural elements that bind those rings, phenolic compounds can be divided into five groups: (i) *flavonoids* (e.g. anthocyanins, flavones, isoflavones), (ii) *phenolic acids*, (iii) *tannins*, (iv) *stilbenes*, and (v) *lignans* (Balasundram *et al.*, 2006; Barros *et al.*, 2012). **Figure 3** shows the classification of polyphenols compounds.

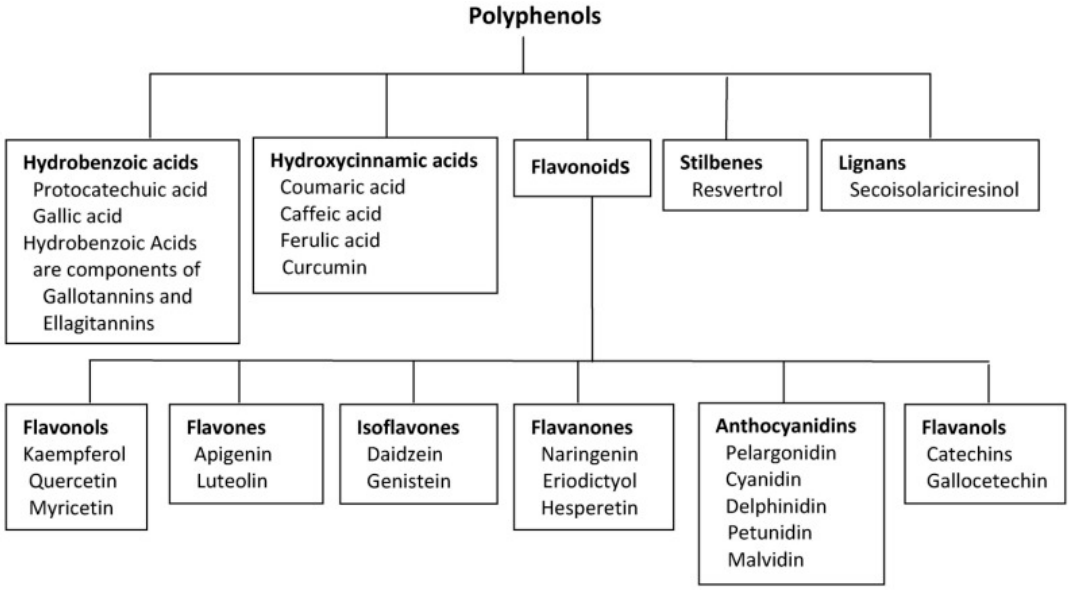


Figure 3. Classification of polyphenols (Hardman, 2014)

Figure 4 shows chemical structures of most important flavonoids and phenolic acids available in herbs. The flavan nucleus form the main structure of flavonoids, this nucleus consists of 15 carbon atoms arranged in 3 rings (C6–C3–C6), labelled A, B and C. Various classes of flavonoid differ in level of oxidation and saturation of ring C, while individual compounds within a class differ in substitution pattern of rings A and B (Wojdyło *et al*., 2007).

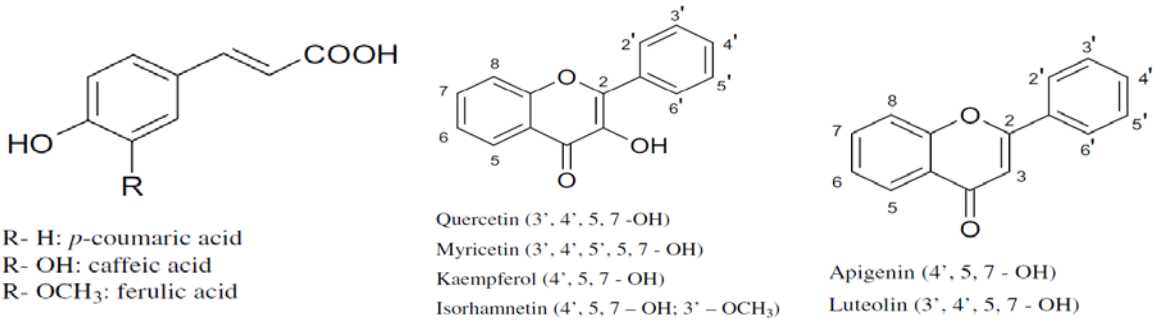


Figure 4. Structures of major phenolic compounds (Wojdyło *et al*., 2007).

Phenolic compounds are soluble in water and they can form glycosides when they combining with sugars, such as glucose, galactose, rhamnose, arabinose, xylose, and rutinose (Justesen *et al*., 1998; Muchuweti *et al*., 2007; Costa *et al*.,

2015). They are present in fruits, vegetables, and other plant products consumed in a normal diet (Justesen & Knuthsen, 2001). Previous studies reported that flavonoids and other phenolic antioxidants, such as rosmarinic acid, are present in many herbs, including basil, dill, oregano, parsley, rosemary, sage, spearmint, and thyme (Baritaux *et al*., 1991; Hoffmann & Hermann, 1982; Justesen, 2000). There are increasing interest on study of phenolics in crude extracts of herbs and their importance in food industry, because they delay oxidative degradation of lipids and thereby improve quality and nutritional value of food (Wojdyło *et al*., 2007). The importance of this topic has grown in the last decade and has got wide attention by the scientific community worldwide. For instance, Nambiar *et al*. (2010) reported that flavonoids, such as quercetin, kaempferol, and acacetin, are present in coriander leaves and also some phenolic acids, such as vanilic, ferulic and *p*-coumaric, were detected in coriander plants. Another study by Yoshikawa *et al*. (2000) recorded the presence of four flavone glycosides apigetrin, apiin, diosmetin 7-*O*-*β*-D-glucopyranoside, and kaempferol-3-*O*-*β*-glucopyranoside in extracts of parsley (*Petroselinum crispum*) shoots. Also, the presence of isorhamnetin, kaempferol, and quercetin were detected by Justesen (2000) in dill (*Anethum graveolens*) extracts as glucuronyl conjugates and quercetin-rhamnoglucoside was also detected as a minor component.

Four phenolic acids (3-*O*-caffeoylquinic acid, caffeic acid, *p*-coumaric and *O*-coumaric acid), 4 flavonoid glycosides (luteolin-7-*O*-glucoside, apigenin-7-*O*-glucoside, quercetin-3-*O*-galactoside and quercetin-3-*O*-rhamnoside), and 3 flavonoid aglycones (luteolin, apigenin and chrysoeriol) were identified in extract of burr parsley (*Caucalis platycarpos* L.) (Plazonić *et al.*, 2009). Hoffmann & Hermann (1982) and Baritaux *et al*. (1991) analyzed some herbs (basil, dill, oregano, parsley, rosemary, sage, spearmint, and thyme) and their results indicated the presence of flavonoids and other phenolic antioxidants, such as rosmarinic acid (Justesen, 2000). Moreover, 15 fresh herbs (basil, chives, coriander, cress, dill, lemon balm, lovage, oregano, parsley, rosemary, sage, spearmint, tarragon, thyme, and watercress) were

analyzed by Justesen & Knuthsen (2001); the results showed that 5 major flavonoid aglycones (apigenin, isorhamnetin, kaempferol, luteolin, and quercetin) were detected.

1.4.2. Analysis of polyphenols

High-performance liquid chromatography (HPLC) techniques are widely used for both separation and quantification of phenolic compounds. Various set-ups, mobile phases, columns, and detectors are available for the analysis of anthocyanins, procyanidins, flavonols, flavan-3-ols, and phenolic acids (Lorrain *et al*., 2013). HPLC equipped with a photodiode array detector (PAD) and mass detector was employed to analysis phenolic compounds in *Brassica rapa*; phenolic compounds related to flavonols and hydroxycinnamic acids were detected (Francisco *et al*., 2009). Recently, an improvement in chromatographic performance has been carried out by the introduction of ultra-performance liquid chromatography (UPLC). Both techniques (HPLC and UPLC) were used to detect pesticides in baby foods and the results showed that UPLC gave narrower peaks and higher speed of analysis (Leandro *et al*., 2006). A total of 21 phenolic compounds in *Lonicera caerulea* L. (cultivar *kamtschatica*) berries were identified by LC-PDA-MS Method, where the quantification was carried out by using UPLC Coupled to PDA and fluorescence (FL) Detector (Wojdyło *et al*., 2013).

1.4.3. Influence of preharvest treatment on polyphenols content

A large number of chemical compounds, such as jasmonic acid, methyl jasmonate, salicylic acid, acetyl salicylic acid, malic acid, oxalic acid, and heavy metals can be applied as preharvest treatments to modify the content of polyphenols in plants (Giri & Zaheer, 2016). Increase from 90.3 to 337 µg g^{-1} of myricetin, from 103 to 219 µg g^{-1} of ellagic acid, and from 65 to 163 µg g^{-1} of quercetin were obtained in red raspberry after preharvest treatment with 0.1 mM methyl jasmonate (Flores & del Castillo, 2014). Also, application of preharvest treatments of oxalic acid after full

blossom of sweet cherry (*Prunus avium* L.) increased the total anthocyanins, total phenolics, and antioxidant activity (Martínez-Esplá *et al*., 2014). Moreover, Giménez *et al*. (2014) reported that total phenolics and total anthocyanins, as well as higher antioxidant capacity, were obtained by applying salicylic and acetylsalicylic acids to two sweet cherry cultivars (Sweet Heart and Sweet Late).

1.5. Antioxidant capacity

An antioxidant can be defined as any substance, present at low concentration, preventing oxidation of oxidizing agents (Rice-Evans *et al*., 1991; Halliwell, 1996; Rubio *et al*., 2016). Antioxidants have a great effect on protecting the body from the harmful effects of free radical damage (Marques *et al*., 2014). Antioxidants provide bioactive mechanisms to reduce free radical. Shortage in natural cell antioxidant capacity or increase amount of reactive oxygen species in organisms will result in oxidative stress. The over production of free radical, caused by unbalancing between oxidants and antioxidants in the body, will damage DNA (Ames *et al*., 1993; Nambiar *et al*., 2010).

According to the properties of polyphenols as antioxidant materials, researchers and food manufactures have paid much attention to the study of polyphenols occurrence in natural sources, such as herbs. Polyphenols play an important role in prevention of various diseases associated with oxidative stress, such as cancer and cardiovascular and neurodegenerative diseases (Nambiar *et al*., 2010). Herbs contain phenolic compounds, so they can be used as an alternative option to synthetic antioxidants in food industry (Madsen & Bertelsen, 1995; Proestos *et al*., 2006).

1.5.1. Measurement of antioxidant capacity

There are several methods that can be used to estimate the total antioxidant capacity (TAC). These methods can be divided into two major groups: (i) assays based on a single electron transfer reaction, which are based on the change in the color according to the redox reaction (reducing of oxidant), and (ii) assays based on

a hydrogen atom transfer reaction, where the antioxidant and the substrate compete for free radicals.

The most commonly assays based on electron transfer reaction are ferric reducing ability of plasma (**FRAP**) assay, copper reduction (**CUPRAC**) assay, the 2,2-diphenyl-1 picrylhydrazyl (**DPPH**) radical scavenging capacity assay, Trolox equivalent antioxidant capacity (**TEAC**) assay and (**ABTS)** assay 2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid).

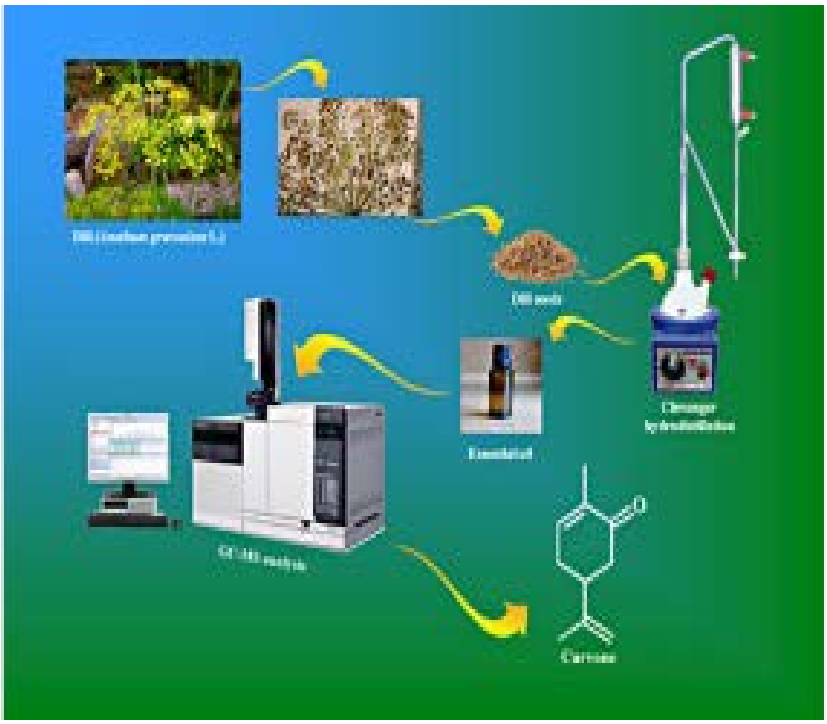
On the other hand, the most popular assays depending on hydrogen atom transfer reaction are the total peroxyl radical-trapping antioxidant parameter (**TRAP**) assay, and the oxygen radical absorbance capacity (**ORAC**) assay (Huang *et al*., 2005; Tabart *et al*., 2009).

In fact, several assays can be applied for the determination of antioxidant capacity of polyphenols compounds. ABTS and DPPH are the most commonly assays used for determination of antioxidant capacity. Comparison between these methods indicates that DPPH does not need any special preparation, where the application of the ABTS method requires generation by enzymes or chemical reaction (Arnao, 2000; Wojdyło *et al*., 2007). Oszmianski *et al*. (2014) studied the antioxidant capacity of *Solanum scabrum* and *Solanum burbankii* berries using ABTS and FRAP assays and they reported that *S. scabrum* was richer in phenolic compounds, especially anthocyanins and was characterized by more powerful antioxidant capacity than *S. burbankii*. Wojdyło *et al*. (2016) applied ABTS and FRAP to estimate the antioxidant capacity of Spanish jujube (*Ziziphus jujube* Mill.) fruits and they reported that some jujube cultivars are excellent sources of natural antioxidants. Furthermore, total equivalent antioxidant capacities of 32 spices extracts from 21 botanical families grown in Poland were investigated and total antioxidant capacity was estimated by ABTS, DPPH and FRAP (Wojdyło *et al*., 2007). The results showed that Polish species were rich in phenolic compounds and demonstrated good antioxidant capacity. The antioxidant capacity of five herbs and spices, namely caraway, turmeric, dill, marjoram and nutmeg, was studied using ABTS and DPPH. Results have pointed out

that the highest antioxidant capacity was present in caraway (Vallverdu-Queralt *et al*., 2015).

The correlation between TAC and the total phenolic compounds was investigated by several authors for different types of food. For instance, a linear positive and significant relationship (R^2=0.71) between the antioxidant capacity and total phenolic contents of Iranian basil (*Ocimum basilicum* L.) had been reported. This result indicated that 71 % of the antioxidant capacity of Iranian basil results from the presence of phenolic compounds (Javanmardi *et al*., 2003). The ABTS, DPPH, FRAP, and ORAC assays were used for analysis of guava fruit extracts. The FRAP technique showed the highest correlation with both ascorbic acid and total phenolic contents (Thaipong *et al*., 2006). In addition, phenolic contents and total equivalent antioxidant capacities (TEAC) of 32 Polish spices extracts were analyzed by Wojdyło *et al*. (2007); 3 assays (ABTS, DPPH, and FRAP) were used to determine the TEAC. The results showed that there was positive relationship between TEAC (ABTS and FRAP) values and total phenolic contents in herbs of *Labiatae* and *Compositae* families.

Chapter 2.- Objectives



2. OBJECTIVES

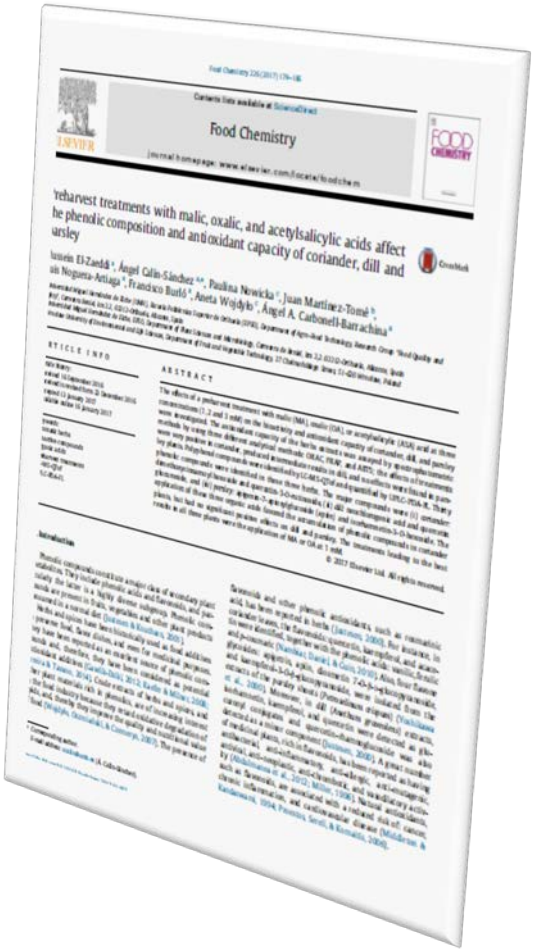
2.1. General aim

The global aim of this Ph.D. dissertation was to analyze the effect of different agricultural practices on production and quality of different aromatic herbs popular in the Mediterranean region of Spain. To reach this main aim, several specific objectives have been considered and will described in section 2.2.

2.2. Specific objectives

- To analyze the volatile composition of essential oils from dill (*Anethum graveolens* L.), parsley (*Petroselinum crispum*), coriander (*Coriandrum sativum*), and mint (*Mentha piperita* L.) at different harvest dates to determine the most suitable harvest time for each of these four herbs.
- To investigate the influence of 3 irrigation treatments and 3 plant density treatments on the production (total yield), volatile composition of essential oil, and sensory quality of parsley.
- To investigate the influence of 3 irrigation treatments and 3 plant density treatments on the production (total yield), volatile composition of essential oil, and sensory quality of dill.
- To study the effects of a preharvest treatment with organic acids (malic, oxalic, or acetylsalicylic) at 3 concentrations (1, 2, and 3 mM) on the bioactivity and antioxidant capacity of coriander, dill, and parsley.

Chapter 3.- Publications



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Preharvest treatments with malic, oxalic, and acetylsalicylic acids affect the phenolic composition and antioxidant capacity of coriander, dill and parsley

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ABSTRACT

Introduction

PUBLICATION 1

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Hussein El-Zaeddi, Juan Martínez-Tomé, Ángel Calín-Sánchez, Francisco Burló and Ángel A. Carbonell-Barrachina

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Article

Volatile Composition of Essential Oils from Different Aromatic Herbs Grown in Mediterranean Regions of Spain

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Abstract: Volatile composition of essential oils from dill, parsley, coriander, and mint were investigated at different harvest dates to determine the most suitable harvest time for each these herbs. Hydrodistillation (HD), using a Deryng system, was used for isolating the essential oils. Isolation and identification of the volatile compounds were performed using gas chromatography-mass spectrometry (GC-MS) instrument. The results of gas chromatography-flame ionization detector (GC-FID) analysis (quantification) showed that the main components in the essential oil of dill shoots were α-phellandrene, dill ether, and β-phellandrene, and the optimal harvest date was D2 (second harvest, fourth week of February 2015). For parsley shoots, the main compounds were 1,3,8-*p*-menthatriene, β-phellandrene, and P1 (first harvest, third week of November 2014) was the sample with the highest essential oil. For coriander, the main compounds were *E*-2-dodecenal, dodecanal, and octane and the highest contents were found at C2 (second harvest, 5 February 2015); while, the main two components of mint essential oil were carvone and limonene, and the highest contents were found at M1 (first harvest, second week of December 2014). The present study was the first one reporting data on descriptive sensory analysis of aromatic herbs at this optimal harvest date according to the content of volatile compounds of their essential oils.

Keywords: dill; parsley; coriander; mint; GC-MS; descriptive sensory analysis

1. Introduction

The production of aromatic herbs, such as oregano, marjoram, rosemary, thyme, lavender, or peppermint, is a rising sector in the Mediterranean countries. These plants have many applications, such as ornamental cropping, perfumery, or food and pharmaceutical industries; these later applications are linked to the beneficial health effects of their essential oils, which have antimicrobial, antifungal, insecticidal, and antioxidant properties [1,2].

Dill (*Anethum graveolens* L.) is an important aromatic herb, which is used as flavoring and seasoning of various foods, such as salads, sauces, soups, sea foods, and especially pickled vegetables [3]. Parsley (*Petroselinum crispum*) and coriander (*Coriandrum sativum*) are two culinary herbs commonly used to enhance the flavor of many dishes of the cuisines of China, Mexico, South America, India, and South East Asia [4]. Peppermint (*Mentha piperita* L.) is a famous aromatic and medicinal herb used in traditional and folk medicines in the world for its antimicrobial and antioxidant properties [5].

In addition, culinary herbal extracts and essential oils have become increasingly popular as alternative sources of natural preservative agents, largely because herbs are widely cultivated, effective, and safe for consumption [5]. Essential oils are extracted from various aromatic plants generally localized in temperate to warm countries, such as the Mediterranean countries [1]. Peppermint (*M. piperita* L.) essential oils have been obtained by steam distillation in a Clevenger-type apparatus [5], while microwave extraction technique of mint essential oil was used by Costa *et al.* [6]. Besides, Huopalahti and Linko [3] isolated aroma compounds of dill (*Anethum graveolens* L.) by using solvent extraction technique.

It is well-known that the presence of essential oils and their composition determine the specific aroma of plants and the flavor of the resulting condiments [7,8]. The main chemical families present in aromatic herbs are: *monoterpenes*, *monoterpenoids*, and *phenylpropanoids*. In lower amount alcohols, *sesquiterpenes*, *sesquiterpenoids*, *aldehydes*, and *esters* were also found [9–12]. The composition and concentrations of essential oils from aromatic herbs depend on many factors, including geographical source, climatic and soil conditions, stage of vegetative cycle, seasonal variation, *etc.* [13–15].

Therefore, the aim of this study was to analyze the volatile composition of the essential oils and the sensory quality of different aromatic herbs (parsley, dill, mint, and coriander) grown in different Mediterranean regions of Spain in order to stablish the best harvest time according to the highest content of essential oils and the optimum sensory quality.

2. Materials and Methods

2.1. Plant Material

Four aromatic herbs (dill, parsley, coriander, and mint) were grown under conventional agricultural practices and conditions according to the recommendations of farmers located at Mediterranean regions of Spain according to their experience. These conditions are described as follows:

Dill seeds (*Anethum graveolens* L. Cv. ELLA) were sown on the 7 September 2014 in expanded polystyrene (EPS) trays and placed in a greenhouse located at Santomera (Murcia, Spain) until 4 October 2014. Then, plantlets were transplanted into a commercial orchard placed at Sucina (Murcia, Spain). Dill samples at the commercial stage were harvested at two different dates within the same plant on the 26 November 2014 (D1: 80 days after sowing) and 28 February 2015 (D2: 174 days after sowing).

Parsley seeds (*Petroselinum crispum* Cv. Gigante Italiano Darkness) were sown on the 2 September 2014 in expanded polystyrene (EPS) trays and placed in a greenhouse located at Santomera (Murcia, Spain) until 24 September 2014. Then, plantlets were transplanted into a commercial orchard placed at Sucina (Murcia, Spain). Parsley samples at commercial stage were harvested at three different dates within the same plant on the 19 November 2014 (P1: 78 days after sowing), 5 January 2015 (P2: 144 days after sowing), and 25 February 2015 (P3: 175 days after sowing).

Coriander seeds (*Coriander sativum* Cv. MARINO) were directly sown on the commercial orchard placed at Sucina (Murcia, Spain). Coriander has only one harvest for each plant and in these regions three crops per year are sown, grown, and harvested. The first crop (C1) was planted on 30 September and harvested on 19 November 2014; the second crop (C2) was planted on 22 October 2014 and harvested on 5 February 2015; and, the third crop (C3) was planted on 29 December and harvested on 25 February 2015.

Mint cuttings (*Mentha piperita* L.) of 5–7 cm were sown on 7 August 2014 in polyethylene trays and placed in a greenhouse located at Santomera (Murcia, Spain) until 6 October 2014. Then, plantlets were transplanted to a commercial orchard placed at Sucina (Murcia, Spain). Mint samples at commercial stages were harvested at two different dates from the same plant on the 11 December 2014 (M1: 133 days after sowing) and 5 February 2015 (M2: 189 days after sowing).

Aromatic herbs were grown using high-frequency drip irrigation systems. The water contribution was carried out using polyethylene pipes of 16 mm of diameter. The emitters were adjusted at 32 cm

of distance with a total flow of 1.6 $L \cdot h^{-1}$. The total volume of water for each crop was as following: 3208 $m^3 \cdot ha^{-1}$ for dill, 3849 $m^3 \cdot ha^{-1}$ for parsley, 3445 $m^3 \cdot ha^{-1}$ for coriander, and 2566 $m^3 \cdot ha^{-1}$ for mint.

The irrigation water was of good quality, highlighting its slightly basic pH (7.91), and its proper electrical conductivity (1.26 $mS \cdot cm^{-1}$), which is suitable for aromatic herbs crops. Soil was uniformly silty-loam in texture, with a low content in organic matter (1.22%), medium salinity conditions (3.35 $mS \cdot cm^{-1}$), and good levels of sulfates (37.83 $meq \cdot L^{-1}$) for aromatic herb development.

Along the development of crops, fertilization was carried out with a total amount of N of 130 $kg \cdot ha^{-1}$, P (P_2O_5) of 60 $kg \cdot ha^{-1}$, and K (K_2O) of 160 $kg \cdot ha^{-1}$ for dill development. For parsley, the total amount of fertilizers was 300 $kg \cdot N \cdot ha^{-1}$, 190 $kg \cdot P \cdot ha^{-1}$ (P_2O_5), and 350 $kg \cdot K \cdot ha^{-1}$ (K_2O). Regarding coriander, the fertilization was carried out with a total amount N of 275 $kg \cdot ha^{-1}$, P (P_2O_5) of 170 $kg \cdot ha^{-1}$, and K (K_2O) of 310 $kg \cdot ha^{-1}$. Finally, the total amount of fertilizers was 200 $kg \cdot N \cdot ha^{-1}$, 130 $kg \cdot P \cdot ha^{-1}$ (P_2O_5), and 230 $kg \cdot K \cdot ha^{-1}$ (K_2O).

2.2. Extraction of Essential Oils

Hydrodistillation (HD), using a Deryng system (the Polish version of the Clevenger apparatus), was used for isolating the essential oil in fresh herbs (dill, parsley, coriander, and mint). About 15.0 g of freshly chopped herbs shoots (aerial part of the plant, including stems and leaves) were put in a 500 mL round bottom flask, together with 1.0 g sodium chloride (NaCl), 150 mL of distilled water, and 50 µL of benzyl acetate as an internal standard (987 $mg \cdot L^{-1}$). After the mixture started boiling, heating was maintained for 1 h. A cold refrigerant was used to condense the vapors, and 1 mL of cyclohexane was added to the Deryng apparatus at the beginning of the hydrodistillation process to retain the essential oil distilled from the samples of herbs shoots. After 60 min of extraction, the solvent, enriched with the volatile compounds, was transferred into a 2.5 mL vial, after drying it over anhydrous sodium sulfate (Na_2SO_4), and kept at −15 °C until the gas chromatography-mass spectrometry (GC-MS) analyses were conducted. The extractions were conducted in triplicate.

2.3. Chromatographic Analyses

Analysis and identification of the volatile compounds were performed using a Shimadzu GC-17A gas chromatograph coupled with a Shimadzu QP-5050A mass spectrometer detector (Shimadzu Corporation, Kyoto, Japan). The GC-MS system was equipped with a TRACSIL Meta.X5 (95% dimethylpolysiloxane and 5% diphenylpolysiloxane) column (60 m × 0.25 mm, 0.25 µm film thickness; Teknokroma S. Coop. C. Ltd, Barcelona, Spain). Analyses were carried out using helium as carrier gas at a column flow rate of 0.3 $mL \cdot min^{-1}$ and a total flow of 3.9 $mL \cdot min^{-1}$ in a split ratio of 1:11 and the following program: (a) 80 °C for 0 min; (b) increase of 3 $°C \cdot min^{-1}$ from 80 °C to 210 °C and hold for 1 min; (c) increase of 25 $°C \cdot min^{-1}$ from 210 °C to 300 °C and hold for 3 min. The temperatures of the injector and detector were 230 °C and 300 °C, respectively.

All compounds were identified using three different analytical methods: (1) comparison of experimental retention indexes (RI) with those of the literature; (2) GC-MS retention times (authentic standards of "all" compounds reported in Tables 1–5 were used for identification purposes); and, (3) mass spectra (authentic chemicals and NIST05 spectral library collection). Only fully identified compounds have been reported in this study.

The semi-quantification of the volatile compounds was performed on a gas chromatograph, Shimadzu 2010, with a flame ionization detector (FID). The column and chromatographic conditions were those previously reported for the GC-MS analysis. The injector temperature was 200 °C and nitrogen was used as carrier gas (1 $mL \cdot min^{-1}$). The quantification was obtained from electronic integration measurements using flame ionization detection (FID). Benzyl acetate (1000 $mg \cdot L^{-1}$) was added as internal standard at the beginning of the distillation procedure to simulate the behavior of volatile compounds; this chemical was used as an internal standard after checking that it was absent in herbs, it separates well from other volatiles, it possesses similar FID and MS response factors to most of the volatiles in the aromatic herb essential oil, it is stable at high temperatures, and does not react with water. Calibration curves were performed with the following compounds (Sigma-Aldrich, Madrid, Spain) as representative of each chemical family: α-phellandrene (monoterpenes), α-terpineol

(terpenoids), *trans*-β-caryophyllene (sesquiterpenes), dill ether (terpene ethers), nonanal (aldehydes), myristicin (phenylpropanoids), bornyl acetate (esters), 1-decanol (alcohols), undecane (alkanes); the correlation coefficients (R^2) for all compounds were >0.995, and results were expressed as mg· kg^{-1} fresh weight, fw.

2.4. Sensory Evaluation with a Trained Panel

A trained panel was used to evaluate the intensity of the main aroma attributes of fresh herbs. Samples were evaluated by seven panelists (five males and two females), with ages between 23 and 56 years old. Panelists belonged to the Food Quality and Safety research group of the Universidad Miguel Hernández de Elche and had over 1000 h of evaluation experience; they had been trained in descriptive evaluation of aromatic herbs [2,8,9].

An odor profile method was used to describe the dill samples. During two preliminary orientation sessions of 90 min, panelists discussed about the main odor characteristics of the herbs and agreed on their use of odor attributes. During these orientation experiments, panelists evaluated different coded samples of Spanish fresh aromatic herbs together with samples from field. Panelists agreed that the odor of the samples could be described using seven attributes: aromatic herb(dill/coriander/mint/parsley)-ID (clean fresh green, bitter, pungent aromatics associated with fresh dill/coriander/mint/parsley; reference: dill/coriander/mint/parsley water; preparation: 25 g chopped fresh dill/coriander/mint/parsley soaked in 300 mL room temperature deionized water for 15 min, filtered), green grass (green aromatics associated with newly cut-grass and leafy plants; reference: hexanal in propylene glycol, 10 g· L^{-1} = 6), citrus (aromatics associated with commonly known citrus fruits, such as lemons, limes, oranges, which could also contain a peely note; reference: McCormick lemon grass = 3.0; preparation: take 0.1 g of lemon grass and place it in a medium snifter together with 100 mL of deionized water, and cover it), pine (aromatics reminiscent of resinous pine tree; can be medicinal or disinfectant in character; reference: *El Corte Inglés* raw pine nuts = 3.0), spicy (sharp aromatics with a physically penetrating sensation in the nose reminiscent of radish and horseradish; reference: fresh radish = 3.0), earthy (humus-like aromatics that may or may not include damp soil, decaying vegetation or cellar-like characteristics; reference: hexanal in propylene glycol, 5 g· L^{-1} = 1.5), and woody (brown, musty aromatics associated with very fibrous plants and bark; reference: *Hacendado* dried parsley = 4.5). The key sensory descriptors (high intensities being related to high quality products) are: herb-ID, green grass, citrus, spicy, and pine; earthy and woody are not positively correlated with the herb quality. Reference products of these attributes with different intensity, were prepared and provided to the panel.

Individual booths with controlled illumination and temperature were used in this study. Three digit numbers were used to code samples, and they were randomly offered to panelists in plastic beakers of 100 mL with lids; samples were left 15 min at room temperature prior to analyses. The intensity of the seven odor attributes was scored using scale from 0 to 10, where 0 = none or not perceptible intensity, and 10 = extremely high intensity.

2.5. Statistical Analysis

To compare the experimental data two consecutive tests were performed: (i) one-way analysis of variance (ANOVA), and (ii) Tukey's multiple range. Homogenous groups and the least significant difference (LSD) were determined at significance level of $p \leqslant 0.05$. Statgraphics Plus 5.0 software (Manugistics, Inc., Rockville, MD, USA) was the program used for the statistical analyses.

3. Results and Discussion

3.1. Volatile Composition of Essential Oil of Dill

Dill was planted on 24 September 2014 and the first harvest date for commercial purpose was on 26 November 2014 (D1) while the second harvest date was on 11 February 2015 (D2). After isolation of the essential oil of dill shoots, 18 compounds were identified by GC-MS (Table 1). The identified volatile compounds can be grouped in eight main chemical groups: monoterpenes (110 compounds),

followed by alkanes (two compounds), terpenoids (one compound), sesquiterpenes (one compound), aldehydes (one compound), phenylpropanoids (one compound), terpenes (one compound), and monoterpene ethers (one compound). The eight main compounds were: α-phellandrene, dill ether, β-phellandrene, limonene, *p*-cymene, α-pinene, *trans*-β-ocimene, and myristicin. In several studies, α-phellandrene was predominating in the "leaf" essential oil of dill from Romania, Egypt, and Finland [3,16–18]. The extraction of the essential oils can be done using different isolation techniques, and this analytical methodology can affect the volatile profile. For example, Huopalahti and Linko [3] found 22 compounds in dill by using a modified Soxhlet technique; however, α-phellandrene, dill ether, β-phellandrene were reported as the major compounds.

Table 1. Identification of essential oils found in dill, parsley, coriander, and mint samples.

Compound	Herb	RT (min)	Retention Indexes (RI)		Descriptor ¶
			Exp. †	Lit. †	
trans-2-Hexenal ‡	dill	11.09	806	800	Green, banana, aldehydic ‡
Octane	coriander	12.12	808	800	
α-Thujene	dill	13.19	873	905	Woody, green, herb
Santene	mint	13.29	879	880	
α-Pinene	dill, parsley, mint	13.58	896	909	Fresh, camphor, sweet, pine, earthy, woody
Camphene	mint	14.38	944	945	Fresh, woody, fir, terpene
Sabinene	dill, parsley	14.89	975	975	Woody, terpene, citrus, pine, spice
Myrcene	dill, parsley, mint	15.15	991	991	Peppery, terpene, spicy
β-Pinene	dill, parsley	15.25	997	990	Dry, woody, pine, hay, green
cis-3-Hexenyl acetate	parsley, coriander, mint	15.70	1008	1009	Fresh, green, sweet, fruity, banana, apple
α-Phellandrene	dill, parsley	16.20	1020	1013	Citrus, herbal, terpene, green, woody, peppery
α-Terpinene	mint	16.68	1031	1018	Woody, terpene, lemon, herbal, citrus
p-Cymene	dill, parsley, mint	16.88	1036	1034	Fresh, citrus, terpene, woody, spice
Limonene	dill, parsley, coriander, mint	17.08	1040	1039	Terpene, pine, herbal, peppery
β-Phellandrene	dill, parsley, coriander	17.25	1044	1036	Mint, terpentine
trans-β-Ocimene	dill, parsley, mint	17.38	1047	1047	Citrus, tropical, green, terpene, woody
γ-Terpinene	parsley, mint	18.20	1066	1066	Woody, terpene, lemon, lime, tropical, herbal
trans-Sabinene hydrate	mint	19.00	1084	1087	Warm, balsamic, woody
Terpinolene	dill, parsley	19.47	1095	1097	Fresh, woody, sweet, pine, citrus.
Undecane	dill, coriander	19.63	1098	1099	Fusel-like
Linalool	coriander, mint	19.82	1103	1103	Citrus, orange, floral, terpy, rose
Nonanal	coriander, mint	20.03	1107	1107	Aldehydic, rose, fresh, orris, orange, peel
1,3,8-*p*-Menthatriene	parsley	20.74	1125	1115	Turpentine, camphor, herbal, woody
cis-Limonene oxide	mint	21.87	1149	1140	Fresh, citrus
trans-Limonene oxide	mint	22.04	1153	1147	Fresh, citrus, mild, green
cis-*p*-Mentha-2.8-dien-1-ol	mint	23.74	1192	1193	
trans-*p*-Mentha-2.8-dien-1-ol	mint	24.07	1199	1196	Fresh, minty
Dill ether	dill	24.40	1206	1187	Herbal, dill, spicy
α-Terpineol	parsley	24.74	1213	1200	Pine, terpene, lilac, citrus, woody, floral
Decanal	coriander	24.79	1214	1207	Sweet, aldehydic, orange, waxy, citrus rind
cis-Carveol	mint	25.05	1220	1221	Caraway, spicy, citrus, fruity
trans-Carveol	mint	25.43	1228	1217	Caraway, green, oily
Carvone	dill, coriander, mint	27.13	1264	1262	Herbaceous, grapefruit, pepper, spicy, woody
E-2-Decenal	coriander	27.54	1273	1278	Earthy, coriander green, mushroom, aldehydic
1-Decanol	coriander	28.45	1292	1287	Floral, orange, sweet, clean watery
Tridecane	dill	29.06	1297	1299	Citrus, fruity, Fusel-like
Bornyl acetate	mint	29.10	1305	1291	Woody, camphor, mentholic, spicy
Undecanal	coriander	29.63	1317	1310	Fresh, citrus, waxy, aldehydic
Carvomenthyl acetate	mint	30.70	1339	1344	
E-2-Undecenal	coriander	32.40	1375	1371	Aldehydic, citrus
1-Undecanol	coriander	33.88	1407	1386	Earthy, soapy, waxy, fatty, honey, coconut
β-Bourbonene	mint	34.08	1412	1407	Herbal, Woody
Decyl acetate	coriander	34.13	1412	1410	Waxy, sweet, fatty, creamy
β-Caryophyllene	mint	34.26	1416	1418	Sweet, woody, spice clove dry
Dodecanal	coriander	34.39	1419	1420	Orange, fatty, herbaceous
trans-β-Caryophyllene	parsley, mint	35.68	1448	1455	Woody, spicy
Z-2-Dodecenal	coriander	36.37	1463	1467	Green, citrus, fruity, mandarin orange, herbal
E-2-Dodecenal	coriander	37.12	1480	1468	Citrus, mandarin orange, aldehydic
α-Humulene	mint	37.48	1489	1489	
E-2-Dodecen-1-ol	coriander	37.78	1495	1483	Oily, fatty
1-Dodecanol	coriander	38.08	1502	1485	Earthy, soapy, waxy, fatty, honey, coconut
Germacrene-D	dill, parsley, mint	38.42	1475	1477	Woody, spice

Table 1. *Cont.*

Compound	Herb	RT (min)	Retention Indexes (RI)		Descriptor ¶
			Exp. †	Lit. †	
Tridecanal	coriander	38.96	1522	1518	Fresh, aldehydic, citrus, grapefruit peel
Nerolidol	parsley	38.97	1525	1528	Floral, green, citrus, woody
Myristicin	dill, parsley	39.97	1543	1532	Spice, warm, balsam, woody
E-2-Tridecenal	coriander	41.58	1582	1571	Citrus, peel tangerine
1-Tetradecanol	coriander	42.81	1615	1618	Fruity, coconut
Tetradecanal	coriander	43.31	1632	1623	Dairy, creamy, fishy with a fruity, pear nuance.

† RT = retention time; Exp. = experimental and Lit. = Literature; ‡ All compounds were identified using retention indexes, mass spectra and retention time of standards; ¶ SAFC (2015); www.pherobase.com; www.thegoodscentscompany.com.

Table 2 shows that 3 compounds (α-phellandrene, dill ether, and β-phellandrene) clearly dominated the dill shoots essential oil, representing 85%–92% of the total concentration of volatile compounds. This experimental finding is supported as well by previous studies by Vokk *et al.* and Radulescu *et al.* [16,19] who reported that α-phellandrene, β-phellandrene, and dill ether were the main compounds of dill essential oil.

Table 2. Volatile composition of dill essential oil at two commercial stages (mg· kg^{-1}· fw).

Compound	ANOVA †	D1	D2
		Concentration, (mg· kg^{-1}· fw)	
trans-2-Hexenal	***	0.18 b ¥	1.81 a
α-Thujene	***	1.35 b	1.81 a
α-Pinene	***	7.84 b	8.70 a
Sabinene	***	0.35 b	0.51 a
Myrcene	***	2.41 b	3.20 a
β-Pinene	***	0.66 a	0.31 b
α-Phellandrene	***	342 b	474 a
p-Cymene	***	12.5 a	3.92 b
Limonene	***	17.6 b	21.4 a
β-Phellandrene	***	46.0 b	60.0 a
trans-β-Ocimene	***	5.13 b	7.50 a
Terpinolene	***	2.53 a	0.33 b
Undecane	***	4.38 a	1.11 b
Dill ether	***	46.2 b	62.9 a
Carvone	NS	0.02 a	0.02 a
Tridecane	***	0.56 a	0.20 b
Germacrene-D	***	4.19 a	1.38 b
Myristicin	***	13.8 a	0.02 b
TOTAL	***	508 b	649 a

† NS = not significant *F* ratio ($p < 0.05$); *** significant at $p < 0.001$. ‡ Treatment means of the ANOVA test (values are the mean value of 3 replications). ¥ Values followed by the same letter, within the same row, were not significant different ($p < 0.05$), Tukey's multiple-range test.

Volatile composition of dill essential oil at two commercial stages were investigated in the current study and the total concentration of volatile compounds was higher in commercial stage of D2 as compared to D1 stage; these trend was also true for the three main components (α-phellandrene, dill ether, and β-phellandrene). For α-phellandrene the concentration changed from 342 mg· kg^{-1} in D1 to 474 mg· kg^{-1} in D2 (an increase of 38.6 %), where the concentration in dill ether changed from 46.2 mg· kg^{-1} in D1 to 62.9 mg· kg^{-1} in D2 (an increase of 36.1 %) and from 46 mg· kg^{-1} (D1) to 60 mg· kg^{-1} (D2) for β-phellandrene (an increase of 30.4 %). Zlatev [20] and El-Gengaihi and Hornok [21] found that the essential oil content in dill increased continuously during the growing period. During the growth of dill, the contents of limonene, 3,6-dimethyl-2,3,3a,4,5,7a-hexahydrobenzofuran, and carvone increased, while those of α-phellandrene, β-pellandrene, myristicin, and apiol decreased. The total amount of aroma compounds varied widely during the growth [3].

3.2. Volatile Composition of Essential Oil of Parsley

Parsley was planted on 24 September 2014, the first harvest date for commercial purposes was on 19 November 2014 (P1), the second harvest date was on 5 January 2015 (P2), and the third harvest date was on 25 February 2015 (P3). Eighteen compounds were identified in the essential oil of parsley and the chemical classification of these 18 compounds was as follows: monoterpenes (nine compounds), sesquiterpenes (three compounds), terpenes (two compounds), monoterpenoids (one compound), monoterpene alcohol (one compound), esters (one compound), and phenylpropanoids (one compound). The main eight compounds of the essential oil of parsley shoots were: 1,3,8-p-menthatriene (38.4%–48.8%), β-phellandrene (22.2%–29.5%), myristcin (6.2%–11.1%), myrcene (5.8%–6.5%), terpinolene (4.2%–5.0%), limonene (2.7%–3.3%), α-pinene (1.6%–2.3%), and α-phellandrene (1.4%–2.7%). Vokk *et al.* [19] used Clevenger distillation method for essential oil isolation and gas chromatography for identifying the extracts and the major constituents of essential oil of parsley leaves were as following: myristicin (30.7%–42.7%), β-phellandrene (21.8%–35.9%), 1,3,8-p-menthatriene (5.4%–10.0%), and β-myrcene (4.5%–8.7%). Essential oils obtained by simultaneous distillation–extraction (SDE) from leaves of parsley plants and the main components were β-phellandrene, 1,3,8-p-menthatriene, α-p-dimethylstyrene, myristicin, β-myrcene, and apiole [15]. The results by Vokk *et al.* [19] and Petropoulos *et al.* [15] agree quite well with the results of this study.

Volatile composition of parsley essential oil at three commercial stages were investigated in the current study and the highest value of total concentration of essential oil was that of the commercial stage P1, 455 mg· kg^{-1}, as compared to 414 and 418 mg· kg^{-1} at P2 and P3 stages, respectively. The main two components were 1,3,8-p-menthatriene and β-phellandrene, which represented 68%–72% of the total concentration of essential oil of parsley. 1,3,8-p-Menthatriene had the highest concentration, 222 mg· kg^{-1}, at the P1 stage, while the highest concentration of β-phellandrene was found at 122 mg· kg^{-1} P2 harvest. For myristicin and myrcene, the highest concentration was in found at P2 stage, while for limonene was P3 and for terpinolene P1 (Table 3). For the essential oil of parsley plant Petropoulos *et al.* [15] recorded that, comparing the relative concentrations of the components for the two sowing dates, important differences were found for β-phellandrene (39.0%–22.0%) and 1,3,8-p-menthatriene (17.4%–45.7%) at the first growth stage and between 1,3,8-p-menthatriene (15.7%–29.0%) and myristicin (27.2%–4.6%) at the second stage.

Table 3. Volatile composition of parsley essential oil at three commercial stages (mg· kg^{-1}· fw).

Compound	ANOVA †	P1	P2	P3
		Concentration, (mg· kg^{-1}· fw)		
α-Pinene	***	7.07 c ¥	8.53 b	9.65 a
Sabinene	***	0.30 b	0.38 b	0.55 a
Myrcene	***	27.0 a	27.1 a	24.3 b
β-Pinene	***	2.47 c	4.14 a	3.64 b
cis-3-Hexenyl acetate	***	1.73 a	0.35 b	0.33 b
α-Phellandrene	***	6.46 c	11.0 a	8.63 b
p-Cymene	***	1.40 b	1.41 b	1.83 a
Limonene	***	12.5 b	11.3 b	13.7 a
β-Phellandrene	***	101 c	122 a	110 b
Trans-β-Ocimene	***	2.89 b	2.33 c	3.69 a
γ-Terpinene	***	0.45 a	0.29 b	0.31 b
Terpinolene	***	22.8 a	17.2 b	18.5 b
1,3,8-*p*-Menthatriene	***	222 a	159 c	192 b
α-Terpineol	***	0.26 c	0.40 b	0.82 a
trans-β-Caryophyllene	***	0.61 c	1.64 b	2.15 a
Germacrene-D	***	0.96 c	1.63 a	1.39 b
Nerolidol	***	0.15 b	0.07 c	0.23 a
Myristcin	***	45.1 a	45.9 a	25.9 b
TOTAL	***	455 a	414 b	418 b

† NS = not significant F ratio ($p < 0.05$); *** significant at $p < 0.001$. ‡ Treatment means of the ANOVA test (values are the mean value of 3 replications). ¥ Values followed by the same letter, within the same row, were not significant different ($p < 0.05$), Tukey's multiple-range test.

3.3. Volatile Composition of Essential Oil of Coriander

The essential oil of three commercial samples of coriander was analyzed by GC-MS after extracted by hydrodistillation technique. The first crop (C1) was planted on 30 September 2014 and the harvest date was 19 November 2014, the second crop (C2) was planted on 22 October 2014 and harvested on 5 January 2015 and the third crop (C3) was planted on 29 December 2014 and harvested on 25 February 2015.

GC-MS was used to identify the chemical composition of essential oil of coriander plant. Twenty-four compounds were identified (Table 1) and the chemical classification of these 24 compounds were as follows: aldehydes (11 compounds), followed by alcohols (five compounds), esters (two compounds), alkanes (two compounds), monoterpenes (two compounds), terpenoids (one compound), and terpenes alcohol (one compound). The main seven compounds of the essential oil of coriander shoots were: decanal (30.7 mg· kg^{-1}, mean of all treatments), *E*-2-dodecenal (mean of 26.9 mg· kg^{-1}), dodecanal (22.0 mg· kg^{-1}), octane (18.6 mg· kg^{-1}), 1-decanol (5.13 mg· kg^{-1}), undecanal (4.30 mg· kg^{-1}), and *E*-2-tridecenal (3.54 mg· kg^{-1}). Given the results in Table 4, decanal, *E*-2-dodecenal, dodecanal, and octane represented a big percentage of the total concentration of volatile compounds in the essential oil of coriander shoots (70.9%–82.5%). The highest concentrations of *E*-2-dodecenal (39.9 mg· kg^{-1}), dodecanal (24.4 mg· kg^{-1}), and octane (20.7 mg· kg^{-1}) were found at C2 harvest, while the maximum value of decanal was at C3 with 36.4 mg· kg^{-1} (Table 4). Nurzyńska-Wierdak [22] reported that the essential oil of the coriander herb contained the highest amount of aliphatic aldehydes, with decanal, *E*-2-dodecanol, and *E*-2-decenol having the highest contents. In coriander (*Coriandrum sativum* L.) the most abundant compounds are *E*-2-decenal, *E*-2-dodecenal, decanal, dodecanal, *E*-2-tridecenal and tetradecenal; these compounds have characteristic green, soapy, and cilantro-like aromas and are particularly important in the overall aroma of the *C. sativum* herb [23,24]. These results are in concordance to a large extent with the current results.

Table 4. Volatile composition of coriander essential oil at three commercial stages (mg· kg^{-1}· fw).

Compound	ANOVA †	C1	C2	C3
		Concentration, (mg· kg^{-1}· fw)		
Octane	***	16.9 c ¥	20.7 a	18.2 b
cis-3-Hexenyl acetate	***	0.64 b	1.17 a	1.11 a
Limonene	***	1.06 a	0.18 b	0.00 c
β-Phellandrene	NS	0.04 c	0.14 b	1.39 a
Undecane	***	0.45 b	1.09 a	0.36 b
Linalool	***	0.06 a	0.14 a	0.05 a
Nonanal	NS	0.01 c	0.92 a	0.22 b
Decanal	***	30.3 b	25.5 c	36.4 a
Carvone	***	3.06 a	0.01 c	0.37 b
E-2-Decenal	***	0.27 b	0.39 b	1.47 a
1-Decanol	***	3.70 c	5.05 b	6.64 a
Undecanal	***	2.23 c	3.93 b	6.70 a
E-2-Undecenal	NS	0.01 c	0.44 b	0.75 a
1-Undecanol	NS	0.05 b	0.11 a	0.05 b
Decyl acetate	NS	0.01 a	0.03 a	0.00 a
Dodecanal	***	24.1 a	24.4 a	17.6 b
Z-2-Dodecenal	***	0.12 b	0.26 a	0.10 b
E-2-Dodecenal	***	15.0 c	39.9 a	25.7 b
E-2-Dodecen-1-ol	***	1.90 a	1.00 b	0.04 c
1-Dodecanol	***	1.80 a	0.21 b	0.01 c
Tridecanal	***	1.90 a	1.18 b	1.22 b
E-2-Tridecenal	***	1.33 b	4.69 a	4.59 a
1-Tetradecanol	***	0.12 b	0.27 a	0.08 b
Tetradecanal	***	1.61 b	1.98 a	0.88 c
TOTAL	***	107 c	134 a	124 b

† NS = not significant F ratio ($p < 0.05$); *** significant at $p < 0.001$. ‡ Treatment means of the ANOVA test (values are the mean value of 3 replications). ¥ Values followed by the same letter, within the same row, were not significant different ($p < 0.05$), Tukey's multiple-range test.

3.4. Volatile Composition of Essential Oil of Mint

Peppermint (*Mentha piperita* L.) was planted on 6 October 2014 and the first harvest date was on 11 December 2014 (M1) while the second harvest date was on 5 February 2015 (M2). After isolation of the essential oil of mint shoots, 27 compounds were identified by GC-MS (Table 1). The identified volatile compounds can be grouped in eight main chemical groups: monoterpenes (six compounds), terpenoid alcohols (three compounds), sesquiterpenes (two compounds), aldehydes (one compound), terpenes (three compounds), esters (two compounds), aldehyde (one compound), terpene alcohols (one compound), and polycyclic alkenes (one compound). The eight main compounds were: carvone, limonene, *cis*-carveol, *trans*-sabinene hydrate, *trans*-caryophyllene, myrcene, santene, and *trans*-β-ocimene.

Volatile composition of mint essential oil at two commercial stages was investigated in the current study and the total concentration of volatile compounds in the essential oil was higher in plants of the commercial stage M1 as compared to those of M2. The contents of the main compound, carvone, also followed this trend (M1 > M2), while the concentrations of limonene and *cis*-carveol were higher in M2 as compared to M1. For carvone the concentration decreased from 2462 mg· kg^{-1} in M1 to 1854 mg· kg^{-1} in (M2) (a decrease of 24.7 %), while the concentration of limonene increased from 590 mg· kg^{-1} in M1 to 735 mg· kg^{-1} in M2 (an increase of 24.6 %) (Table 5).

Table 5. Volatile composition of mint essential oil at two commercial stages (mg· kg^{-1}· fw).

Compound	ANOVA †	M1	M2
		Concentration, (mg· kg^{-1}· fw)	
Santene	***	22.5 a ¥	24.05 a
Camphene	NS	2.17 b	3.50 a
β-Pinene	***	12.3 a	13.1 a
Myrcene	***	23.6 b	28.3 a
cis-3-Hexenyl acetate	NS	0.56 a	0.62 a
p-Cymene	NS	1.29 b	2.55 a
α-Terpinene	NS	0.54 b	2.90 a
Limonene	***	590 b	735 a
trans-β-Ocimene	***	19.1 a	18.2 a
γ-Terpinene	NS	1.31 b	5.17 a
trans-Sabinene hydrate	***	34.3 b	73.6 a
Nonanal	***	10.6 a	12.0 a
Linalool	NS	1.29 b	2.23 a
cis-Limonene oxide	NS	1.28 a	1.23 a
trans-Limonene oxide	NS	2.84 a	1.79 b
cis-*p*-Mentha-2,8-dien-1-ol	NS	6.43 a	6.39 a
trans-*p*-Mentha-2,8-dien-1-ol	NS	4.45 b	11.4 a
cis-Carveol	***	65.3 b	85.8 a
trans-Carveol	NS	8.12 a	9.73 a
Carvone	***	2462 a	1854 b
Bornyl acetate	NS	0.51 a	0.43 a
Carvomenthyl acetate	***	12.0 b	14.7 a
β-Bourbonene	***	14.6 a	14.0 a
β-Caryophyllene	NS	2.30 b	3.59 a
trans-Caryophyllene	***	22.1 b	35.5 a
Alloaromadendrene	NS	1.74 b	2.57 a
α-Humulene	NS	3.58 b	5.11 a
TOTAL	***	3326 a	2968 b

† NS = not significant F ratio ($p < 0.05$); *** significant at $p < 0.001$. ‡ Treatment means of the ANOVA test (values are the mean value of 3 replications). ¥ Values followed by the same letter, within the same row, were not significant different ($p < 0.05$), Tukey's multiple-range test.

According the previous studies, the harvest date and harvest time can affect the water content, the concentrations of menthol and menthofuran and the yield of limonene, menthol, and menthofuran

in Mentha canadensis [25,26]. Besides, in Japanese mint (*Mentha arvensis* L.) the content of menthol was not affected by the planting date or harvesting schedule but menthone signficantly decreased with the delay in harvesting [27].

The major components of peppermint essential oil were menthol (30.35 %), menthone (21.12 %), and trans-carane (10.99 %) according to previous studies [5]. These results did not agree with the results obtained in the current study. *Mentha piperita* (peppermint) showed in the composition of its essential oil a higher content of monoterpenes D-carvone (58.79 %) and limonene (28.29 %) [28]. Rohloff [29] found increased levels of other oxygenated monoterpenes and limonene in their work compared to *Mentha piperita* grown in Norway [28]. These results correspond to a large extent with the results in the current study.

3.5. Descriptive Sensory Evaluation

Volatiles directly affect the sensory quality of fresh fruits, vegetables and aromatic herbs. Within the sensory quality, the odor (perception of volatile compounds with the food outside the mouth) [30] plays an important role, especially in essential oils of aromatic herbs; the aroma is formed by a complex group of chemical substances, which includes aldehydes, alcohols, ketones, esters, lactones, terpenes, among other volatile compounds. The concentration of these volatile compounds is generally low (mg· kg^{-1}) and can be affected by a number of agronomic (variety, climatological conditions, ripening stage) [31,32] and technological (harvest, post-harvest treatments, storage and processing conditions) factors [33]. The quality of the vegetal products can be affected by both too high or too low concentrations of the volatile compounds; thus an equilibrium among them is necessary. Thus, all the information related to descriptive sensory evaluation (DSA), which is going to be shown, corresponds to those samples with the highest concentrations of the volatile compounds present in each essential oil (Tables 2–5); of course, this is a first step that will need further research but it is a very important step, which is done by the first time and will provide very practical information for farmers. According to this statement, the DSA was carried out with the following samples: D2 (dill harvested at the second commercial stage); P1 (parsley harvested at the first commercial stage); C2 (coriander harvested at the second commercial stage); and M1 (mint harvested at the third commercial stage).

Figure 1 shows the DSA profiles of different aromatic herb samples. The descriptors selected for the DSA were successfully used by this research group in previous studies [2,8,9].

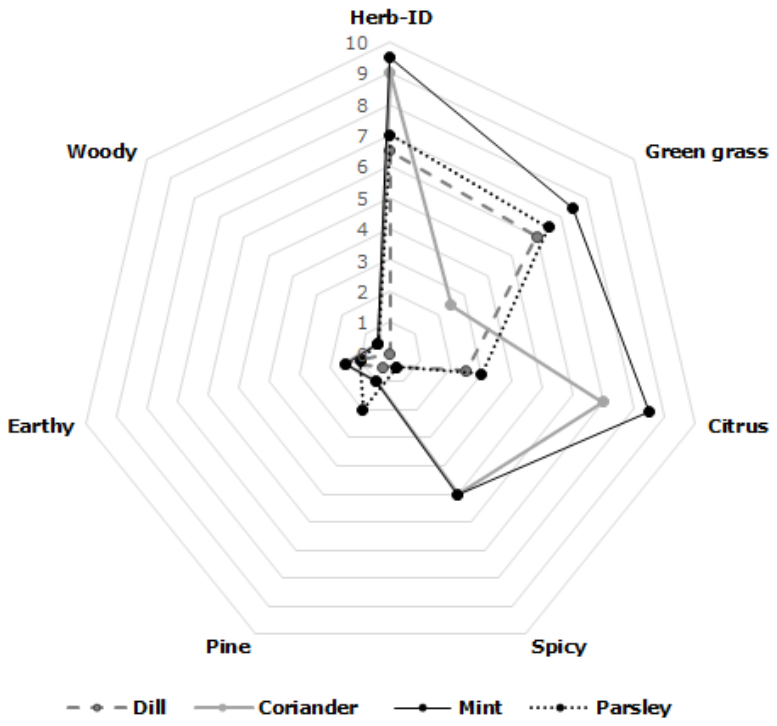

Figure 1. Descriptive sensory analysis of Spanish aromatic herbs.

Dill samples (D2), with a total number of 18 volatile compounds accounting a total concentration of 649 mg· kg^{-1} of its essential oil, were characterized by high intensity of dill-ID (herb-ID in Figure 1) (6.5), green grass (6.0), and citrus (2.5) notes (Figure 1). On the other hand, dill samples scored low values of attributes such as spicy (0.5), earthy (1.5), pine (0.5), or woody (0) (Figure 1).

Parsley samples (P1), with total number of 18 volatile compounds accounting a total concentration of 455 mg· kg^{-1} of its essential oil, were characterized by high intensity of parsley-ID (herb-ID in Figure 1) (7), citrus (3), and green grass (6.5) notes (Figure 1), while undesirable parsley attributes scored low values, for instance spicy (0.5), earthy (1.0), pine (2.0), or woody (0.5) (Figure 1).

Coriander samples (C2), with a total number of 24 volatile compounds accounting a total concentration of 134 mg· kg^{-1} of its essential oil, were characterized by high intensity of coriander-ID (herb-ID in Figure 1) (9), citrus (7), and spicy (5) and low values of green grass (2.5), earthy (1.5), pine (1.0), or woody (0.5) (Figure 1).

Finally, mint samples (M1), with the highest number of volatile compounds (27) and the higher concentration of these compounds in its essential oils (3326 mg· kg^{-1}), were characterized by high intensity of mint-ID (herb-ID in Figure 1) (9.5), green grass (7.5), citrus (8.5), and spicy (5).

The present study was the first one reporting data on descriptive sensory analysis of aromatic herbs at their optimal harvest time according to the content of volatile compounds of their essential oils. This information is valuable for farmers because the reported data shows the optimal date according to the highest productions of essential oils and high sensory quality.

4. Conclusions

Considering all the data generated in this study, the final recommendation according to the essential oil content and sensory quality is to harvest at the following dates: for dill (11 February 2015) however, for parsley (19 November 2014) while, for coriander (5 January 2015), and for mint (11 December 2014), there are relevant aspects which must subjected to further studies such as, different irrigation treatments, different plant densities, or fertilization conditions. In addition, the effect of pre-harvest treatments with organic compounds may be employed.

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PUBLICATION 2

Irrigation dose and plant density affect the essential oil content and sensory quality of parsley (*Petroselinum sativum*)

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Irrigation Dose and Plant Density Affect the Essential Oil Content and Sensory Quality of Parsley (*Petroselinum sativum*)

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Running title: Essential oil and sensory quality of parsley

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ABSTRACT

In the current study the influence of 3 irrigation treatments (ID0 as a control, ID1, and ID2), and 3 plant density treatments (PD0 as a control, PD1, and PD2) were investigated on the production (total yield), volatile composition of essential oil, and sensory quality of parsley (*Petroselinum sativum*). The results showed that the highest plant yield was obtained when using the highest values of both irrigation dose (ID2=1788 m^3 ha^{-1}) and plant density (7.41 plants m^{-2}). Hydrodistillation technique was used to extract the essential oil of parsley shoots and GC-MS and GC-FID were used to identify and quantify the components of the essential oil, respectively. The results showed that the main compounds of the essential oil were *β*-phellandrene, *1,3,8-p*-menthatriene, myristicin, myrcene, terpinolene, limonene, *α*-pinene, *and α*-phellandrene. The treatment ID1 (861 m^3 ha^{-1}) led to the highest concentrations of most of the main compounds: 1,3,8-*p*-menthatriene (150 mg kg^{-1}), myristicin (46.8 mg kg^{-1}), and myrcene (33.7 mg kg^{-1}); a similar pattern was found for the plant density PD0 (5.56 plants m^{-2}), with contents being *1,3,8-p*-menthatriene (143 mg kg^{-1}), *β*-phellandrene (130 mg kg^{-1}), and myristicin (38.1 mg kg^{-1}). Aroma attributes, such as parsley-like, citrus, and green grass significantly had the highest intensities in ID1 and PD0 plants. The final recommendation based on all data generated is to use the irrigation dose of 861 m^3 ha^{-1} (ID1) and the plant density of 5.56 plants m^{-2} (PD0) for better yield and quality of the final product under the assayed conditions.

Keywords: descriptive sensory analysis, hydrodistillation, *Petroselinum sativum*, plant yield, volatile compounds.

Introduction

Parsley is a very popular herb, native of the Mediterranean region, and widely used as herb or spice in Europe, America, and Middle Eastern countries. The varieties of parsley differ according to which organs or parts of the plant are used. There are plain and curly-leafed varieties, which are basically used for their leaves, as well as turnip-rooted varieties, such as Hamburg type varieties, which are used for their fleshy roots (Petropoulos et al., 2006, 2008; Najla et al., 2012). Parsley is native to Europe and Western Asia and is cultivated worldwide as an annual crop for its aromatic and attractive leaves (Simon and Quinn, 1988; Zhang et al., 2006). Parsley, leaves and seeds, is commonly used as food condiment in many dishes, such as meats, fishes, salads, creams, or soups. It is also a rich source of essential oil, which can be also extracted from leaves and seeds and used as a flavoring agent or fragrance in perfumes (Zhang et al., 2006). There are different techniques for the extraction of essential oils from parsley, for instance, Vokk et al. (2011) used hydrodistillation with Clevenger apparatus to isolate the essential oils of dried plant material of dill and parsley, while Petropoulos et al. (2004) employed simultaneous distillation–extraction (SDE) for the same objective. However, other isolation techniques have been successfully applied in aromatic herbs, such as hydrodistillation with Deryng apparatus (Calín-Sánchez et al., 2015). Moreover, microwave extraction process was applied by Costa et al. (2014) for the extraction of essential oil of mint and volatile compounds of fresh coriander leaves, which were extracted using solid-phase microextraction (SPME) (Fan and Sokorai, 2002).

Vokk et al. (2011) reported that the major constituents in the essential oil of Estonian parsley leaves were myristicin, *β*-phellandrene, *p*-1,3,8-menthatriene and *β*-myrcene; while the results obtained by Petropoulos et al. (2004) indicated that the main compounds of essential oil of Greek parsley plants were *β*-phellandrene, 1,3,8-*p*-menthatriene, *p*-dimethylstyrene, myristicin, *β*-myrcene, and apiole.

In fact, there are many factors affecting the composition of essential oil and also the yield of herbs, such as irrigation dose, plant density, sowing date, climate

of the area, among others. For instance, Khazaie et al. (2008) studied the effect of irrigation frequency and planting density on herbage biomass and oil production of thyme; Callan et al. (2007) studied the effect of plant density on the dill oil composition, and Petropoulos et al. (2004) studied the effect of sowing date on the parsley essential oil composition.

The optimization of irrigation for the production of parsley fresh leaves is essential because, as in other horticultural crops, water is a major component of the fresh plant material, and significantly affects both weight and quality (Jones and Tardieu, 1998; Petropoulos et al., 2008). Providing a permanent source of water is a priority to increase the production and improve the quality of the cultivated vegetables; besides, parsley is classified as a sensitive plant to water stress (Najla et al., 2012). Khazaie et al. (2008) reported that optimum planting density is a key factor to achieve maximum crop production, especially when water is a limiting factor, as it is the case of the Spanish agriculture. Several authors discussed the effect of plant density on the yield production and essential oil yield. For instance, Shalaby and Razin (1992) reported that herbage biomass and essential oil production of thyme increased at lower planting distances (Khazaie et al., 2008). El-Gendy et al. (2001) showed that the lowest planting distance (15 cm) resulted in higher biomass and essential oil yield compared to a 45 cm planting distance in sweet basil (Khazaie et al., 2008).

The aim of this study was to optimize the irrigation dose and plant density to obtain the highest production (yield, kg ha^{-1}) and highest quality (essential oil content and sensory quality) of parsley.

Material and methods

Plant material, irrigation doses and plant density

Parsley seeds (*Petroselinum sativum* L.), cultivar *Gigante Italiano Darkness* (plain type) were sown on the 19th of September 2014 in expanded polystyrene (EPS) trays (41 cm × 65 cm, with 260 cells) and placed in a greenhouse located at Santomera (Murcia, Spain) until 17th of October. Then, plantlets were transplanted to a commercial parsley orchard located at Sucina (Murcia, Spain) with a total surface of 2.5 ha. Parsley plants were grown using a high-frequency drip irrigation system.

Irrigation was carried out according to 3 irrigation doses consisting on the following total water amounts: (i) control treatment, ID0, normal irrigation conditions, with 1300 m^3 ha^{-1}; (ii) treatment 1, ID1, with lower than normal irrigation conditions, 861 m^3 ha^{-1}; and, (iii) treatment 2, ID2, with higher than normal irrigation conditions, 1788 m^3 ha^{-1}. The plant densities of the 3 treatments was reached by using plant lines separated by 0.9 m and a distance of 0.20 m between plants of the same line, leading to a plant density of 5.56 plants m^{-2} (e.g. 56 plants in a surface of 10 m^2). The total volume of water was settled according to the irrigation time. The water contribution was carried out using polyethylene pipes of 16 mm of diameter. The drippers were adjusted at both different flows and drippers distance to fit the established volume of water. At ID0 irrigation conditions, 16 mm pipes, separated by 0.9 m, were used, with a distance between two consecutive drippers of 0.32 m; the flow was 1.6 L h^{-1} for each emitter with an irrigation surface of 0.29 m^2; the total number of drippers per ha was 34722, with a total volume of water of 53.87 m^3 ha^{-1} (~24 h of irrigation). ID1 was the lowest irrigation dose and at these conditions, 16 mm pipes, separated by 0.9 m, were used, with an emitter distance of 0.50 m; the flow was 1.6 L h^{-1} for each emitter with an irrigation surface of 0.45 m^2; the total number of drippers per ha was 22222, with a total volume of water of 35.55 m^3 ha^{-1} (~24 h of irrigation). ID2 was the highest irrigation dose, and at these conditions, 16 mm pipes, separated by 0.9

m, were used, with emitter distance of 0.32 m; the flow was 2.2 L h^{-1} for each emitter with an irrigation surface of 0.29 m^2; the total number of drippers per ha were 34722, with a total volume of water of 74.07 m^3 ha^{-1} (~24 h of irrigation).

Regarding plant density, 3 treatments were assayed. The water contribution was carried out using polyethylene pipes of 16 mm of diameter, with a distance between drippers of 0.33 m. The flow was 1.6 L ha^{-1} for each emitter, making a total volume of water applied of 1290 m^3 ha^{-1}, according to the irrigation time. Plant densities under study were as following: (i) control treatment, PD0 (5.56 plants m^{-2}), or normal plant density, with plant lines separated by 0.9 m, a distance of 0.20 m between plants of the same line; (ii) treatment 1, PD1 (4.44 plants m^{-2}), with plant lines separated by 0.9 m, a distance of 0.25 m between root balls; and, (iii) treatment 2, PD2 (7.41 plants m^{-2}), with lines separated by 0.9 m, 0.15 m between root balls. Thirty-six plants of each treatment were assayed in the following surfaces: PD0 required a total surface of 6.48 m^2, PD1 surface was of 8.10 m^2, and finally PD2 needed a total surface of 4.86 m^2. All the field treatments were run in triplicate.

The irrigation water was of good quality, highlighting its slightly basic pH (7.91), and its proper electrical conductivity (1.26 mS cm^{-1}), which is suitable for growing aromatic herbs crops. Soil was uniformly silty-loam in texture, with a low content in organic matter (1.22%), medium salinity conditions (3.35 mS cm^{-1}) and appropriate levels of sulfates (37.83 meq L^{-1}) for parsley development. Along the development of parsley plants, fertilization was carried out with a total amount of N of 130 kg ha^{-1}, P (P_2O_5) of 60 kg ha^{-1}, and K (K_2O) of 160 kg ha^{-1}.

Extraction of essential oil

Hydrodistillation (HD), using a Deryng system (the Polish version of the Clevenger apparatus), was used for isolating the essential oil in fresh parsley. About 15.0 g of fresh chopped parsley shoots (aerial part of the plant, including stems and leaves) were put in a 500 mL round bottom flask, together with 1.0 g

sodium chloride (NaCl), 150 mL of distilled water, and 50 µL of benzyl acetate as internal standard. After the mixture started boiling, heating was maintained for 1 h. A cold refrigerant was used to condense the vapors, and 1 mL of cyclohexane was added to the Deryng apparatus at the beginning of the hydrodistillation process to retain the essential oil distilled from the samples of parsley shoots. After 60 min of extraction, the solvent, enriched with the volatile compounds, was transferred into a 2.5 mL vial, after drying it over anhydrous sodium sulfate (Na_2SO_4), and kept at -15 ºC until the GC-MS and GC-FID analyses were conducted. The extractions were conducted in triplicate.

Chromatographic analyses

Isolation and identification of the volatile compounds were performed using a Shimadzu GC-17A gas chromatograph coupled with a Shimadzu QP-5050A mass spectrometer detector (Shimadzu Corporation, Kyoto, Japan). The GC-MS system was equipped with a TRACSIL Meta.X5 (95% dimethylpolysiloxane and 5% diphenylpolysiloxane) column (60 m × 0.25 mm, 0.25 µm film thickness; Teknokroma S. Coop. C. Ltd, Barcelona, Spain). Analyses were carried out using helium as carrier gas at a flow rate of 0.3 mL min^{-1} in a split ratio of 1:11 and the following program: (a) 80 ºC for 0 min; (b) increase by 3 ºC min^{-1} from 80 to 210 ºC, and hold for 1 min; (c) increase by 25 ºC min^{-1} from 210 to 300 ºC, and hold for 3 min. The temperatures of the injector and detector were 230 and 300 ºC respectively. Data handling was made through GCMSsolution 1.01 (Shimadzu).

Most compounds were identified using three different analytical methods: (i) retention indexes, RI; (ii) GC-MS retention times (authentic standards of all compounds reported were used for identification purposes); and, (iii) mass spectra (authentic chemicals and NIST05 spectral library collection).

The semi-quantification of the volatile compounds was performed on a gas chromatograph, Shimadzu 2010, with a flame ionization detector (FID). The column and chromatographic conditions were those previously reported for the GC-MS

analysis. The injector temperature was 250 ºC and N_2 was used as carrier gas (1 mL min^{-1}). Data handling was carried out by means of GCsolution 2.3 (Shimadzu).

For the semi-quantification of the volatile compounds, benzyl acetate was added as internal standard at a concentration of ~1.0 g L^{-1} (50 µL); this chemical was used as internal standard after checking that it was absent in the extracts of parsley and under the proposed conditions, it separates well from other volatile compounds. Data included in this study should be considered as semi-quantitative, because no standard curves were carried out for each one of the quantified volatile compounds. However, relative values are useful to compare differences among farming treatments.

Sensory evaluation with a trained panel

A trained panel was used to quantify the intensity of the main aroma attributes of fresh parsley. Samples were evaluated by 7 panelists (5 males and 2 females), with ages between 23 and 56 years old. Panelists belonged to the Food Quality and Safety research group of the Universidad Miguel Hernández de Elche and had over 500 h of evaluation experience; they had been trained in descriptive evaluation of aromatic herbs (Calín-Sánchez et al., 2011; Calín-Sánchez et al., 2015).

An odor profile method was used to describe the parsley samples. During two preliminary orientation sessions of 90 min, panelists discussed about the main odor characteristics of parsley and agreed on their use of odor attributes. During these orientation experiments, panelists evaluated different coded samples of Spanish fresh parsley together with samples from the current study. Panelists agreed that the odor of parsley samples could be described using 7 attributes: parsley-like, citrus, green grass, pine, earthy, spicy, and woody aroma. Reference products of these attributes with different intensity, were prepared and provided to the panel.

Individual booths with controlled illumination and temperature were used in this study (AENOR, 1997). Three digit numbers were used to code samples, and

they were randomly offered to panelists in plastic beakers of 100 mL with lids; samples were left for 15 min at room temperature prior to analyses.

The intensity of the 7 odor attributes was scored using scale from 0 to 10, where 0 = none or not perceptible intensity, and 10 = extremely high intensity.

Statistical analysis

To compare the experimental data two consecutive tests were performed: (i) one-way analysis of variance (ANOVA) and (ii) Tukey's multiple range. Two different factors: (a) irrigation dose, and (b) plant density, were assayed in two independent studies. Homogenous groups and the least significant difference (LSD) were determined at significance level of $p \leq 0.05$. Statgraphics Plus 5.0 software (Manugistics, Inc., Rockville, MD, U.S.A.) was the program used for the statistical analyses.

Results and discussion

Yield and plant morphology

Data presented in **Table 1** shows the effect of three levels of irrigation "irrigation dose, ID" on the yield (kg ha^{-1}), length of the main stem (cm), and weight of the main stem (g) of parsley. There were no significant changes after decreasing the water volume from 1300 m^3 ha^{-1} (control treatment, ID0) to 861 m^3 ha^{-1} (ID1) in any of the three morphological parameters studied (yield, stem length and stem weight); however, there was a significant effect on the yield when the dose of water was increased to 1788 m^3 ha^{-1} (ID2). From these data, it can be stated that the highest yield can be obtained by applying ID2 dose for the applied plant density (5.6 plants m^{-2}; normal value for commercial farms). It must be added that there was no statistically significant effect of the irrigation dose on neither the stem length nor the stem weight of parsley; although the highest values were always found at the highest dose (ID2). In several studies, the effect of irrigation dose on the yield of some herbs was assayed. For instance, Hassan and Ali (2014) showed that the vegetative growth parameters of coriander plants were improved as a result of applying higher irrigation levels. Petropoulos et al. (2008) reported that the plant growth of parsley (foliage and root weight, leaf number) was significantly reduced by water stress.

The effect of "plant density, PD" on the yield, stem length and weight of parsley was also investigated (**Table 1**). Three different plant densities were used [PD0 (5.56 plants m^{-2}), PD1 (4.44 plants m^{-2}), and PD2 (7.41 plants m^{-2})]. In fact, there was not significant effect of plant density on the stem weight; however, there were slight differences, but not statistically significant, on the production (yield) and stem length of parsley, with treatment PD2 giving the highest production of parsley (10640 kg ha^{-1}) and the longest stems (21.36 cm). Khazaie et al. (2008) studied the effect of plant density on the yield of thyme plant, and reported that the only significantly ($p>0.05$) difference for herb biomass was obtained at the highest planting density (10 plants m^{-2}) compared to the two other planting densities (8.0

and 6.6 plants m^{-2}). For dill plant, Callan et al. (2007) recorded that leaves and stems comprised a larger proportion of the biomass at high density, while the umbel development was greater at low density.

Volatile composition of essential oil

Hydrodistillation technique, using a Deryng apparatus, was used to isolate the essential oil of the aerial portion of parsley (shoots and leaves). Essential oil of different herbs, such as dill, thyme, and rosemary was successfully extracted by this same technique (Calín-Sánchez et al., 2013; Szumny et al., 2010). The identification of the chemical composition of essential oil was done by GC-MS, using retention times, mass spectra, and retention indexes (**Table 2**). Eighteen compounds were identified and the chemical classification was as follows: monoterpenes (8 compounds), terpenes (3 compounds), sesquiterpenes (3 compounds), monoterpenoids (1 compound), monoterpene alcohols (1 compound), esters (1 compound), and phenylpropanoids (1 compound). The main 8 compounds of the essential oil of parsley shoots were: *β*-phellandrene, 1,3,8-*p*-menthatriene, myristicin, myrcene, terpinolene, limonene, *α*-pinene, and *α*-phellandrene (**Table 3**). Vokk et al. (2011) used Clevenger distillation method for the essential oil isolation and gas chromatography for identifying the compounds found in the extracts. The major constituents of essential oil of parsley leaves were: myristicin (30.7–42.7%), *β*-phellandrene (21.8–35.9%), 1,3,8-*p*-menthatriene (5.4–10.0%), and *β*-myrcene (4.5–8.7%). On another study by Petropoulos et al. (2011), the essential oil obtained by simultaneous distillation–extraction (SDE) from leaves of parsley plants were mainly formed by *β*-phellandrene, 1,3,8-*p*-menthatriene, *α*-*p*-dimethylstyrene, myristicin, *β*-myrcene, and apiole. Thus, the results obtained by Vokk et al. (2011) and Petropoulos et al. (2004) were consistent with the results of this study to a large extent.

The concentrations of individual volatile compounds of parsley essential oil was clearly affected by the different irrigation doses used in this study (ID0, ID1,

and ID2). In general, the irrigation dose ID1 led to essential oils with the highest concentrations of most of the key compounds: *β*-phellandrene (118 mg kg^{-1}), 1,3,8-*p*-menthatriene (150 mg kg^{-1}), myristicin (46.8 mg kg^{-1}), limonene (14.4 mg kg^{-1}), *α*-pinene (7.70 mg kg^{-1}), and *α*-phellandrene (6.78 mg kg^{-1}), while the highest concentrations of myrcene (34.4 mg kg^{-1}) and terpinolene (22.5 mg kg^{-1}) were obtained at the ID2 dose. These results seem to indicate that the lowest irrigation dose ID1 (860 m^3 ha^{-1}), for the applied plant density (5.56 plants m^{-2}), increased the concentrations of most of the key and main components of the essential oil of parsley shoots (**Table 3**). Petropoulos et al. (2004) studied the effect of water stress on the essential oil of parsley and reported that the water stress increased the yield of essential oil from the leaves of plain-leafed and curly-leafed parsley samples; this result was compatible with the obtained results in the current study. Similar results have been reported by other authors stating that water stress led to increased contents of essential oils in different plants, such as sweet basil, savory, lavender, and absinthium (Simon et al., 1992; Baher et al., 2002; Karamzadeh et al., 2003; Khazaiea et al., 2008). These results agree quite well with those found in the current study, where a decrease in the irrigation dose resulted in increased concentrations of most of the volatiles compounds, leading to the highest total concentration (416 mg kg^{-1} in ID1 plants) (**Table 3**).

The plant density significantly affected the concentrations of individual volatile compounds of parsley essential oil (**Table 3**). The plant density PD0 (5.56 plants m^{-2}) led to the highest concentrations of all key compounds, including *β*-phellandrene (130 mg kg^{-1}), 1,3,8-*p*-menthatriene (143 mg kg^{-1}), myristicin (38.1 mg kg^{-1}), myrcene (34.0 mg kg^{-1}), terpinolene (19.8 mg kg^{-1}), limonene (10.2 mg kg^{-1}), *α*-pinene (4.76 mg kg^{-1}), and *α*-phellandrene (14.2 mg kg^{-1}). However, results from the lowest (PD1, 4.44 plants m^{-2}) and highest (PD2, 7.41 plants m^{-2}) plant densities were statistically similar, and equivalent concentrations of most of the components of the essential oils were found. Finally, it must be highlighted that the control treatment, PD0, led to the highest total content of volatile compounds,

409 mg kg^{-1} as compared to treatments with the lowest (PD1, 277 mg kg^{-1}) or the highest (PD2, 279 mg kg^{-1}) plant densities, respectively (**Table 3**).

Callan et al. (2007) investigated the effect of plant density on the composition of dill essential oil, and the results showed that the higher the plant density, the higher the content of essential oil. This result agreed with the increase in the total content of volatiles reported in the PD1 plants as compared to control parsley plants (PD0); however, this behavior was not followed by PD2 plants, indicating that a threshold (5.56 plants m^{-2}) was reached, for the applied irrigation dose (1290 m^3 ha^{-1}), and that above this threshold, plant density did not improve the total content of volatile compounds found in parsley essential oil. Khazaie et al. (2008) studied the effect of plant density on the oil production of thyme and hyssop plants and reported that thyme plants oil production was lower at the highest planting density, while hyssop plants showed no response to planting density; these results of thyme plant were compatible with the results found for the highest PD treatment of the current study.

The total amount of volatile compounds produced [volatile yield= plant yield (**Table 1**) × total concentration of volatile compounds (**Table 3**)] by these treatments was also calculated. According to the calculated data, the best irrigation dose was ID2 (3528 g ha^{-1}), because it led simultaneously to the highest plant yield (9459 kg ha^{-1}) and intermediate but high content of total volatile compounds (373 mg kg^{-1}); this treatment was followed by ID1 (2911 g ha^{-1}), which was characterized by the highest yield of the total amount of volatiles (416 mg kg^{-1}). In the case of the plant density, the highest value of the total amount of volatile compounds produced was reached for the PD0 treatment (3674 g ha^{-1}), especially by the contribution of the highest total content of volatile compounds. The second best plant density treatment was PD2 (2969 g ha^{-1}), which is characterized by the highest plant yield (10640 kg ha^{-1}).

Descriptive sensory evaluation

Volatile compounds directly affect the sensory quality of fresh fruits, vegetables, and aromatic herbs. Within the sensory quality, the aroma plays an important role, especially in aromatic herbs; the aroma is formed by a complex group of chemical substances, which includes aldehydes, alcohols, ketones, esters, lactones, terpenes, among other volatile compounds. The concentration of these volatile compounds is generally low (µg *per* L) and can be affected by a number of agronomic (variety, climatological conditions, ripening stage) (Vendramini and Trugo, 2000; Melgarejo et al., 2012; Melgarejo et al., 2014), and technological (harvest, post-harvest treatments, storage and processing conditions) factors (Gironés-Vilaplana et al., 2015).

Figure 1 shows the DSA (descriptive sensory analysis) profiles of different parsley samples.

Parsley samples grown under the lowest irrigation dose (ID1, 861 m^3 ha^{-1}) and intermediate plant density (PD0, 5.56 plants m^{-2}) were characterized by high intensity of parsley-like (7.0 and 6.5, respectively), citrus (2.5 and 3, respectively), and green grass (6.5 and 7.5, respectively) notes (**Figure 1A** and **1B**). These samples were related to the highest concentrations of essential oil (**Table 3**).

In general, the irrigation dose and plant density significantly affected the intensities of the key sensory attributes of parsley. Attributes, such as parsley-like, citrus, and green grass significantly could be optimized by decreasing the irrigation dose from 1300 m^3 ha^{-1} (normal conditions at commercial orchards in Spain) to 861 m^3 ha^{-1}, at the applied plant density of 5.56 plants m^{-2}; however, the plant density was fully optimized at the normal plant density (5.56 plants m^{-2}) and under the applied irrigation dose (1290 m^3 ha^{-1}).

The present study was the first one reporting data on descriptive sensory analysis of parsley as affected by different agricultural practices. Sensory data confirmed that ID1 was the best one because led to parsley plants with the highest intensities of the key attributes (parsley-like, citrus, and green grass). Regarding

plant density, PD0 plants presented the highest intensities of parsley-like, citrus, and green grass notes.

CONCLUSIONS

The main purpose of this study was to optimize two key farming practices: (i) irrigation dose, and (ii) plant density in parsley farms. This optimization process was based on plant yield but also on the quality of parsley (volatile composition of essential oil and sensory quality). The highest plant yields were obtained when using ID2 (1788 m^3 ha^{-1}) and PD2 (7.41 plants m^{-2}). The highest total concentrations of volatile compounds were found in ID1 (861 m^3 ha^{-1}) and PD0 (5.56 plants m^{-2}) plants. The use of descriptive sensory analysis helped in reaching a final decision and the parsley plants with the highest sensory quality were those of the ID1 and PD0 treatments. Considering all the data generated in this study, the final recommendation is to use: (i) the irrigation dose of 861 m^3 ha^{-1} (ID1) at the applied plant density of 5.56 plants m^{-2}, and (ii) the plant density of 5.56 plants m^{-2} (PD0) at the applied irrigation dose of 1290 m^3 ha^{-1}, if the objective is to produce parsley samples with the highest aromatic and sensory quality. However, if the only objective is to produce high amounts of parsley, the best options are ID2 (1788 m^3 ha^{-1}) and PD2 (7.41 plants m^{-2}); ID2 also leads to the highest "production" of volatile compounds (3528 g ha^{-1}).

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Table 1. Yield (kg ha^{-1}), average stem length (cm), and average stem weight (g) of parsley as affected by irrigation dose and plant density.

Treatment	Yield (kg ha^{-1})	Stem length (cm)	Stem weight (g)
Irrigation Dose, ID			
ID0	7035 a‡	20.8 a	4.44 a
ID1	6997 a	20.6 a	4.74 a
ID2	9459 b	21.0 a	5.33 a
ANOVA†	***	NS	NS
Plant Density, PD			
PD0	8982 a	20.79 a	5.33 a
PD1	9859 a	19.74 a	4.44 a
PD2	10640 a	21.36 a	4.74 a
ANOVA†	NS	NS	NS

*†NS = not significant F ratio ($p < 0.05$); *, **, and ***, significant at $p < 0.05$, 0.01, and 0.001, respectively. ‡ Values followed by the same letter, within the same column and factor (irrigation dose, ID, or plant density, PD), were not significant different ($p < 0.05$), Tukey's multiple-range test.*

Table 2: Identification of the compounds found in the essential oil of parsley samples

Compound	Retention time (min)	Retention Indexes (RI)		Sensory Descriptors[‡]
		Exp.[†]	Lit.[†]	
α-Pinene	13.58	896	909	fresh camphor sweet pine earthy woody
Sabinene	14.89	975	975	woody terpene citrus pine spice
Myrcene	15.13	990	990	peppery terpene spicy balsam plastic
β-Pinene	15.23	995	990	dry woody resinous pine hay green
cis-3-Hexenyl acetate	15.70	1008	1009	fresh green sweet fruity banana apple grassy
α-Phellandrene	16.13	1019	1013	citrus herbal terpene green woody peppery
p-Cymene	16.93	1037	1034	fresh citrus terpene woody spice
Limonene	17.06	1040	1039	terpene pine herbal peppery
β-Phellandrene	17.23	1044	1034	mint terpentine
trans-*β*-Ocimene	17.40	1047	1045	citrus tropical green terpene woody green
γ-Terpinene	18.20	1066	1066	oily woody terpene lemon/lime tropical herbal
Terpinolene	19.44	1095	1097	fresh woody sweet pine citrus
1,3,8-*p*-Menthatriene	20.74	1125	1115	turpentine camphor herbal woody
α-Terpineol	24.74	1213	1200	pine terpene lilac citrus woody floral
trans-*β*-Caryophyllene	35.68	1448	1455	sweet woody spice clove dry
Germacrene-D	38.38	1510	1496	woody spice
Nerolidol	38.97	1525	1528	floral green waxy citrus woody
Myristicin	39.82	1543	1532	spice warm balsam woody

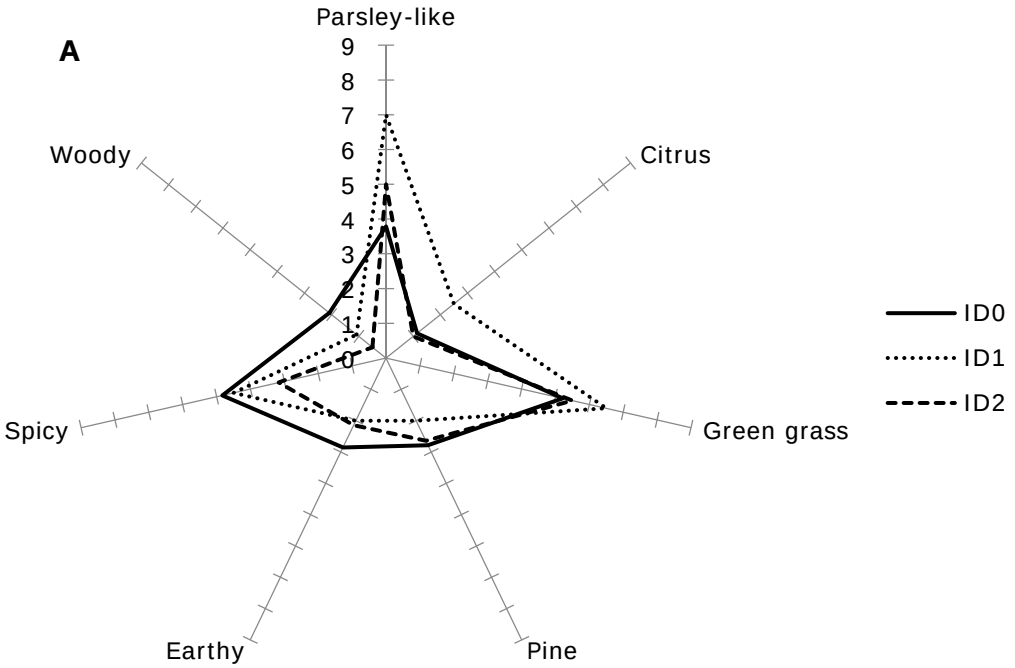
[†] *Exp.=experimental and Lit.=Literature;* [‡] *SAFC (2013).*

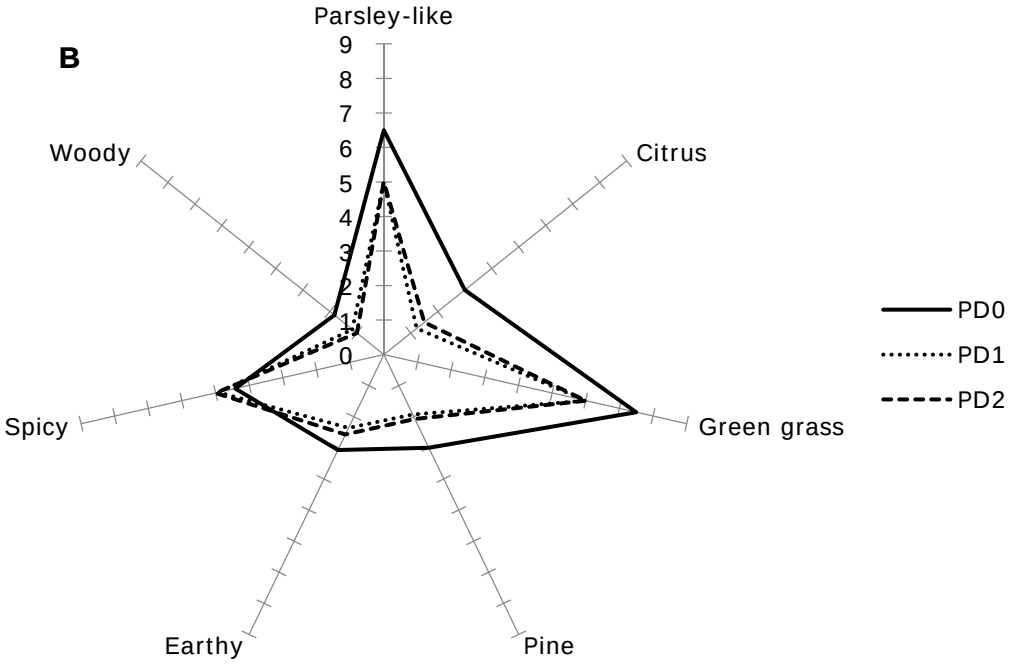
Table 3. Concentration (mg kg^{-1}) of volatile compounds from parsley leaves as affected by irrigation dose (ID) and plant density (PD)

Compound	ANOVA[†]	Irrigation Dose, ID			ANOVA[†]	Plant Density, PD		
		ID0	ID1	ID2		PD0	PD1	PD2
		Concentration (mg kg^{-1})				Concentration (mg kg^{-1})		
α-Pinene	***	5.77[‡] c[¥]	7.70 a	6.34 b	***	4.76 a	3.71 b	3.09 c
Sabinene	***	0.31 b	0.35 b	0.41 a	***	0.21 a	0.14 b	0.19 a
Myrcene	***	26.1 b	33.7 a	34.4 a	***	34.0 a	16.6 b	18.4 b
β-Pinene	***	2.87 b	3.10 a	2.34 b	***	1.96 a	1.01 b	1.06 b
cis-3-Hexenyl acetate	***	0.39 c	0.66 a	0.55 b	***	0.27 a	0.22 b	0.30 a
α-Phellandrene	***	4.90 b	6.78 a	6.63 a	***	14.2 a	4.52 b	4.85 b
p-Cymene	***	1.01 b	1.79 a	1.38 ab	***	1.31 a	0.95 b	0.72 c
Limonene	***	9.89 b	14.4 a	12.8 a	***	10.2 a	6.82 b	6.70 b
β-Phellandrene	***	102 b	118 a	118 a	***	130 a	85.1 c	92.7 b
trans-*β*-Ocimene	***	0.97 c	2.96 a	2.20 b	***	3.05 a	2.07 b	1.65 c
γ-Terpinene	***	0.34 a	0.36 a	0.35 a	***	0.09 b	0.39 a	0.33 a
Terpinolene	***	15.7 b	20.6 a	22.5 a	***	19.8 a	14.8 b	17.5 ab
1,3,8-*p*-Menthatriene	***	79.4 c	150 a	115 b	***	143 a	103 b	97.5 b
α-Terpineol	***	0.15 b	0.32 a	0.32 a	NS	0.05	0.10	0.01
trans-*β*-Caryophyllene	***	1.23 b	2.01 a	2.15 a	***	2.31 a	1.47 b	1.77 ab
Germacrene-D	***	0.99 b	1.58 a	1.50 a	***	0.97 a	1.02 a	0.91 a
Nerolidol	***	0.15 b	0.21 a	0.28 a	NS	0.04	0.05	0.06
Myristicin	***	40.8 b	46.8 a	40.9 b	***	38.1 a	31.0 b	26.3 c
TOTAL		**298 bc**	**416 a**	**373 b**		**409 a**	**277 b**	**279 b**

*[†] NS = not significant F ratio ($p < 0.05$); *, **, and ***, significant at $p < 0.05$, 0.01, and 0.001, respectively. [‡] Treatment means of the ANOVA test (values are the mean value of 3 replications). [¥] Values followed by the same letter, within the same row and factor (irrigation dose, ID, or plant density, PD) were not significant different ($p < 0.05$), Tukey's multiple-range test.*

Figure 1. Descriptive sensory analysis of parsley as affected by different irrigation doses (figure 1A) and plant densities (figure 1B).





PUBLICATION 3

Irrigation dose and plant density affect the volatile composition and sensory quality of dill (*Anethum graveolens* L.)

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Irrigation Dose and Plant Density Affect the Volatile Composition and Sensory Quality of Dill (*Anethum graveolens* L.)

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Running title: Volatile profile and sensory quality of dill

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ABSTRACT

BACKGROUND: Two independent field experiments were carried out to investigate the influence of (i) 3 irrigation treatments (ID0 =1585 m^3 ha^{-1}, considered as a control; ID1 = 1015 m^3 ha^{-1}; and, ID2 = 2180 m^3 ha^{-1}), or (ii) 3 plant density treatments (PD0 = 5.56 plants m^{-2}, considered as a control; PD1= 4.44 plants m^{-2}; and, PD2 = 7.41 plants m^{-2}) on the production, volatile composition of essential oil, and sensory quality of dill.

RESULTS: The results showed that the highest plant yield was obtained with intermediate conditions of both irrigation dose (ID0) and plant density (PD0). The main compounds of the essential oil were *α*-phellandrene, dill ether, and *β*-phellandrene. The highest irrigation dose (ID2) produced the highest concentrations of most of the main compounds: *α*-phellandrene (49.5 mg 100 g^{-1}), *β*-phellandrene (6.89 mg 100 g^{-1}) and limonene (2.49 mg 100 g^{-1}); a similar pattern was found for the highest plant density (PD2), *α*-phellandrene (71.0 mg 100 g^{-1}), dill ether (16.7 mg 100 g^{-1}) and *β*-phellandrene (9.70 mg 100 g^{-1}). The use of descriptive sensory analysis helped in reaching a final decision and the dill plants with the highest sensory quality were those of the ID2 and PD0 treatments.

CONCLUSION: The final recommendation is to use the irrigation dose of ID2 and the plant density of PD2 if the objective is to produce dill samples with the highest aromatic and sensory quality; however, if the only objective is to produce high amounts of dill, the best options are ID0 and PD0.

Keywords: *Anethum graveolens* L., descriptive sensory analysis, GC-MS, hydrodistillation, plant yield, volatile compounds

INTRODUCTION

Dill (*Anethum graveolens* L.) is used as fresh herb, spice, and carminative and/or antispasmodic product.[1-3] Dill seeds are usually sown in early spring for seed production.[1,3] The steam-distilled essential oil of European dill is widely used as flavoring for foods and beverages. Most of the oil is produced in the flowers, although leaves and stems also contain oil. A sweet dill herb oil can be steam-distilled from the freshly cut stalks of the plant bearing leaves and flowers, while a seed oil is obtained from the separated dry mature fruits.[4] The best yield of oil, with maximal carvone content, was obtained when the seeds of the primary umbels showed the first red coloration. Limonene and phellandrene were found in stems, leaves, and primary and secondary umbels, but their highest contents were in stems and leaves.[5] Steam distilled plant essential oils have been widely used instead of ordinary culinary herbs not only as food flavoring but also for bactericidal, fungicidal, and medicinal applications and in fragrances.[6,7] The principal components of dill leaf oil were *α*-phellandrene (47.7–62.5%), myristicin (1.7–28.2%), dill ether (0.9–14.8%), *β*-phellandrene (7.4–7.5%), and limonene (3.7–3.8%).[7]

In a large part of the agricultural areas in the world, water is an important factor limiting plant growth and productivity.[3] It is well known that agricultural practices, development stage, and processing have great influence on the composition of vegetables; perhaps, the essential oil content of dill, and its quality, can be increased partly by a moderate water deficit during the vegetative stage (stem elongation).[8] Plants grown at low density had a more extensive development of umbellate fruiting structures and a lower proportion of leaf and stem tissue than plants at high density. One of the initial hypotheses of this study is that a high plant density is desirable if herbaceous oil characteristics (the different phellandrene isomers are described as having minty and herbaceous flavor notes) are desired, while a low plant density is suitable when growing dill for seed or for a high-carvone oil (carvone is described as having caraway, minty and herbaceous flavor notes).

An increase in plant density is expected to result in an essential oil enriched in compounds that are more abundant in leaves and stems (*α*-phellandrene, *β*-phellandrene, and dill ether) than in seeds (carvone).[4]

It is well known that volatiles directly affect the sensory quality of fresh fruits, vegetables and aromatic herbs. Within sensory quality, aroma plays an important role, especially in aromatic herbs; the aroma is formed by a complex group of chemical substances, which includes aldehydes, alcohols, ketones, esters, lactones, terpenes, among other volatile compounds. The concentration of these compounds can be affected by a number of agronomic (variety, climatological conditions, ripening stage or agricultural practices)[9-11] and technological (harvest, post-harvest treatments, storage and processing conditions) factors.[12,13]

Therefore, the aim of this study was to determine the optimal (i) irrigation dose, and (ii) plant density, according to the volatile composition of the essential oil and sensory quality of dill. To reach this aim, gas chromatography-mass spectrometry (GC-MS) and descriptive sensory analysis (DSA) were used together with plant yield at different irrigation or plant density treatments.

MATERIALS AND METHODS

Plant material, irrigation doses and plant density

Dill seeds (*Anethum graveolens* L., cultivar *N3*) were sown on the 18th of September 2014 in expanded polystyrene (EPS) trays (41 cm × 65 cm, with 260 cells) and placed in a greenhouse located at Santomera (Murcia, Spain) until 20th of October 2014. Then, plantlets were transplanted to a commercial dill orchard placed at Sucina (Murcia, Spain) with a total surface of 2.5 ha. Dill plants were grown using high-frequency drip irrigation systems. Plants were harvest on 21-01-2015 for both irrigation and plant density experiments.

Two independent experiments were conducted in this study; the first one evaluated the effects of the *irrigation dose* on the volatile profile and sensory quality of dill, while the second one evaluated the effects of the *plant density* on the same parameters.

Irrigation was carried out according to 3 irrigation doses consisting on the following total water amounts: control treatment (ID0), normal irrigation conditions, with 1585 m^3 ha^{-1}; treatment 1 (ID1), with lower than normal irrigation conditions, 1015 m^3 ha^{-1}; and, treatment 2 (ID2), with higher than normal irrigation conditions, 2180 m^3 ha^{-1}. The plant density of the 3 irrigation treatments was reached by using plant lines separated by 0.9 m and a distance of 0.20 m between plants of the same line, leading to a plant density of 5.56 plants m^{-2} (e.g. 56 plants in a surface of 10 m^2). The total volume of water was settled according to the irrigation time. The water contribution was carried out using polyethylene pipes of 16 mm of diameter. The drippers were adjusted at both different flows and drippers distance to fit the established volume of water. At ID0 irrigation conditions, 16 mm pipes, separated by 0.9 m, were used with a distance between two consecutive drippers of 0.32 m; the flow was 1.6 L h^{-1} for each emitter with an irrigation surface of 0.29 m^2; the total number of drippers per ha was 34722, with a total volume of water of 55.55 m^3 ha^{-1} h^{-1} (~28.5 h of irrigation). ID1 was the lowest irrigation dose and at these conditions, 16 mm pipes, separated by 0.9 m,

were used with an emitter distance of 0.50 m; the flow was 1.6 L h^{-1} for each emitter with an irrigation surface of 0.45 m^2; the total number of emitters per ha were 22222, with a total volume of water of 35.55 m^3 ha^{-1} h^{-1} (~28.5 h of irrigation). ID2 was the highest irrigation dose, and at these conditions, 16 mm pipes, separated by 0.9 m, were used with drippers distance of 0.32 m; the flow was 2.2 L h^{-1} for each emitter with an irrigation surface of 0.29 m^2; the total number of drippers per ha were 34722, with a total volume of water of 76.39 m^3 ha^{-1} h^{-1} (~28.5 h of irrigation). To make reproducibility of the irrigation treatments possible in other climates or regions, the ET_0 (reference evapotranspiration) has been calculated according to Allen et al.[14], and it is provided, together with key climatic parameters, for the 4 months of the experiment (October-2014, November, December, and January-2015):

- ET_0: 83.00, 55.05, 52.66, and 31.95 mm, respectively;
- Accumulated radiation: 426, 283, 291, and 208 MJ m^{-2}, respectively;
- Mean temperature: 20.7, 15.1, 11.3, and 10.4 ºC, respectively;
- Mean wind speed: 1.33, 1.56, 1.83, and 1.33 m s^{-1}, respectively;
- Number of sun hours: 252, 212, 225, and 154 h, respectively; and,
- Vapor pressure deficit: 1.08, 0.61, 0.59, and 0.63 kPa, respectively.

Regarding plant density, 3 treatments were assayed. The water contribution was carried out using polyethylene pipes of 16 mm of diameter, with a distance between drippers of 0.33 m. The flow was 1.6 L ha^{-1} for each emitter, making a total volume of water applied of 1290 m^3 ha^{-1}, according to the irrigation time. Plant densities under study were as follow: (i) control treatment, PD0 (5.56 plants m^{-2}), or normal plant density, with plant lines separated by 0.9 m, a distance of 0.20 m between plants of the same line; (ii) treatment 1, PD1 (4.44 plants m^{-2}), with plant lines separated by 0.9 m, a distance of 0.25 m between root balls; and, (iii) treatment 2, PD2 (7.41 plants m^{-2}), with lines separated by 0.9 m, 0.15 m between root balls. Thirty-six plants of each treatment were assayed in the following surfaces: PD0 required a total surface of 6.48 m^2, PD1 surface was of

8.10 m^2, and finally PD2 needed a total surface of 4.86 m^2. All the field treatments were run in triplicate.

The irrigation water was of good quality, highlighting its slightly basic pH (7.91), and its proper electrical conductivity (1.26 mS cm^{-1}), which is suitable for aromatic herbs crops. Soil was uniformly silty-loam in texture, with a low content in organic matter (1.22%), medium salinity conditions (3.35 mS cm^{-1}) and good levels of sulfates (37.83 meq L^{-1}) for dill development. Along the development of dill plants, fertilization was carried out with a total amount of N of 130 kg ha^{-1}, P (P_2O_5) of 60 kg ha^{-1}, and K (K_20) of 160 kg ha^{-1}.

Extraction of essential oil

Hydrodistillation (HD), using a Deryng system (the Polish version of the Clevenger apparatus), was used for isolating the essential oil in fresh dill. About 15.0 g of fresh chopped dill shoots (aerial part of the plant, including stems and leaves) were put in a 500 mL round bottom flask, together with 1.0 g sodium chloride (NaCl), 150 mL of distilled water, and 50 µL of benzyl acetate as internal standard. After the mixture started boiling, heating was maintained for 1 h. A cold refrigerant, consisting of ethanol kept at -3 ºC, was used to condense the vapors, and 1 mL of cyclohexane was added to the Deryng apparatus at the beginning of the hydrodistillation process to retain the essential oil distilled from the samples of dill shoots. After 60 min of extraction, the solvent, enriched with the volatile compounds, was transferred into a 2.5 mL vial, after drying it over anhydrous sodium sulfate (Na_2SO_4), and kept at -15^{0}C until the GC-MS analyses were conducted. The extractions were conducted in triplicate.

Chromatographic analyses

Isolation and identification of the volatile compounds were performed using a Shimadzu GC-17A gas chromatograph coupled with a Shimadzu QP-5050A mass spectrometer detector (Shimadzu Corporation, Kyoto, Japan). The GC-MS system

was equipped with a TRACSIL Meta.X5 (95% dimethylpolysiloxane and 5% diphenylpolysiloxane) column (60 m × 0.25 mm, 0.25 µm film thickness; Teknokroma S. Coop. C. Ltd, Barcelona, Spain). Analyses were carried out using helium as carrier gas at a flow rate of 0.3 mL min^{-1} in a split ratio of 1:11 and the following program: (a) 80 ºC for 0 min; (b) increase of 3 ˚C min^{-1} from 80 to 210 ºC and hold for 1 min; (c) increase of 25 C min^{-1} from 210 to 300 ^{0}C and hold for 3 min. The temperatures of the injector and detector were 230 and 300 ^{0}C respectively.

Most compounds were identified using three different analytical methods: (1) retention indexes (RI); (2) GC-MS retention times (authentic standards of all compounds reported were used for identification purposes); and, (3) mass spectra (authentic chemicals and NIST05 spectral library collection).

The semi-quantification of the volatile compounds was performed on a gas chromatograph, Shimadzu 2010, with a flame ionization detector (FID). The column and chromatographic conditions were those previously reported for the GC-MS analysis. The injector temperature was 250 ºC and N_2 was used as carrier gas (1 mL min^{-1}). Data handling was carried out by means of GCsolution 2.3 (Shimadzu).

For the semi-quantification of the volatile compounds, benzyl acetate was added as internal standard at a concentration of ~1.0 g L^{-1} of chloroform (50 µL); this chemical was used as internal standard after checking that it was absent in the extracts of parsley and under the proposed conditions, it separates well from other volatile compounds. Data included in this study should be considered as semi-quantitative, because no standard curves were carried out for each one of the quantified volatile compounds. However, relative values are useful to compare differences among farming treatments.

Sensory evaluation with a trained panel

A trained panel was used to quantify the intensity of the main aroma attributes of fresh dill. Samples were evaluated by 7 panelists (5 males and 2

females), with ages between 23 and 56 years old. Panelists belonged to the Food Quality and Safety research group of the *Universidad Miguel Hernández de Elche* and had over 500 h of evaluation experience; they had been trained in descriptive evaluation of aromatic herbs.[15,16] An odor profile method was used to describe the dill samples. During two preliminary orientation sessions of 90 min, panelists discussed about the main odor characteristics of dill and agreed on their use of odor attributes. During these orientation experiments, panelists evaluated different coded samples of Spanish fresh dill together with samples from field. Panelists agreed that the odor of dill samples could be described using 7 attributes: dill-like, citrus, green grass, pine, earthy, spicy, and woody. Reference products of these attributes with different intensity, were prepared and provided to the panel.

Individual booths with controlled illumination and temperature were used in this study.[17] Three digit numbers were used to code samples, and they were randomly offered to panelists in plastic beakers of 100 mL with lids; samples were left 15 min at room temperature prior to analyses.

The intensity of the 7 odor attributes was scored using scale from 0 to 10, where 0 = none or not perceptible intensity, and 10 = extremely high intensity.

Statistical analysis

To compare the experimental data two consecutive tests were performed: (i) one-way analysis of variance (ANOVA), and (ii) Tukey's multiple range. Homogenous groups and the least significant difference (LSD) were determined at significance level of $p \leq 0.05$. Statgraphics Plus 5.0 software (Manugistics, Inc., Rockville, MD, U.S.A.) was the program used for the statistical analyses.

RESULTS AND DISCUSSION

Yield and plant morphology

Exposure of dill plants to 3 different levels of "irrigation dose, ID" caused a significant (p<0.001) change in the yield (t ha^{-1}), length of the main stem (cm), and main stem weight (g) of dill (**Table 1**). When the total amount of water was decreased, from 1585 m^3 ha^{-1} (control treatment, ID0) to 1015 m^3 ha^{-1} (ID1), the yield, stem length, and stem weight also decreased. However, an increase of the irrigation dose to 2180 m^3 ha^{-1} (ID2), as compared to ID0, also resulted in a reduction of plant yield and stem length (**Table 1**). From these data, it can be stated that the highest production or yield of dill plants (25.51 t ha^{-1}) can be reached by adjusting the irrigation dose up to a level of 1585 m^3 ha^{-1}, under the studied conditions (e.g. 5.56 plants m^{-2}). This result agrees with the findings of Islam *et al*.[18] and Hassan and Ali.[19] For example, an increase in irrigation water [from 40% to 120% of ETP (potential evapotranspiration)], also improved the dry matter content and yield of *Coriandrum sativum* L. plants.[19] In any case, it is essential to state that the volume of irrigation water cannot be increased unlimited, and the maximum threshold should be established at the ID0 level. In fact, this is the dose being applied by many of the most important and big farms in the Mediterranean region of Spain.

In the second experiment, three different quantities of dill seeds (PD0, PD1, and PD2 with 5.56, 4.44, and 7.41 plants m^{-2}, respectively) were sown to study the effect of the "plant density, PD" on plant yield and the morphology of dill plants. PD0 produced the highest yield (27.12 t ha^{-1}) and stem length (32.0 cm), while PD1 led the highest leaves weight (2.30 g) (**Table 1**).

Regarding plant density, it must be mentioned that there is a strong effect of on dill yield and plant morphology. The current results reported that plant yield was maximum at a plant density of 5.56 plants m^{-2} (PD0); this statement agrees quite well with previous results obtained by Callan *et al*.[4] who stated that largest dill plants were found under low plant density. Besides, the PD0 treatment led to the

tallest plants but not to those with the heaviest stems, which were found at a lower plant density (PD1). These experimental observations meant that PD0 plants produced more leaves than stems as compared to PD1 and PD2 plants.

Volatile composition of essential oil

Essential oil of the aerial portion of dill plants (shoots) was obtained by hydrodistillation. This distillation technique, using a Deryng apparatus, has been successfully used in previous studies to extract the essential oils of different aromatic herbs, such as marjoram,[16] thyme, rosemary, basil, and oregano.[20-23]

After isolation of the essential oil of dill shoots, 18 compounds were identified by GC-MS, using retention times, mass spectra, retention indexes, and pure standards (**Table 2**). The identified volatile compounds can be grouped in 6 main chemical groups: *monoterpenes* (11 compounds), *monoterpenoids* (2 compounds), *alkanes* (2 compounds), *sesquiterpenes* (1 compound), *aldehydes* (1 compound), and *phenylpropanoids* (1 compound) (**Table 3**). The 7 main compounds were: *α*-phellandrene (47.0±3.6 mg 100 g^{-1}, mean of all treatments), dill ether (8.4±1.2 mg 100 g^{-1}, mean of all treatments), *β*-phellandrene (6.2±1.1 mg 100 g^{-1}, mean of all treatments), limonene (2.2±0.7 mg 100 g^{-1}, mean of all treatments), *p*-cymene (1.9±0.6 mg 100 g^{-1}, mean of all treatments), *α*-pinene (0.8±0.2 mg 100 g^{-1}, mean of all treatments), and *trans*-*β*-ocimene (0.8±0.2 mg 100 g^{-1}, mean of all treatments). In several studies, *α*-phellandrene was predominating in the “leaf” essential oil of dill from Romania,[24,25] Egypt,[26] and Finland.[27] Other separations techniques were also applied to isolate the dill essential oils. For example, Huopalahti and Linko [27] found 22 compounds in dill by using a modified Soxhlet technique; *α*-phellandrene, dill ether, *β*-phellandrene were also reported as the major compounds.

Table 3 shows that 3 compounds (*α*-phellandrene, dill ether, and *β*-phellandrene) clearly dominated the dill shoots essential oil, representing 85–90% of the total concentration of volatile compounds. This experimental finding is

supported as well by previous studies[7,24] by Vokk *et al*. and Radulescu *et al*., who reported that *α*-phellandrene, *β*-phellandrene, and dill ether were the main compounds of dill essential oil. Pino *et al*. and Callan *et al*.[4,28] reported that the properties of dill oil depend largely on the proportions of carvone and α-phellandrene. The odor of carvone is described as caraway-like and cooling, while α-phellandrene can be described as dill-like, fragrant, and fresh. In this way, the characteristic dill aroma of this essential oil predominates if the carvone content is less than 35% lead, above that content the caraway notes predominate.[28,4] In the samples under analysis in this study, the carvone content was very low, below 0.2 mg 100 g^{-1}, thus the predominating note was dill-like.

The "irrigation dose" significantly affected the concentrations of individual volatile compounds of dill essential oil. In general, the highest irrigation dose ID2 led to essential oils with the highest concentrations of most of the key compounds: *α*-phellandrene (49.5 mg 100 g^{-1}), *β*-phellandrene (6.89 mg 100 g^{-1}), limonene (2.49 mg 100 g^{-1}), *p*-cymene (2.89 mg 100 g^{-1}), *α*-pinene (0.95 mg 100 g^{-1}), and *trans*-*β*-ocimene (0.88 mg 100 g^{-1}). In addition to that, dill plants irrigated with the ID0 dose had higher concentrations for most of the compounds than ID1 plants; with the exception of *p*-cymene, myrcene, undecane, and myristicin. It is important to mention that the contents of several compounds were not significantly influenced by the irrigation dose treatments; these compounds included sabinene, *β*-pinene and terpinolene, and of especial importance were dill ether and carvone (key compounds for the dill essential oil odor/aroma/flavor) (**Table 3**).

Khamssi[8] studied the influence of water deficit on the essential oil content of dill and found that it can be increased by a moderate water deficit during the vegetative stage (stem elongation). Similar results have been reported by other authors[29-32] stating that water stress led to increases in the essential oil content of different plants, such as sweet basil (*Ocimum basilicum*), savory (*Satureja hortensis*), lavender (*Lavandula officinalis*) and absinthium (*Artemisia absinthium*). These results do not agree with those found in the current study, where an increase

in the irrigation dose resulted in increased of concentrations of most of the volatiles compounds, leading to the highest total concentration (73.3 mg kg^{-1}) (**Table 3**).

On the other hand, Singh and Ramesh,[33] Zehtab-Salmasi *et al*.,[34] and Petropoulos *et al*.[35] reported that water deficit decreased the oil yield of rosemary (*Rosmarinus officinalis* L.) and anise (*Pimpinella anisum* L.); these results in two aromatic herbs are comparable to the results obtained here for dill.

The "plant density" significantly affected the concentrations of individual volatile compounds of dill essential oil (**Table 3**). The highest plant density PD2 gave the highest concentrations of most of the key compounds, including *α*-phellandrene (71.0 mg 100 g^{-1}), dill ether (16.7 mg 100 g^{-1}) *β*-phellandrene (9.70 mg 100 g^{-1}), limonene (3.35 mg 100 g^{-1}), *p*-cymene (3.00 mg 100 g^{-1}), *α*-pinene (1.20 mg 100 g^{-1}), and *trans*-*β*-ocimene (0.94 mg 100 g^{-1}). One of the initial working hypothesis of this study was that an increase in plant density will create a water deficit, and, then, the plant will react by activating defense or adaptation mechanisms increasing the concentrations of volatile compounds; however, this hypothesis was true for the most intense treatment (PD2), but the opposite trend was observe for the PD1 dill plants. In this way, the concentrations of several compounds were higher in the PD1 treatment (4.44 plants m^{-2}) as compared to control treatment (PD0=5.56 plants m^{-2}); these compounds included *α*-phellandrene, limonene, *β*-phellandrene, and germacrene-D (**Table 3**).

Callan *et al*.[4] investigated the effect of plant density on the composition of dill essential oil, and the results showed that the higher the plant density, the higher the content of essential oil and especially the content of phellandrene (*α* and *β*). These results agreed well with the trend showed by dill plants from the PD2 treatment as compared to PD0 and PD1.

A possible explanation for the trend observed when comparing the compositions of PD0 and PD1 plants is that the PD0 plant density (5.56 plants m^{-2}) is high enough to slightly reduce the generation of volatile compounds, but not high enough to trigger the defensive/adaptive mechanisms of the dill plant. In this way,

it can be stated the PD0 plant density only creates “soft or moderate” water and nutrients deficit, but the PD2 plant density (7.41 plants m^{-2}) is intense enough to create “strong” water and nutrients stresses in the dill plants and they react by increasing the generation of bioactive compounds to improve their adaptive strategies against the stress. Up to now, no explanation has been stated in previous studies providing plausible explanations for reported relationships among plant density, irrigation dose, and content or composition of herbs essential oils. Consequently, more interdisciplinary research is needed to find acceptable justification. However, this is outside the aim of this study, which was to optimize the irrigation dose and the plant density in order to get the highest concentrations of the main components of essential oil in dill shoots.

To finish the discussion of the sections on yield and volatile compounds of dill plants as affected by irrigation dose and plant density, the “yield of total volatile compounds” [volatile yield= plant yield (**Table 1**) × total concentration of volatile compounds (**Table 3**)] by these treatments was calculated. This parameter (yield of total volatile compounds) has been evaluated instead of the essential oil content because the dill plants are intended for fresh consumption; if the dill was intended for industrial production, essential oil content should be evaluated and will be in a near future. The mean values of the total amount of volatile compounds were: 15.97, 8.75, and 15.00 kg ha^{-1} for ID0, ID1, and ID2, respectively, and 15.54, 17.40, and 18.89 kg ha^{-1} for PD0, PD1, and PD2, respectively. According to these data, the best irrigation dose was ID0, because it led simultaneously to the highest plant yield (25.51 t ha^{-1}) and to the highest yield of total volatile compounds (15.97 kg ha^{-1}). The situation was not so clear for the plant density, because PD0 led to the highest plant yield (27.12 t ha^{-1}) but PD2 led to the highest yield of total volatile compounds (18.89 kg ha^{-1}). With this situation, perhaps PD1 might also be a good option because has a quite high plant yield (25.44 t ha^{-1}) and an intermediate volatile yield, 17.40 kg ha^{-1}.

Descriptive sensory evaluation

Figure 1 shows the DSA (descriptive sensory analysis) profiles of different dill samples. The descriptors selected for the DSA of dill were previously used while studying the best drying method in other herbs, such as marjoram,[16] rosemary,[15] basil,[22] and thyme.[20]

Dill samples grown under the highest irrigation dose (ID2) and plant density (PD2) were characterized by high intensity of dill-like or dill-ID (6.5 and 6.5, respectively), citrus (2.0 and 2.5, respectively), and green grass (6.0 and 8.5, respectively) notes (**Figure 1A** and **1B**). These samples were those also having the highest total content of volatile compounds (**Table 3**). On the other hand, dill samples with intermediate irrigation dose (ID0) or plant density (PD0) increased the scores of attributes such as spicy, earthy or woody (**Figure 1A and 1B**).

In general, the "irrigation dose" and "plant density" significantly affected the intensities of the key sensory attributes of dill shoots. Attributes, such as dill-like, citrus, and green grass significantly increased ($p<0.01$) as the irrigation dose and plant density increased; these are the key sensory attributes to describe the quality of dill. The trends of the other attributes was not so clear, although and in general, the attributes spicy, earthy, and woody increased as the irrigation dose decreased.

The present study was the first one reporting data on descriptive sensory analysis of dill as affected by different agricultural practices. Sensory data confirmed that ID2 was the best one because led to dill plants with the highest intensities of the key attributes (dill-like, citrus, and green grass). Regarding "plant density" even though PD2 plants presented the best sensory profiles, PD0 plants presented statistically equivalent intensities of dill-like, citrus, and green grass notes and even slightly higher intensity of the spicy note as compared to PD2 dill samples. Consequently and considering that the plant yield of PD0 plants was much higher than that of PD2 plants, the final recommendation regarding plant density is that the best option for this parameter is PD0 (5.56 plants m^{-2}).

CONCLUSION

The main purpose of this study was to optimize two key farming practices: (i) irrigation dose, and (ii) plant density, in dill intensive orchards. This optimization process was based on plant yield but also on the quality of dill (volatile composition of essential oil and sensory quality). The highest plant yields were obtained when using ID0 (1585 m^3 ha^{-1}) and PD0 (5.56 plants m^{-2}). The highest total concentrations of volatile compounds were found in ID2 (2180 m^3 ha^{-1}) and PD2 (7.41 plants m^{-2}). The use of descriptive sensory analysis helped in reaching a final decision and the dill plants with the highest sensory quality were those of the ID2 and PD0 treatments (statistically equivalent to PD2). Considering all the data generated in this study, the final recommendation is to use: (i) the irrigation dose of 2180 m^3 ha^{-1} (ID2) at the applied plant density of 5.56 plants m^{-2}, and (ii) the plant density of 5.56 plants m^{-2} (PD0) at the applied irrigation dose of 1290 m^3 ha^{-1}, if the objective is to produce dill samples with the highest aromatic and sensory quality. However, if the only objective is to produce high amounts of dill, the best options are ID0 (1585 m^3 ha^{-1}) and PD0 (7.41 plants m^{-2}).

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Table 1. Yield (kg ha^{-1}), average stem length (cm) and average stem weight (g) of dill as affected by irrigation dose and plant density.

Treatment	Yield (t ha^{-1})	Stem length (cm)	Stem weight (g)
Irrigation Dose, ID			
ID0	25.51 b[ξ]	29.8 a	1.62 a
ID1	17.61 a	26.6 a	1.05 b
ID2	20.47 ab	27.5 a	1.62 a
ANOVA[†]	***	NS	***
Plant Density, PD			
PD0	27.12 a	32.0 a	1.72 b
PD1	25.44 a	31.6 a	2.30 a
PD2	17.49 b	24.7 b	0.91 c
ANOVA[†]	***	***	***

*[†] NS = not significant F ratio ($p < 0.05$); *, **, and ***, significant at $p < 0.05$, 0.01, and 0.001, respectively. [ξ] Values followed by the same letter, within the same column and factor (irrigation dose, ID, or plant density, PD), are not significant different ($p < 0.05$), Tukey's multiple-range test.*

Table 2: Identification of essential oils found in dill samples

Compound	Retention time (min)	Retention Indexes (RI)		Descriptor‡
		Exp.†	Lit.†	
trans-2-Hexenal	11.09	806	800	Almond, apple, green, plum, sweet
α-Thujene	13.19	899	905	Woody, green, herb
α-Pinene	13.58	906	909	Camphor, sweet, pine, earthy, woody
Sabinene	14.89	975	975	Woody, terpene, citrus, pine, spice
Myrcene	15.15	991	991	Peppery, terpene, spicy, balsam, plastic
β-Pinene	15.25	997	990	Dry, woody, resinous, pine, hay, green
α-Phellandrene	16.20	1020	1013	Citrus, herbal, terpene, green, woody, peppery
p-Cymene	16.88	1036	1034	Citrus, terpene, woody, spice
Limonene	17.08	1040	1039	Terpene, pine, herbal, peppery
β-Phellandrene	17.25	1044	1034	Mint, turpentine
trans-*β*-Ocimene	17.38	1047	1047	Citrus, tropical, green, terpene, woody
Terpinolene	19.47	1095	1097	Fresh, woody, sweet, pine, citrus
Undecane	19.63	1100	1100	
Dill ether	24.40	1196	1187	Herbal, dill, spicy
Carvone	27.13	1264	1262	Herbaceous, grapefruit, pepper, spicy, woody
Tridecane	29.06	1300	1300	
Germacrene-D	38.42	1510	1516	Woody, spice
Myristicin	39.97	1535	1533	Spice, warm balsam, woody

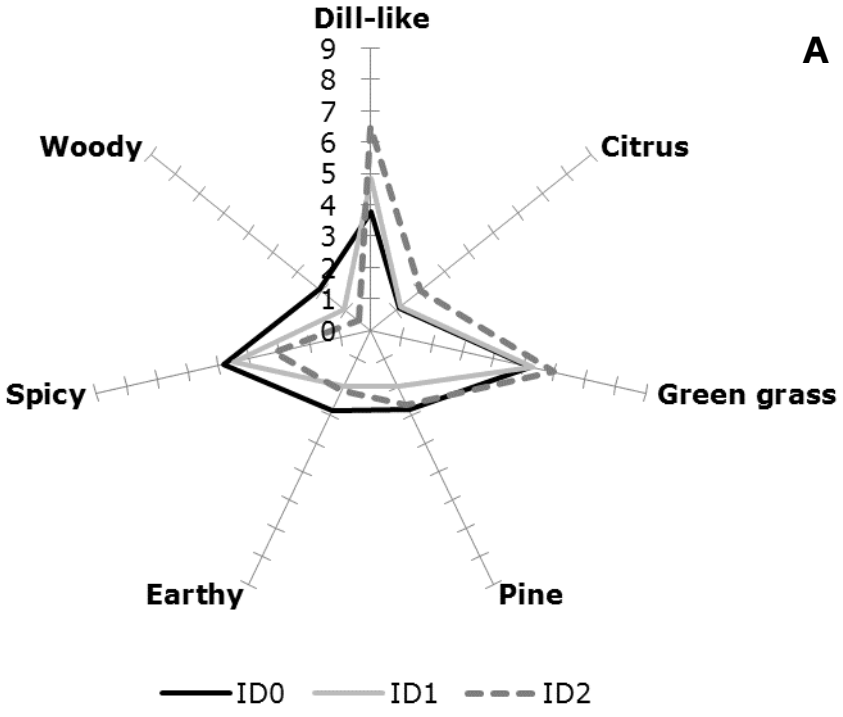
† *RT=retention time; Exp.=experimental and Lit.=Literature;* ‡ *SAFC (2012) and The Good Scents Company (2016).*

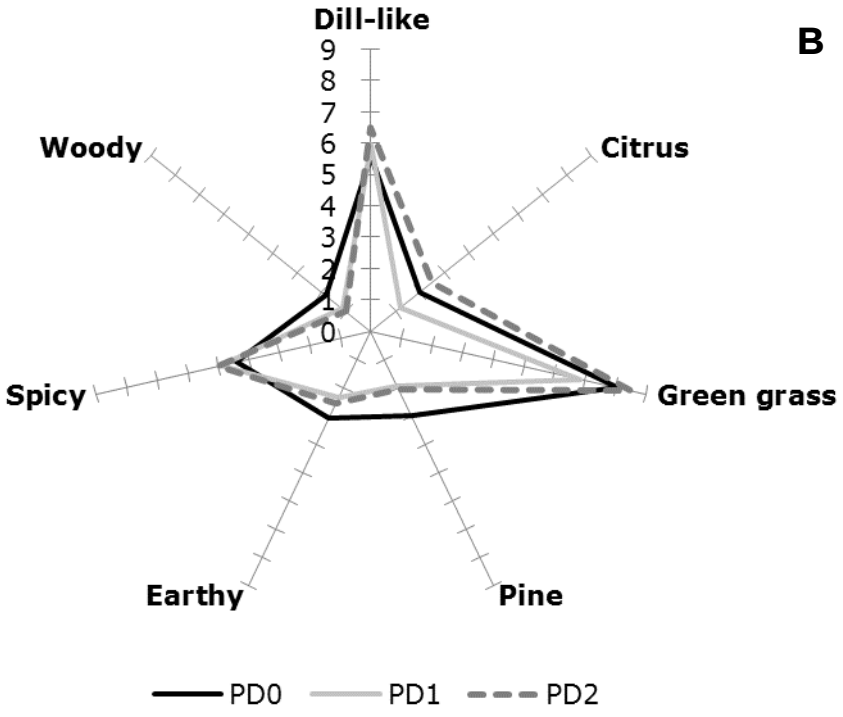
Table 3. Concentration (mg kg^{-1}) of dill shoots affected by different conditions of irrigation dose (ID) and plant density (PD)

Compound	ANOVA†	Irrigation Dose, ID			ANOVA†	Plant Density, PD		
		ID0	ID1	ID2		PD0	PD1	PD2
		Concentration (mg 100 g^{-1})				Concentration (mg 100 g^{-1})		
trans-2-Hexenal	***	0.62 a¥	0.43 b	0.15 c	***	0.52 a	0.41 b	0.29 c
α-Thujene	***	0.11 b	0.10 b	0.20 a	***	0.12 b	0.14 b	0.21 a
α-Pinene	***	0.60 ab	0.49 b	0.95 a	***	0.62 b	0.81 b	1.20 a
Sabinene	NS	0.04	0.04	0.06	NS	0.05	0.05	0.07
Myrcene	***	0.14 b	0.21 ab	0.37 a	***	0.28 b	0.30 b	0.43 a
β-Pinene	NS	0.06	0.05	0.07	NS	0.04	0.03	0.04
α-Phellandrene	***	44.8a	31.7 b	49.5 a	***	37.4 c	47.6 b	71.0 a
p-Cymene	***	1.35 b	2.03 a	2.89 a	***	1.32 b	1.14 b	3.00 a
Limonene	***	1.55 b	1.54 b	2.49 a	***	1.86 c	2.20 b	3.35 a
β-Phellandrene	***	4.50 b	4.39 b	6.89 a	***	5.24 c	6.24 b	9.70 a
trans-*β*-Ocimene	***	0.60 b	0.54 b	0.88 a	***	0.66 b	0.88 b	0.94 a
Terpinolene	NS	0.31	0.24	0.29	NS	0.05	0.05	0.05
Undecane	***	0.18 b	0.28 b	0.44 a	***	0.30 b	0.35 a	0.29 b
Dill ether	NS	6.33	6.05	5.98	***	7.84 b	7.44 b	16.7 a
Carvone	***	0.02 a	n.d.	0.04 a	***	n.d.	n.d.	0.18 a
Tridecane	***	0.06 b	0.06 b	0.19 a	NS	0.05	0.02	0.03
Germacrene-D	***	0.62 a	0.60 a	0.37 b	***	0.49 b	0.62 a	0.049 b
Myristicin	***	0.71 b	0.93 ab	1.49 a	***	0.31 a	0.15 b	0.05 c
TOTAL	*******	**62.6b**	**49.7c**	**73.3a**	*******	**57.3c**	**68.4b**	**108a**

† *NS = not significant F ratio ($p < 0.05$); *, **, and ***, significant at $p < 0.05$, 0.01, and 0.001, respectively.* ‡ *Treatment means of the ANOVA test (values are the mean value of 3 replications).* ¥ *Values followed by the same letter, within the same row and factor (irrigation dose, ID, or plant density, PD) are not significant different ($p < 0.05$), Tukey's multiple-range test.*

Figure 1. Descriptive sensory analysis of dill as affected by different irrigation doses (figure 1A) and plant densities (figure 1B).





PUBLICATION 4

Preharvest treatments with malic, oxalic and acetylsalicylic acids affect the phenolic composition and antioxidant capacity of coriander, dill and parsley

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Preharvest Treatments with Malic, Oxalic, and Acetylsalicylic Acids Affect the Phenolic Composition and Antioxidant Capacity of Coriander, Dill and Parsley

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Running title: Elicitation of aromatic herbs

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ABSTRACT:

The effects of a preharvest treatment with malic (MA), oxalic (OA), or acetylsalicylic (ASA) acid at three concentrations (1, 2 and 3 mM) on the bioactivity and antioxidant capacity of coriander, dill, and parsley were investigated. The antioxidant capacity of the herbs extracts was assayed by spectrophotometric methods by using three different analytical methods: ORAC, FRAP, and ABTS; the effects of treatments were very positive in coriander, produced intermediate results in dill, and no effects were found in parsley plants. Polyphenol compounds were identified by LC-MS-QTof and quantified by UPLC-PDA-FL. Thirty phenolic compounds were identified in these three herbs. The major compounds were (i) *coriander*: dimethoxycinnamoyl hexoside and quercetin-3-*O*-rutinoside, (ii) *dill*: neochlorogenic acid and quercetin glucuronide, and (iii) *parsley*: apigenin-7-apiosylglucoside (apiin) and isorhamnetin-3-*O*-hexoside. The application of these three organic acids favored the accumulation of phenolic compounds in coriander plants, but had no significant positive effects on dill and parsley. The treatments leading to the best results in all three plants were the application of MA or OA at 1 mM.

KEYWORDS: aromatic herbs, bioactive compounds, organic acids, preharvest treatments, LC-MS-QTof, UPLC-PDA-FL.

INTRODUCTION

Phenolic compounds constitute a major class of secondary plant metabolites. They include phenolic acids and flavonoids, and particularly the latter is a highly diverse subgroup. Phenolic compounds are present in fruits, vegetables and other plant products consumed in a normal diet (Justesen and Knuthsen 2001).

Herbs and spices have been historically used as food additives to preserve food, flavor dishes, and even for medicinal purposes. They have been reported as an excellent source of phenolic compounds and, therefore, they have been considered as potential antioxidant additives (Kaefer and Milner 2008, Gawlik-Dziki 2012; Pereira and Tavano 2014). Crude extracts of herbs and spices, and other plant materials rich in phenolics, are of increasing interest in the food industry because they retard oxidative degradation of lipids, and, thereby they improve the quality and nutritional value of food (Wojdyło, Oszmiański & Czemerys, 2007). The presence of flavonoids and other phenolic antioxidants, such as rosmarinic acid, has been reported in herbs (Justesen 2000). For instance, in coriander leaves, the flavonoids: quercetin, kaempferol, and acacetin were identified, together with the phenolic acids: vanilic, ferulic and *p*-coumaric (Nambiar, Daniel & Guin, 2010). Also, four flavone glycosides: apigetrin, apiin, diosmetin *7-O*-β-D-glucopyranoside, and kaempferol-*3-O*-β-glucopyranoside, were isolated from the extracts of the *parsley* shoots (*Petroselinum crispum*) (Yoshikawa, Uemura, Shimoda, Kishi, Kawahara & Matsuda, 2000). Moreover, in *dill* (*Anethum graveolens*) extracts, isorhamnetin, kaempferol, and quercetin were detected as glucuronyl conjugates and quercetin-rhamnoglucoside was also detected as a minor component (Justesen 2000). A great number of medicinal plants, rich in flavonoids, has been reported as having antibacterial, anti-inflammatory, anti-allergic, anti-mutagenic, antiviral, anti-neoplastic, anti-thrombotic, and vasodilatory activity (Miller, 1996; Abdulmanea, Prokudina, Lanková, Vaníčková, Koblovská, Zelený & Lapčík, 2012). Natural antioxidants,

such as flavonoids, are associated with a reduced risk of: cancer, chronic inflammation, and cardiovascular disease (Middleton & Kandaswami, 1994; Proestos, Sereli & Komaitis, 2006).

Enhancement of secondary metabolites by *elicitation* is one of the few strategies that have recently found commercial application. Elicitors are compounds, of microbial origin or non-biological origin, which upon contact with higher plant cells trigger the increased production of pigments, flavones, phytoalexins, and other defense related compounds (Bagherifard, Bagheri, Sabourifard, Bagherifard & Najar, 2015). For example, antioxidant enzyme activities and proline content were enhanced in ginger plants treated with a low concentration (10^{-5} M) of salicylic acid (Ghasemzadeh and Jaafar 2013). Martínez-Esplá, Zapata, Valero, García-Viguera, Castillo & Serrano (2014) investigated the effect of preharvest application of oxalic acid on bioactive compounds, and antioxidant capacity in sweet cherry cultivars and they reported that at harvest, the content of bioactive phenolics, including anthocyanins, flavonols, and chlorogenic acid derivatives were significantly higher in fruits from treated trees as compared to control fruits. Moreover, Bagherifard et al. (2015) reported that a treatment with salicylic acid caused significant increases of phenolic compounds, including flavonoids, in the leaves of artichoke plants; the recommended level leading to the highest accumulation of phenolics, was 1 mM.

However, no literature is available regarding the effects of preharvest treatments with organic acids as elicitors on the content of phenolic compounds in commercial fields of herbs. Consequently, the main aim of this study was to evaluate the preharvest treatments of malic, oxalic, or acetylsalicylic acid on the antioxidant and phenolic profile and contents in three herbs (dill, coriander, and parsley), grown under commercial plant density and irrigation strategies.

MATERIALS AND METHODS

Chemicals

The following chemicals of UPLC and LC/MS purity were bought from Sigma-Aldrich (Steinheim, Germany): acetonitrile, methanol formic acid, leucine enkephalin, 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), 6-hydroxy-2,5,7,8-tetra-methylchroman-2-carboxylic acid (Trolox), 2,4,6-tri(2-pyridyl)-*s*-triazine (TPTZ), and ammonium formate, whereas some standards of flavonol glycosides such as quercetin-3-*O*-rutinoside, quercetin-3-*O*-glucuronide, kaempferol-3-*O*-rutinoside, isorhamnetin-3-*O*-rutinoside, isorhamnetin-3-*O*-galactoside, *p*-coumaric acid, sinapic acid, chlorogenic acid, neochlorogenic acid, and cryptochlorogenic acid were bought from Extrasynthese (Lyon, France) and TRANS MIT GmbH (Giessen, Germany). An HLP SMART 1000s system (Hydrolab, Gdańsk, Poland) was used to prepare UPLC-grade water, this water was immediately filtered through a 0.22 µm membrane before use.

Plant material and preharvest treatments

Three aromatic herbs (coriander, dill, and parsley) were grown under conventional "commercial" agricultural conditions, typical of fields located in the Mediterranean region of Spain. These conditions are described below.

Coriander seeds (*Coriander sativum,* cultivar "*Marino*") were directly sown on 25 December 2015 on a commercial orchard placed at Librilla (Murcia, Spain). The plant density was reached by using plant lines separated by 1.50 m and a distance of 0.07 cm between plants of the same line. Coriander plants were grown using high frequency drip irrigation systems. The water contribution was carried out using polyethylene pipes of 16 mm of diameter. The drips were adjusted at 32 cm of distance, with a total flow of 1.6 L h^{-1}. The total volume of water for coriander was 1507 m^3 ha^{-1}.

Dill seeds (*Anethum graveolens* L., cultivar "*Ella*") were sown on 4 December 2015 in expanded polystyrene (EPS) trays, and placed in a

greenhouse located at Santomera (Murcia, Spain), until January 2016. Then, plantlets were transplanted into a commercial orchard placed at Sucina (Murcia, Spain). The plant density was reached by using plant lines separated by 0.50 m and a distance of 0.20 cm between plants of the same line. Dill plants were grown using high frequency drip irrigation systems. The water contribution was carried out using polyethylene pipes of 16 mm of diameter. The drips were adjusted at 32 cm of distance, with a total flow of 1.6 L h^{-1}. The total volume of water for coriander was 1954 m^3 ha^{-1}.

Parsley seeds (*Petroselinum crispum,* cultivar "*Gigante Italiano Darkness*") were sown on 25 November 2015 in expanded polystyrene (EPS) trays, and placed in a greenhouse located at Santomera (Murcia, Spain), until 30 December 2015. Then, plantlets were transplanted into a commercial orchard placed at Sucina (Murcia, Spain). The plant density was reached by using plant lines separated by 0.50 m and a distance of 0.20 cm between plants of the same line. Parsley plants were grown using high frequency drip irrigation systems. The water contribution was carried out using polyethylene pipes of 16 mm of diameter. The drips were adjusted at 32 cm of distance, with a total flow of 1.6 L h^{-1}. The total volume of water for coriander was 2062 m^3 ha^{-1}.

Along the development of the crops, fertilization was carried out using the following amounts: (i) *coriander*: a total of 135 kg N ha^{-1}, 75 kg P ha^{-1} (P_2O_5), and 150 kg K ha^{-1} (K_2O); (ii) *dill*: 176 kg N ha^{-1}, 97 kg P (P_2O_5) ha^{-1}, and 195 kg K (K_2O) ha^{-1}; and, (iii) *parsley*: 185 kg N ha^{-1}, 100 kg P ha^{-1} (P_2O_5), and 205 kg K ha^{-1} (K_2O).

The water which was employed for irrigation has the following specifications: pH (7.9), and electrical conductivity (1.26 mS cm^{-1}); while the soil was uniformly silty-loam with medium salinity (3.35 mS cm^{-1}), low organic matter content (1.22 %) and good level of sulphates (37.83 meq L^{-1}). Both water and soil characteristics are suitable for aromatic herbs crops.

The application of malic (MA), oxalic (OA), or acetylsalicylic acids (ASA), as a preharvest treatment, was carried out simultaneously, using the same concentrations and under the same conditions for the three crops under study. These conditions were as follow: the application was carried out on 6 March 2016. Forty plots of 1 m^2 were randomly selected for each acid treatment and crop. Three concentrations (1, 2 and 3 mM) and 4 replicates of each acid were assayed. The remaining 4 plots were used as control treatments. The different organic acids doses were applied by foliar pulverization with a total volume of 1000 L ha^{-1}, and therefore 100 cm^3 of each concentration was applied in each plot of 1 m^2. The harvest of the three herbs was done on 13 March 2016.

The aerial parts of coriander, dill and parsley (leaves and stems) were used for all analyses conducted in the present study.

UPLC-PDA−Q/TOF-MS analysis, identification of phenolic compounds

According to previous study by Wojdyło, Oszmiański & Laskowski (2008) and Wojdyło, Oszmianski & Bielicki (2013), the extraction of phenolic compounds was performed. Briefly, grounded freeze-dried vegetative parts (leaves and stems) (~1 g) were weighed into a test tube. A total of 20 mL of 80% of aqueous methanol with 1% of HCl was added, and the suspension was slightly stirred. Tubes were sonicated for 15 min twice and left for 24 h at room temperature in darkness (~20 °C). The extract was centrifuged for 10 min (10 min, 20.878×*g*), and supernatants were collected at 4 °C, and were analyzed within 24 h. An ACQUITY ultra-performance LC system (UPLC) with binary solvent manager (Waters Corp., USA), and a Micromass Q-Tof Micro mass spectrometer (Waters; Manchester, UK) equipped with an electrospray ionization (ESI) source operating in negative mode were used for the identification of polyphenols compounds in coriander, dill, and parsley extracts. The UPLC system was used for the separation of individual polyphenols with UPLC BEH C18 column (1.7 μm, 2.1 ×

100 mm, Waters Corp.; Milford, USA) at 30 °C. The flow rate was 0.45 mL min^{-1} and 10 µL of the samples were injected during 15 min to complete the elution of the sample. Solvent A (2.5 % formic acid, v/v) and solvent B (100 % acetonitrile) were the mobile phase. The elution was as following: 0−1 min, isocratic elution with 99 % A; and then a linear gradient was used until 12 min, lowering A to 0 %; from 12.5 to 13.5 min, the initial composition (99 % A) was used, and, then, it was held constant to re-equilibrate the column. Full scan from *m/z* 100 to 1500 was used for the analysis of the samples. The internal reference compound (leucine encephalin) was introduced via the LockSpray channel and the lock mass correction was ±1.000 for the mass window. All Q/TOF-MS chromatograms are presented as the base peak intensity (BPI) chromatograms and scaled to 12.400 counts per second (cps) (=100 %). The eluent was passed to the electrospray source with the following conditions: capillary voltage of 2500 V, cone voltage of 30 V, source temperature of 130 °C, desolvation temperature of 350 °C and desolvation gas (nitrogen) flow rate 300 L h^{-1}. The retention time and the accurate molecular masses were characterization of the single components and each compound was estimated according to its molecular mass $[M-H]^-$ in the negative mode before and after fragmentation. MassLynx 4.0 ChromaLynx Application Manager software was employed to scan different samples according to data obtained from UPLC/MS and the runs were monitored at the following wavelengths: hydroxycinnamates at 320 nm and flavonol glycosides at 360 nm. The spectra of Photodiode detector (PDA) was measured in range range 200−600 nm, the retention time and spectra were compared with standards. The calibration curves of the standards were plotted at 0.05 to 5 mg mL^{-1}.

Antioxidant capacity

The extracts for the analysis of the antioxidant capacity were prepared as previously described by Wojdyło et al. (2008 and 2013). The ORAC, ABTS, and FRAP assays were determined as previously described by Ou, Huang, Hampsch-

Woodill, Flanagan & Deemer (2002), Re, Pellegrini, Proteggente, Pannala, Yang & Rice-Evans (1999), and Benzie and Strain (1996), respectively. All antioxidant capacity was expressed as millimoles of Trolox *per* 100 g of dry matter (dm). ORAC assay was performed using a RF5301 PC spectrofluorometer (Shimadzu, Kyoto, Japan). Determinations by ABTS and FRAP methods were performed using a UV2401 PC spectrophotometer (Shimadzu, Kyoto, Japan).

Statistical analysis

Results are given as the mean of, at least, three independent determinations. Analysis of variance (ANOVA) and multiple range test (Tukey's HSD test) were carried out. All analyses were performed in triplicate.

RESULTS AND DISCUSSION

Identification of phenolic compounds

The vegetative parts of coriander, dill, and parsley plants were analyzed by LC-MS-PDA-QToF systems to determine their phenolic profiles. In total, 30 polyphenolic compounds were detected in these three herbs. The phenolic compounds were identified by comparing their UV−vis spectra, λ_{max}, MS spectra, and retention times (R_t) to those of all available standards. From a total of 30 compounds, 16 were flavonols, such as quercetin, kaempferol, and isorhamnetin derivatives, 4 flavones and 10 phenolic acids derivatives.

The data in **Table 1** showed the identification of phenolic compounds in *coriander* vegetative parts. Compounds C6, C7, and C8 had a characteristic MS/MS fragment at *m/z* 301. Compound C6 (R_t = 6.13 min, λ_{max} = 353 nm) was identified as a quercetin derivative on the basis of a $[M-H]^-$ at *m/z* 609, and a MS/MS fragment at *m/z* 301. Compound C7 (R_t = 7.24 min, λ_{max} = 352 nm) was identified as quercetin-3-*O*-rutinoside on the basis of a $[M-H]^-$ at *m/z* 609, and a MS/MS fragment at *m/z* 301. Compound C8 (R_t = 6.51 min, λ_{max} = 354 nm) was identified as quercetin-3-*O*-glucuronide on the basis of a $[M-H]^-$ at *m/z* 477, and a MS/MS fragment at *m/z* 301. Compound C9 had an UV spectrum similar to that of kaempferol, and a $[M-H]^-$ at *m/z* 593, with MS/MS fragment at *m/z* 285; the loss of 308 mass units (rutinoside moiety) allowed its identification as kaempferol-3-*O*-rutinoside. Barros, Dueñas, Dias, Sousa, Santos-Buelga & Ferreira (2012) reported that the main compounds of the vegetative parts of coriander (*Coriandrum sativum* L.) were flavonol derivatives (quercetin and kaempferol derivatives) and hydroxycinnamic acids derivatives. Five phenolic acids were found in the aerial parts of coriander. Compounds C1, C2, C3, C4, and C5 showed UV spectra typically for hydroxycinnamic acid derivatives. Compound C2 showed a λ_{max} at 328 nm, a molecular ion $[M-H]^-$ at *m/z* 369; thus, the compound was tentatively identified as dimethoxycinnamoyl hexoside. This compound (C2) was previously identified by Barros et al. (2012) and

Alonso-Salces, Guillou, & Berrueta (2009) in coriander and green coffee beans, respectively. Compound C1 presented a molecular ion $[M-H]^-$ at *m/z* 353, and λ_{max} in the UV spectrum at 325 nm, which is related to chlorogenic acid. Compound C4 showed λ_{max} at 315 nm, and a molecular ion $[M-H]^-$ at 341 *m/z*, showing a MS/MS fragment at 179 *m/z* (indicative of a loss of 162 mass units corresponding to a hexose moiety). Using all previous information, the compound (C4) was tentatively identified as caffeic acid-*O*-4-hexoside. Chlorogenic acid and caffeic acid-*O*-hexoside were detected by Vallverdú-Queralt, (2014) in rosemary, thyme, oregano, bay, cinnamon, and cumin. Compound C3 had a MS/MS fragment at 164 *m/z*, and it was tentatively identified as sinapoylmalic acid. Compound C5 showed an UV spectrum similar to that of ferulic acid with λ_{max} at 310 nm; besides, it presented a molecular ion $[M-H]^-$ at *m/z* 355, and it released a MS/MS fragment at *m/z* 193 (loss of 162 amu, indicative of a hexose moiety) attributed to ferulic acid. Using all previous information, this compound (C5) was tentatively identified as ferulic acid hexoside.

Table 1 also shows the identification of phenolic compounds in *dill* vegetative parts. Compounds D1, D2, and D3 had MS/MS fragments at m/z 191, and their molecular ion $[M-H]^-$ at *m/z* 353, and consequently they were identified as neochlorogenic acid, chlorogenic acid, and cryptochlorogenic acid, respectively. Neochlorogenic, cryptochlorogenic and chlorogenic (*m/z* 353) were detected in ground dried dill (*Anethum graveolens*) by Vallverdú-Queralt, Regueiro, Rinaldi Alvarenga, Martinez-Huelamo, Neto Leal & Lamuela-Raventos (2015). Compound D4 presented a molecular ion $[M-H]^-$ at *m/z* 367 with an MS/MS fragment at *m/z* 191, and it was identified as feruloylquinic acid. Zheng and Cliford (2008) reported this compound (D4) in the stem of sweet potato. Quercetin glucuronide and kaempferol-3-*O*-glucuronide were compounds D5 and D8, respectively according to their MS/MS fragments at *m/z* 301 and 285, respectively. These two compounds (D5 and D8) were found by Justesen (2000)

in dill (*Anethum graveolens*). Compounds D6, D9, and D10 had a MS/MS fragment at *m/z* 315, which is indicative of isorhamnetin derivatives, and they were identified as isorhamnetin-3-*O*-rutinoside, isorhamnetin-3-*O*-galactoside or glucoside and isorhamnetin-3-*O*-glucuronide, respectively. Isorhamnetin derivatives were previously reported by Justesen (2000) in chives, cress, and dill, and also by Brito, Ramirez, Areche, Sepúlveda & Simirgiotis (2014) in citrus fruits. Finally, the compound D7 showed MS/MS at *m/z* 623 with a fragment at *m/z* 285; thus, it was tentatively identified as kaempferol connected by two sugar moieties, kaempferol-galactoside-glucoside.

Table 1 shows the identification of phenolic compounds in the *parsley* vegetative parts. Compound P1 had a MS/MS fragment at *m/z* 163, which is related to hydroxycinnamic acid derivatives, and thus it was identified as *p*-coumaroyl-hexoside. No literature references reporting the presence of *p*-coumaroyl-hexoside in parsley were found; so, this is the first study reporting this compound in parsley. Compounds P3, P5, P6, P7, P8, and P10 had a MS/MS fragments at *m/z* 315, which is indicative of the presence of isorhamnetin derivatives; thus, all these 6 compounds were identified as isorhamnetin derivatives as can be seen in **Table 1**. Some isorhamnetin derivatives have been previously reported as constituents of coriander, chives, cress, dill, and citrus fruits (Barros et al., 2012; Justesen, 2000; Brito et al., 2014). Compound P9 showed a molecular ion $[M-H]^-$ at *m/z* 563, and it was identified as apigenin-7-apiosylglucoside (apiin). Justesen (2000) reported that apiin (*Mr* 564) is a major component in parsley; this statement is fully compatible with the results of the current study. Compounds P2 and P4 were identified as luteolin-di-glucoside and apigenin-6,8-*O*-di-glucoside (vicenin 2), respectively; these two compounds were typically for citrus fruit juices (Abad-García, Garmón-Lobato, Sánchez-Ilárduya, Berrueta, Gallo, Vicente & Alonso-Salces, 2014). Diosmetin-apiosylglucoside (compound P11) was present in parsley vegetative part as

reported in previous study in parsley flakes by Luthria, Mukhopadhyay & Kwansa (2006).

Quantification of phenolic compounds

Data in **Table 2** showed that the main phenolic compounds in the *coriander* shoots were dimethoxycinnamoyl hexoside (C2) and quercetin-3-*O*-rutinoside (C7). Experimental data proved that coriander leaf spray with malic acid (MA), oxalic acid (OA), and acetylsalicylic acid (ASA) had a significantly impact on the concentrations of phenolic compounds of coriander plant. All samples of coriander treated with organic acids as preharvest treatment significantly ($p<0.001$) increased their phenolic contents as compared with the control sample. The total concentration of the phenolic compounds reached its maximum value after the application of these organic acids at a rate of 2 mM of MA (7523 mg kg^{-1}) and 2 mM of ASA (10584 mg kg^{-1}), respectively. However, the highest concentration (3 mM) of the OA treatment led to the highest total concentration of polyphenols (13290 mg kg^{-1}). A previous study by Giménez, Valverde, Valero, Guillén, Martínez-Romero, Serrano & Castillo (2014) showed that increasing in the concentration of ASA from 0.5 mM to 1.0 mM increased the content of bioactive compounds, and antioxidant properties of sweet cherry. In addition, flavonols, and chlorogenic acid derivatives were also increased by OA preharvest treatment of sweet cherry trees of the cultivars "*Sweet Heart*" and "*Sweet Late*" (Martínez-Esplá et al. 2014).

The main compounds detected in dill samples were neochlorogenic acid (D1) and quercetin glucuronide (D5) (**Table 3**), while the main compounds found in parsley samples were apigenin-7-apiosylglucoside (P9) and isorhamnetin-3-*O*-hexoside (P10) (**Table 4**). In fact, the preharvest treatment of both dill and parsley with organic acids (MA, OA and ASA) did not significantly improve the quality of these herbs, as defined by their phenolic content. Experimental results showed that the control samples had the highest contents

of total phenolic compounds in parsley (total, 11324 mg kg^{-1}) and dill (total, 2047 mg kg^{-1}). Eraslan, Inal, Gunes & Alpaslan (2007) reported that exogenous application of salicylic acid enhanced growth, physiological process, and antioxidant activity of carrot plants grown under salinity stress (Javaheri, Mashayekhi, Dadkhah & Tavallaee, 2012). Bagherifard et al. (2015) reported that after the treatment of artichokes (*Cynara Scolymus* L.) with salicylic acid, the flavonoids and antioxidant activity increased.

Antioxidant capacity of preharvest treated aromatic herbs

There are different methods for evaluating the antioxidant capacity of foods. This variety of methods is because none of them by itself is able to determine exactly the total antioxidant potential in a food system. For this reason, the antioxidant capacity of coriander, dill, and parsley samples as affected by preharvest treatments with organic acids (MA, OA, and ASA) was evaluated using 3 different analytical methods: ORAC, FRAP, and ABTS.

To the best to our knowledge, there are no available references of MA, OA and ASA as preharvest treatment of aromatic herbs and antioxidant capacity; however, these compounds have been successfully applied in fruits. For instance, MA is thought to inhibit the reactive oxygen species (ROS) over-production, increasing the antioxidant capacity (Velioglu, Mazza, Gao & Oomah, 1998; Huang, Jian, Jiang, Duan & Qu, 2016). Recently, Martínez-Esplá et al. (2014) suggested that OA employed as a preharvest treatment in sweet cherries increased the activities of different antioxidant enzymes, such as superoxide dismutase, catalase, ascorbate peroxidase and polyphenol oxidase. These antioxidant enzymes, together with the antioxidant compounds (phenolic acids, flavonoids, etc.) are involved in scavenging free radicals and ROS, which are generated during the development of the crops (Hodges, Lester, Munro & Toivonen, 2004). Regarding ASA, several authors have reported an increment in the antioxidant capacity when applied as pre- or post-harvest treatments

(Valero, Díaz-Mula, Zapata, Castillo, Guillén, Martínez-Romero & Serrano, 2011; Sayyari, Castillo, Valero, Díaz-Mula & Serrano, 2011). The mechanisms in which ASA increases the antioxidant capacity is not clear; however, the increment in the antioxidant capacity of fruits and vegetables treated with preharvest treatments could be attribute to the simple accumulation of ASA in the plant tissues (Giménez et al., 2014).

Both ORAC and FRAP values in coriander samples were significantly affected by both organic acids (MA, OA, and ASA), and their concentration (1, 2, and 3 mM) in coriander samples. *Coriander* antioxidant capacity values assayed by the ORAC method ranged from 2.85 mmol Trolox/100 g dm in control samples up to 9.34 mmol Trolox/100 g dm in coriander treated with 3 mM OA (**Table 5**). In this way, the organic acid leading to the highest ORAC values in coriander was OA, followed by ASA, MA, and finally control samples. Regarding the FRAP method, coriander samples showed a similar trend to that previously reported for the ORAC values. FRAP values ranged from 4.60 mmol Trolox/100 g dm in control treatment up to 10.59 mmol Trolox/100 g dm in coriander plants treated with 3 mM OA (**Table 5**). Slightly difference trend was observed in the ABTS assay, where the only organic acid leading to higher values was OA; besides, the concentration factor did not affect the values of this antioxidant method. ABTS values ranged from 0.85 mmol Trolox/100 g dm in coriander plants treated with 3 mM MA up to 3.30 mmol Trolox/100 g dm in coriander plants treated with 3 mM OA. In general, it can be stated that the antioxidant capacity of coriander can be significantly enhanced by a preharvest treatment with MA, OA, and ASA, and results are optimized when these organic acids are applied at a concentration of 3 mM. Besides, the higher antioxidant capacity showed by samples treated with 3 mM OA were positively correlated with the highest contents of phenolic compounds (**Table 2**).

It can be stated that the preharvest application of organic acids in *dill* plants only affected significantly ($p<0.001$) the FRAP values, at any of the

concentrations assayed (1, 2, and 3 mM) (**Table 5**). The ORAC values were not significantly (p>0.05) affected by neither the organic acid nor the concentration; they ranged from 6.37 mmol Trolox/100 g dm in dill plant treated with 3 mM ASA concentration up to 10.0 mmol Trolox/100 g dm in dill treated with 1 mM MA. Regarding FRAP method, antioxidant capacity values ranged from 0.83 mmol Trolox/100 g dm in control treatment up to 3.92 mmol Trolox/100 g dm in dill plants treated with 1 mM MA (**Table 5**). FRAP assay reported significant differences for both factors under evaluation (i) organic acids and (ii) concentration. The application of MA led to the highest values at any of the concentrations assayed (1-3 mM). Finally, the ABTS assay showed values ranging from 1.87 mmol Trolox/100 g dm in dill plants treated with 3 mM MA up to 28.4 mmol Trolox/100 g dm in dill plants treated with 1 mM MA. In this last antioxidant method, only the application of MA at 1 mM provided better results than the control plants; all other treatments were similar or worse than the control plants. At this point, it must be highlighted the 1 mM MA reported the higher values of antioxidant capacity assayed by three different methods. These higher values of antioxidant capacity found in dill plants treated with 1 mM MA was not related to the highest total content of phenolic compounds, but to the second highest, only below the value of control plants. These results, suggest that not only phenolic compounds are responsible for the antioxidant capacity of dill plants (**Table 3**). Other compounds, such as vitamin C and chlorophylls, could contribute to the dill antioxidant capacity.

Parsley plants presented a completely different behavior to that of coriander or dill plants, with none of the factors studied (organic acid or concentration) significantly (p>0.05) affecting the antioxidant capacity values as measured by any of the methods assayed. Only the application of MA at any concentration (1-3 mM) increased the values of the FRAP activity; all other treatments led to antioxidant activity values equivalent to those of the control

parsley plants. Finally, no positive relationship was found among the antioxidant capacity values (**Table 5**) and the phenolic compounds contents (**Table 4**).

Summarizing the main findings dealing with the antioxidant capacity, some general and particular aspects must be highlighted

i. *The three herbs studied showed a differential response after the preharvest application of organic acids*. While the application of all organic acids was very positive in coriander (ORAC, FRAP and ABTS values were improved at all concentrations, 1-3 mM, with the exception of ABTS), only the FRAP values were improved in dill plants, and none of the treatments led to significant better results in parsley plants as compared to control plants.

ii. The FRAP method was the most sensitive to the application of organic acids, and, thus, it is the recommended method to evaluate the effects of these factors (organic acid and concentration) on the antioxidant capacity.

iii. Only the coriander antioxidant capacity showed a positive relationship with the contents of the phenolic compounds. Then, other parameters or components should be active in determining the antioxidant capacity of dill and parsley plants.

CONCLUSION

The results showed that leaf spray of organic acids (malic, oxalic, and acetylsalicylic acids) was very positive in coriander plants, in which they increased the contents of most of the phenolic compounds and the antioxidant activity (ORAC, FRAP, and ABTS), led to intermediate improvements in dill plants, where only the treatment with malic acid at 1 mM showed positive results, and finally no significant beneficial effects were observed in parsley plants. Besides, experimental results proved that the antioxidant capacity responding better to the effects of these preharvest treatments in herbs was FRAP. Finally, it must be concluded that other parameters, such as vitamin C and/or chlorophylls, are expected to positively contribute to the antioxidant capacity of herbs, because no positive relationships were found among the contents of phenolic compounds and the antioxidant capacity values, with the exception of coriander plants.

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Table 1. Identification of phenolic compounds in coriander, dill, and parsley by using LC-MS-QTof.

Compound	Compound code	R_t (min)	λ_{max} (nm)	MS $[M-H]^-$ (*m/z*)	MS/MS (*m/z*)
Coriander					
Chlorogenic acid	C1	3.77	325	353	191
Dimethoxycinnamoyl hexoside	C2[†]	3.93	328	369	189
Sinapoylmalic acid	C3[†]	4.24	320	339	223/164
Caffeic acid-*O*-4-hexoside	C4[†]	4.78	315	341	179
trans-Ferulic acid hexoside	C5[†]	4.89	310	355	193
Quercetin-3-*O*-robinobioside	C6	6.13	254/353	609	477/301
Quercetin-3-*O*-rutinoside	C7	6.28	254/354	609	463/301
Quercetin-3-*O*-glucuronide	C8	6.51	254/354	477	301
Kaempferol-3-O-rutinoside	C9	7.09	264/344	593	469/285
Dill					
Neochlorogenic acid	D1	2.81	325	353	191
Chlorogenic acid	D2	3.73	325	353	191
Cryptochlorogenic acid	D3	4.68	315	353	191
Feruloylquinic acid	D4	5.29	324	367	191
Quercetin glucuronide	D5	6.44	238/352	477	301
Isorhamnetin-3-*O*-rutinoside	D6	7.14	237/264/346	623	315
Kaempferol-galactoside-glucoside	D7[†]	7.29	237/264/346	623	285
Kaempferol-3-*O*-glucuronide	D8	7.40	252/352	461	285
Isorhamnetin-3-*O*-galactoside	D9	7.53	237/264/346	477	315
Isorhamnetin-3-*O*-glucuronide	D10	7.60	250/268/342	491	315
Parsley					
p-Coumaroyl-hexoside	P1	3.98	310	325	163
Luteolin-di-glucoside	P2	4.62	237/342	609	447/285
Isorhamnetin-3-*O*-galactoside	P3	4.82	252/351	477	315
Apigenin-6,8-*O*-di-glucoside (vicenin 2)	P4[‡]	4.90	237/332	593	473/353/303
Isorhamnetin-di-*O*-hexoside	P5[‡]	4.95	252/351	639	477/315
Isorhamnetin-3-*O*-glucoside	P6[‡]	5.00	237/348	477	315
Isorhamnetin-3-*O*-galactoside	P7	5.06	237/348	477	315
Isorhamnetin-di-pentosyl-rhamnoside	P8	5.53	238/350	725	519/ 315
Apigenin-7-apiosylglucoside (apiin)	P9	7.38	237/337	563	440/323/269
Isorhamnetin-3-*O*-hexoside	P10	7.52	237/336	477	315
Diosmetin-apiosylglucoside	P11	7.83	237/346	593	447/300

[†]Tentatively identified; [‡]Compounds P4-P6 did overlap in the chromatograms.

Table 2. Quantification of individual phenolic compounds (mg/kg dry matter, dm) in coriander as affected by field treatment with organic acids.

Treatment	Compounds from Table 1									TOTAL
	C1	C2	C3	C4	C5	C6	C7	C8	C9	
	Concentration (mg/kg dm)									
Control	172	1481	173	23.1	112	86.3	1108	69.0	56.6	**3282**
Malic 1 mM	354	2593	195	27.0	126	113.7	1604	80.6	73.4	**5166**
Malic 2 mM	638	3866	152	34.2	191	170.6	2264	128.1	79.0	**7523**
Malic 3 mM	208	2224	299	19.3	121	112.4	1360	73.1	56.2	**4472**
Oxalic 1 mM	859	5550	168	45.3	279	292.3	3656	212.1	118.8	**11181**
Oxalic 2 mM	699	6053	124	42.4	337	339.1	4348	248.5	141.6	**12334**
Oxalic 3 mM	1045	6357	165	66.5	333	368.3	4573	251.5	131.5	**13290**
Acetyl salicylic 1 mM	285	2886	331	34.7	176	127.3	2108	109.9	86.4	**6145**
Acetyl salicylic 2 mM	809	4952	181	66.2	242	309.6	3677	213.3	133.3	**10584**
Acetyl salicylic 3 mM	547	3769	171	53.6	171	222.4	2901	159.0	98.2	**8093**
	ANOVA Test[†]									
Organic acid	***	***	*	**	***	***	***	***	***	*******
Concentration	*	*	*	NS	*	*	*	*	*	*****
	Multiple Range Test (Tukey)[‡]									
ORGANIC ACID										
Control	172 c	1481 c	173 ab	23.1 b	112 c	86.3 c	1108 c	69.0 c	56.6 c	**3282 c**
Malic acid	400 bc	2894 bc	215 ab	26.9 b	146 c	132 c	1742 c	94.0 c	69.6 b	**5720 c**
Oxalic acid	868 a	5987 a	152 b	51.4 a	316 a	333 a	4192 a	237 a	131 a	**12268 a**
Acetyl salicylic acid	547 b	3869 b	228 a	51.3 a	196 b	220 b	2896 b	161 b	106 a	**8274 b**
CONCENTRATION										
Control	172 b	1481 b	173 ab	23.1	112 b	86.3 b	1108 b	69.0 b	56.6 b	**3282 b**
1 mM	499 ab	3676 ab	231 a	35.7	194 ab	178 ab	2456 ab	134 ab	92.9 ab	**7497 ab**
2 mM	715 a	4957 a	152 b	46.4	257 a	273 a	3429 a	197 a	117 a	**10147 a**
3 mM	600 ab	4117 a	211 ab	47.6	208 ab	234 ab	2944 ab	161 ab	95.3 ab	**8618 a**

*[†]NS = not significant F ratio (p<0.05); *, **, and ***, significant at p<0.05, 0.01, and 0.001, respectively. [‡]Values followed by the same letter, within the same column and factor (organic acid or concentration), were not significant different (p<0.05), Tukey's multiple-range test.*

Table 3. Quantification of individual phenolic compounds (mg/kg dm) in dill as affected by field treatment with organic acids.

Treatment	Compounds from Table 1										TOTAL
	D1	D2	D3	D4	D5	D6	D7	D8	D9	D10	
	Concentration (mg/kg dm)										
Control	7500	170	266	130	2178	131.4	403	37.1	35.1	474	**11324**
Malic 1 mM	7009	138	261	121	1568	96.8	298	33.6	28.5	348	**9903**
Malic 2 mM	5604	136	197	102	1078	73.6	224	22.7	24.1	299	**7761**
Malic 3 mM	4224	88	148	104	1222	78.8	229	26.4	25.9	357	**6504**
Oxalic 1 mM	3145	83	109	89	556	33.8	194	18.9	12.6	166	**4408**
Oxalic 2 mM	5286	121	171	125	1168	77.5	170	25.1	22.5	293	**7458**
Oxalic 3 mM	4690	116	153	123	939	62.3	121	26.6	19.1	240	**6491**
Acetyl salicylic 1 mM	4129	103	162	97	973	65.3	139	20.6	21.6	248	**5959**
Acetyl salicylic 2 mM	4437	121	164	103	577	33.9	118	20.1	11.9	159	**5745**
Acetyl salicylic 3 mM	3626	70	117	76	680	39.1	100	15.9	13.2	195	**4933**
	ANOVA Test[†]										
Organic acid	**	**	***	*	***	***	***	***	***	***	***
Concentration	*	***	*	NS	**	**	***	**	*	**	**
	Multiple Range Test (Tukey)[‡]										
ORGANIC ACID											
Control	7500 a	170 a	266 a	130 a	2178 a	131 a	403 a	37.1 a	35.1 a	474 a	**11324 a**
Malic acid	5612 ab	121 ab	202 b	109 a	1290 b	83.1 b	250 b	27.6 b	26.2 b	335 b	**8056 b**
Oxalic acid	4374 bc	107 b	145 c	112 a	887 c	57.9 c	162 c	23.5 b	18.1 c	233 c	**6119 bc**
Acetyl salicylic acid	4064 c	98.4 b	147 c	92.0 b	743 c	46.1 c	119 d	18.9 c	15.6 c	201 c	**5545 c**
CONCENTRATION											
Control	7500 a	170 a	266 a	130	2178 a	131 a	403 a	37.1 a	35.1 a	474 a	**11324 a**
1 mM	4761 b	108 bc	177 b	102	1032 b	65.3 b	210 b	24.4 b	20.9 b	254 b	**6756 b**
2 mM	5109 ab	126 b	177 b	110	941 b	61.7 b	171 b	22.6 b	19.5 b	250 b	**6988 b**
3 mM	4180 b	91.6 c	139 b	101	947 b	60.1 b	150 b	23.0 b	19.4 b	264 b	**5976 b**

*[†]NS = not significant F ratio (p<0.05); *, **, and ***, significant at p<0.05, 0.01, and 0.001, respectively. [‡]Values followed by the same letter, within the same column and factor (organic acid or concentration), were not significant different (p<0.05), Tukey's multiple-range test.*

Table 4. Quantification of individual phenolic compounds (mg/kg dm) in parsley as affected by field treatment with organic acids.

Treatment	Compounds from Table 1									TOTAL
	P1	P2	P3	P4-P6	P7	P8	P9	P10	P11	
	Concentration (mg/kg dm)									
Control	38.6	64.8	397	8.3	97.9	17.2	1240	60.1	123	**2047**
Malic 1 mM	80.5	43.0	379	5.6	78.4	28.1	1215	82.5	155	**2067**
Malic 2 mM	29.3	43.7	305	5.4	63.4	44.6	881	50.9	144	**1567**
Malic 3 mM	68.1	38.8	285	6.7	69.1	14.1	1116	66.9	111	**1776**
Oxalic 1 mM	60.2	49.9	326	5.3	63.2	25.1	1104	66.5	106	**1806**
Oxalic 2 mM	60.3	43.1	330	4.9	75.3	27.6	828	46.5	144	**1560**
Oxalic 3 mM	50.0	40.8	297	3.1	68.9	12.6	654	42.2	165	**1335**
Acetyl salicylic 1 mM	89.3	36.0	282	5.6	71.5	12.7	830	44.5	170	**1542**
Acetyl salicylic 2 mM	33.8	34.0	242	4.5	56.9	30.8	823	52.5	198	**1476**
Acetyl salicylic 3 mM	32.9	33.1	236	4.4	55.3	29.9	800	51.1	193	**1436**
	ANOVA Test†									
Organic acid	NS	***	***	***	***	NS	**	*	***	****
Concentration	**	***	**	**	**	*	*	NS	NS	****
	Multiple Range Test (Tukey)‡									
ORGANIC ACID										
Control	38.6	64.8 a	397 a	8.3 a	98.0 a	17.3	1240 a	60.1 ab	123 b	**2008 a**
Malic acid	59.3	41.8 b	323 b	5.9 b	70.3 b	28.9	1071 a	66.8 a	137 b	**1744 a**
Oxalic acid	56.8	44.6 b	318 b	4.4 c	69.2 ab	21.7	862 b	51.7 b	138 b	**1510 b**
Acetyl salicylic acid	52.0	34.3 c	253 c	4.8 c	61.2 c	24.5	818 b	49.4 b	187 a	**1433 b**
CONCENTRATION										
Control	38.6 b	64.8 a	397 a	8.3 a	97.8 a	17.3 b	1240 a	60.1	123	**2008 a**
1 mM	76.6 a	43.0 b	329 b	5.5 b	71.1 b	21.9 b	1050 a	64.5	143	**1728 a**
2 mM	41.1 b	40.3 b	292 bc	4.9 b	65.2 b	34.3 a	844 b	50.0	162	**1493 b**
3 mM	50.3 b	37.6 b	273 c	4.7 b	64.5 b	18.9 b	857 b	53.4	157	**1465 b**

*†NS = not significant F ratio (p<0.05); *, **, and ***, significant at p<0.05, 0.01, and 0.001, respectively. ‡Values followed by the same letter, within the same column and factor (organic acid or concentration), were not significant different (p<0.05), Tukey's multiple-range test.*

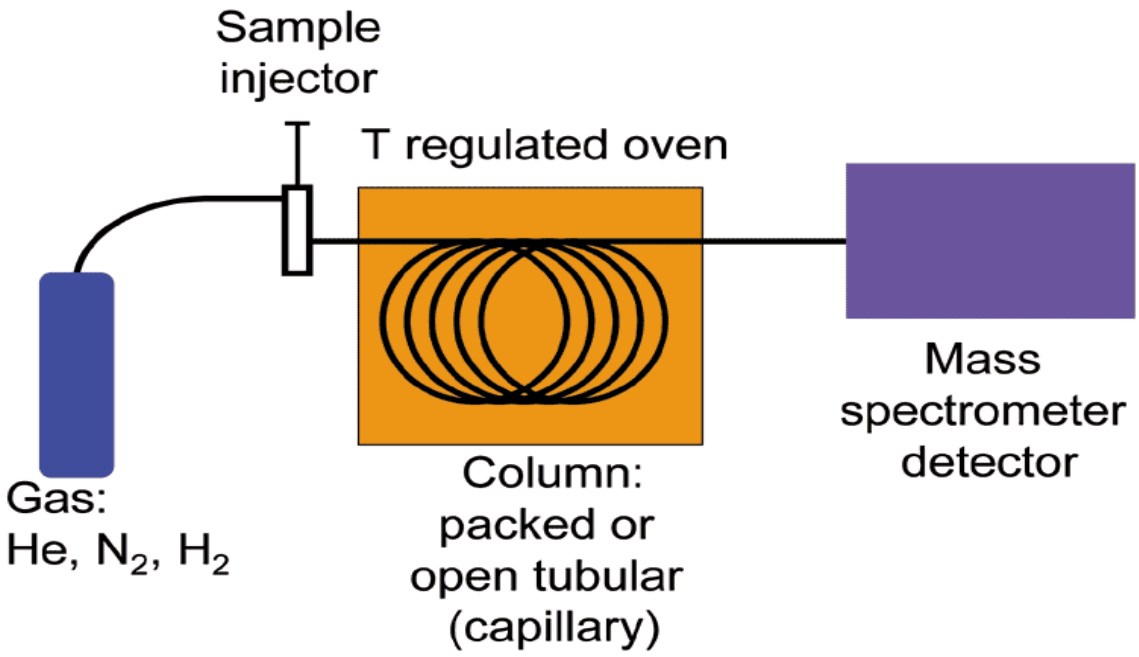
Table 5. Antioxidant capacity (mmol Trolox/100 g dm) of coriander, dill and parsley samples as affected by field treatment with organic acids.

Treatment	Coriander			Dill			Parsley		
	ORAC	FRAP	ABTS	ORAC	FRAP	ABTS	ORAC	FRAP	ABTS
	(mmol Trolox/100 g dm)								
Control	2.85	4.60	1.55	7.61	0.83	6.74	16.27	0.34	0.87
Malic 1 mM	4.24	6.32	2.37	10.0	3.92	28.4	16.68	0.67	0.80
Malic 2 mM	3.30	7.24	1.96	8.10	3.45	6.05	14.05	0.65	0.84
Malic 3 mM	5.77	5.12	0.85	8.01	3.30	1.87	14.78	0.39	0.95
Oxalic 1 mM	7.43	7.50	2.27	7.85	2.73	3.15	14.99	0.13	0.91
Oxalic 2 mM	8.47	9.48	2.28	8.04	3.28	3.60	19.55	0.47	0.80
Oxalic 3 mM	9.34	10.59	3.30	9.86	3.00	2.76	17.88	0.38	0.73
Acetyl salicylic 1 mM	5.98	5.63	1.41	7.25	2.25	3.93	19.10	0.34	0.95
Acetyl salicylic 2 mM	7.95	8.65	1.66	9.34	3.41	5.20	18.25	0.63	0.86
Acetyl salicylic 3 mM	5.84	6.65	1.66	6.37	3.41	5.20	12.01	0.63	0.86
	ANOVA Test[†]								
Organic acid	***	**	***	NS	***	*	NS	*	NS
Concentration	*	**	NS	NS	***	NS	NS	NS	NS
	Multiple Range Test (Tukey)[‡]								
ORGANIC ACID									
Control	2.85 c	1.38 c	1.55 b	7.61	0.83 c	6.74 ab	16.27	0.34 ab	0.87
Malic acid	4.44 c	1.87 bc	1.72 b	8.71	3.56 a	12.10 a	15.17	0.57 a	0.86
Oxalic acid	8.41 a	2.76 a	2.61 a	8.58	3.00 b	3.17 b	17.47	0.33 b	0.81
Acetyl salicylic acid	6.59 b	2.30 ab	1.58 b	7.65	3.03 b	4.77 ab	16.45	0.54 ab	0.89
CONCENTRATION									
Control	2.85 b	1.38 b	1.55	7.61	0.83 b	6.74	16.27	0.34	0.87
1 mM	5.88 ab	1.95 ab	2.02	8.33	2.97 a	11.82	16.92	0.38	0.89
2 mM	6.57 a	2.54 a	1.97	8.49	3.38 a	4.95	17.28	0.59	0.84
3 mM	6.98 a	2.44 a	1.94	8.08	3.24 a	3.28	14.89	0.47	0.85

*[†]NS = not significant F ratio (p<0.05); *, **, and ***, significant at p<0.05, 0.01, and 0.001, respectively. [‡]Values followed by the same letter, within the same column and factor (organic acid or concentration), were not significant different (p<0.05), Tukey's multiple-range test*

Chapter 4.

Resumen de Materiales, Métodos, Resultados y Discusión



4. RESUMEN DE MATERIALES, MÉTODOS, RESULTADOS Y DISCUSIÓN

4.1. Publicación 1

Volatile composition of essential oils from different aromatic herbs grown in Mediterranean regions of Spain

Hussein El-Zaeddi, Juan Martínez-Tomé, Ángel Calín-Sánchez, Francisco Burló, Ángel A. Carbonell-Barrachina

Foods 2016; 5, 41

El **objetivo** de este estudio fue evaluar el contenido en aceites esenciales en cilantro, perejil, eneldo y menta, recolectadas en diferentes estados vegetativos. Además, se llevó a cabo un análisis del perfil sensorial de las muestras que presentaron los niveles de aceites esenciales más elevados.

4.1.1. Resumen de materiales y métodos

Material vegetal. Las cuatro hierbas aromáticas estudiadas [eneldo (*Anethum graveolens* L., cultivar "ELLA"), perejil (*Petroselinum crispum*, cultivar "Gigante Italiano Darkness"), cilantro (*Coriander sativum*, cultivar "Marino"), y menta (*Mentha piperita* L.)] fueron cultivadas empleando las prácticas agronómicas tradicionales de la zona mediterránea, y que han sido optimizadas durante siglos.

Diseño experimental. Inicialmente las semillas de estas cuatro plantas se sembraron en bandejas de poliestireno expandido y germinaron en un invernadero de Santomera (Murcia, España). Posteriormente, las plántulas se trasplantaron a un huerto comercial en Sucina (Murcia, España). Las muestras comerciales de estas plantas fueron recolectadas en las siguientes fechas:

- las de eneldo transcurridos 80 y 174 días desde su siembra (4ª semana de noviembre y 4ª semana de febrero; D1 y D2 respectivamente);

- las de perejil tras 78, 144 y 175 días (3ª semana de noviembre, 1ª semana de enero y 4ª semana de febrero; P1, P2 y P3 respectivamente);
- las de cilantro a los 50, 106 y 58 días (3ª semana de noviembre, 1ª semana de febrero y 4ª semana de febrero; C1, C2 y C3 respectivamente), pero empleando en este caso, diferentes meses de siembra: septiembre, diciembre y octubre, respectivamente; y,
- las muestras de menta se recolectaron a los 133 y 189 días tras su siembra (2ª semana de diciembre y 1ª semana de enero; M1 y M2 respectivamente).

El agua de riego total aportada en cada uno de los cultivos fue: eneldo 3208 m^3/ha, perejil 3849 m^3/ha, cilantro 3445 m^3/ha, y menta 2566 m^3/ha. El agua de riego era de buena calidad, pH 7,91, y conductividad eléctrica 1,26 mS/cm. El suelo tenía una textura lodo-limosa, con un contenido bajo de materia orgánica (1,22 %), condiciones medias de salinidad (3,35 mS/cm); todas estas condiciones son adecuadas para el cultivo de plantas aromáticas. Por supuesto, se utilizó fertilización para estos cuatro cultivos, aportando los niveles necesarios de nitrógeno, fósforo (P_2O_5) y potasio (K_2O), para cada uno de ellos.

Extracción del aceite esencial o la fracción volátil. Las fracciones volátiles (o aceites esenciales) de las hierbas aromáticas en estudio (perejil, eneldo, cilantro y menta) se extrajeron por hidrodestilación con un aparato Deryng. Entre 10 y 15 g de la parte aérea, comestible, de plantas recién recolectadas se cortaron finamente y se introdujeron en un matraz de 500 mL, junto con bolitas de cristal, *boiling beads* (para conseguir una ebullición controlada), NaCl (~ 1 g), agua destilada (150 mL) y patrón interno (acetato de bencilo). Después de que la mezcla comenzó a hervir, se añadió 1 mL de ciclohexano en el Deryng para recoger los compuestos volátiles que se destilaban de la mezcla. Tras 1 hora, el disolvente enriquecido con los compuestos volátiles se transfirió a un vial de 2,5 mL y se secó sobre Na_2SO_4 anhidro. El extracto se mantuvo a -15 ºC hasta que se realizó el análisis cromatográfico. El proceso de extracción se realizó por triplicado.

Análisis cromatográfico. La configuración del sistema cromatográfico (GC-MS) utilizada para la separación, identificación y semicuantificación de los compuestos volátiles (**Figura 5**) estuvo constituida por:

- un cromatógrafo de gases Shimadzu GC-17A (Shimadzu Corporation, Kyoto, Japón),
- un detector de espectrometría de masas Shimadzu QP-5050A, y
- una columna TRACSIL Meta.X5 (95 % dimetilpolisiloxano y 5 % difenilpolisiloxano) (60 m × 0,25 mm, espesor de película 0,25 µm; Teknokroma S. Coop. C. Ltd, Barcelona, España).

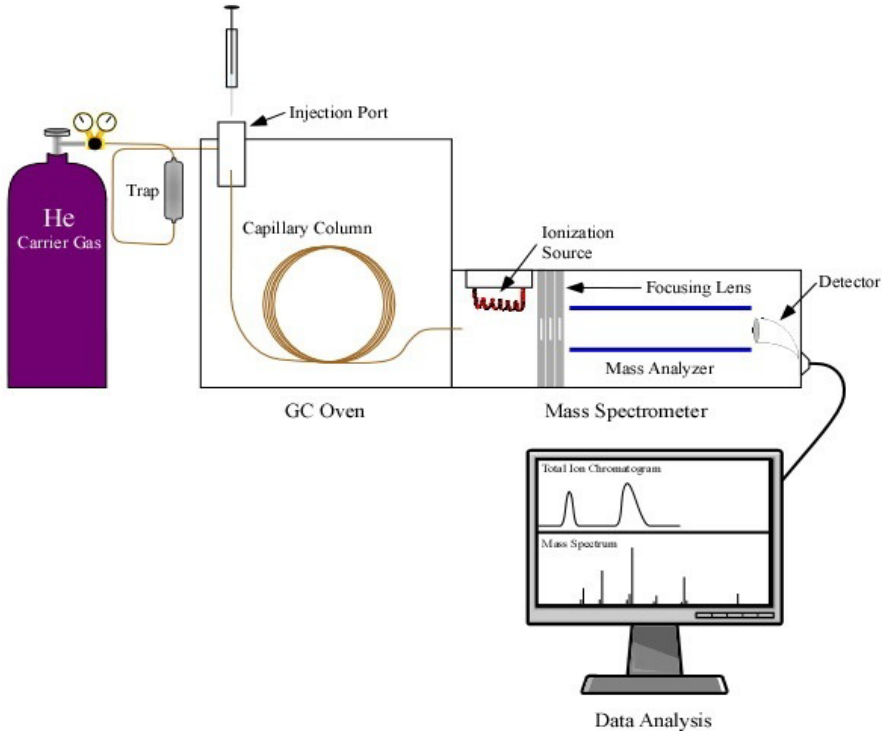


Figura 5. Representación esquemática del sistema cromatográfico empleado en este estudio (GC-MS, del inglés *gas chromatogramphy-mass spectrometry*) (Dunnivant & Ginsbach, 2017).

Se usó helio (He) como gas portador (0,3 mL/min) usando un split de aproximadamente 1:10. Las temperaturas del detector y el inyector fueron 230 y 330 ºC, respectivamente, y el programa de temperatura para el horno fue el siguiente:

- 80 ºC, 0 min;
- rampa de 3 ºC/min desde 80 a 210 ºC, y se mantuvieron los 20 ºC durante 1 min; y
- rampa de 25 ºC/min desde 210 a 300 ºC, manteniendo esta temperatura durante 3 min.

La identificación de los compuestos volátiles se realizó utilizando 4 métodos principales:

- índices de retención de los compuestos volátiles a identificar;
- tiempos de retención de los estándares de los compuestos identificados;
- espectros de masas de los estándares; y,
- la biblioteca espectral NIST05.

Análisis sensorial. Para el estudio sensorial descriptivo de las muestras, se empleó un panel entrenado de 7 panelistas (5 hombres y 2 mujeres), de edades comprendidas entre 23 y 56 años. El panel perteneció al grupo de investigación Calidad y Seguridad Alimentaria, CSA, de la Universidad Miguel Hernández de Elche, y tenía una experiencia superior a 1000 horas. El panel estaba entrenado en la evaluación descriptiva de hierbas aromáticas y se empleó en estudios diversos (Calín-Sánchez *et al.*, 2012, 2013, 2015). Se usó el método de perfil del olor para describir las muestras con mayor contenido en compuestos volátiles. El panel tras 2 sesiones de orientación, decidió trabajar con 7 atributos para desarrollar el perfil aromático de estas hierbas aromáticas: atributos herbal característico de cada especie (eneldo, perejil, cilantro y menta), vegetal (césped verde), cítrico, especiado, pino, terroso y amaderado. Los 5 primeros atributos se consideran como positivos, mientras que los últimos dos se consideran como defectos y se asocian a una humedad excesiva o un excesivo secado, respectivamente. Se usó una escala de 0 a 10 puntos, donde 0 es una intensidad no perceptible y 10 es una intensidad extremadamente elevada.

Tratamiento estadístico. Todos los resultados incluidos en esta Tesis Doctoral se han presentado como el valor medio de, al menos, tres repeticiones independientes (todos los análisis se realizaron por triplicado) ± error estándar, que

se calculó como la desviación estándar dividida por raíz cuadrada del número de muestras. Inicialmente, se realizó la prueba ANOVA (análisis de varianza), y si los resultados mostraron diferencias estadísticamente significativas, la prueba de rango múltiple de Tukey se llevó a cabo para agrupar las muestras de acuerdo con su valor medio.

4.1.2. Resumen de resultados y discusión

Se identificaron un total de 18 compuestos volátiles presentes en el aceite esencial de eneldo, de los cuales (en función de su familia química) 11 fueron monoterpenos, 2 alcanos, 1 aldehído, 1 sesquiterpeno, 1 fenilpropano, 1 cetona monoterpénica y 1 éter monoterpénico. Los compuestos principales encontrados fueron α-felandreno, éter de eneldo (*dill ether*), β-felandreno, limoneno, *p*-cimeno, α-pineno y *trans*-β-ocimeno. De estos compuestos, el α-felandreno, el éter de eneldo y el β-felandreno representaron entre el 85 y 92 % del total de la concentración de compuestos volátiles. Estos resultados concuerdan con resultados previos obtenidos en aceite esencial de eneldo cultivado en Rumanía, Egipto y Finlandia (Orhan *et al*., 2013; Huopalahti & Linko, 1983; Rãdulescu *et al*., 2010).

Comparando los diferentes tiempos de recolección, en general, D2 tuvo más concentración de compuestos aromáticos que D1. La concentración de α-felandreno, el compuesto mayoritario, se incrementó desde 342 mg/kg (D1) hasta 474 mg/kg (D2). Este mismo comportamiento fue el observado para los otros dos compuestos mayoritarios, el éter de eneldo y el β-felandreno, cuyas concentraciones variaron desde ~ 46 (D1) hasta ~ 62 mg/kg (D2). Estos resultados coinciden con los obtenidos por El-Gengaihi & Hornok (1978) y Huopalahti & Linko (1983) que demostraron que la composición volátil de eneldo incrementa a la par que lo hace su crecimiento.

Tras el estudio de la composición volátil de perejil, se aislaron y cuantificaron 18 compuestos aromáticos. En este caso se estudió la composición volátil total y la de los tallos y las hojas por separado. En el caso de la composición volátil del aceite esencial obtenido de los tallos, 1,3,8-p-mentatrieno (38,4-48,8 % del total de

compuestos), β-felandreno (22,2-29,5 %) y miristicina (6,2-11,1 %) fueron los compuestos mayoritarios, mientras que en el análisis de la composición volátil de las hojas de perejil, se encontraron mayoritariamente estos tres mismos compuestos pero en diferente orden de concentración siendo, en este caso, miristicina el compuesto mayoritario (30,7-42,7 %), seguido de β-felandreno (21,8-35,9 %) y de 1,3,8-p-mentatrieno (5,4-10,0 %). Estos resultados corroboran los obtenidos por Petropoulos *et al*. (2004) y Vokk *et al*. (2011). Teniendo en cuenta los diferentes tiempos de recolección estudiados, el tiempo de recolección P1 (455 mg/kg) obtuvo una concentración de compuestos aromáticos mayor que la de los otros dos tiempos estudiados P2 (414 mg/kg) y P3 (418 mg/kg). Petropoulos *et al*. (2004) obtuvieron, al igual que en nuestro estudio, que la concentración relativa de los compuestos aromáticos de perejil cambia en función de la fecha de recolección, siendo más alta en la primera etapa de crecimiento.

Tras el análisis de la composición volátil de los aceites esenciales de cilantro, se identificaron y cuantificaron un total de 24 compuestos aromáticos (10 aldehídos, 6 alcoholes, 2 ésteres, 2 alcanos, 2 monoterpenos, 1 cetona monoterpénica y 1 alcohol monoterpénico). Decanal, *E*-2-dodecenal y dodecanal fueron los compuestos mayoritarios con concentraciones de 30,7, 26,9 y 22,0 mg/kg, respectivamente, representando entre los tres compuestos entre el 71 y 83 % de la concentración total. Nurzyńska-Wierdak (2013) demostró que el cilantro tiene gran cantidad de aldehídos alifáticos, entre los que destacan por su concentración decanal, *E*-2-dodecanol y *E*-2-decenol. El contenido más alto de decanal se observó en el tiempo de recolección C3 con una concentración de 36,4 mg/kg, mientras que el tiempo de recolección C2 obtuvo valores más altos de *E*-2-dodecenal (39,9 mg/kg), dodecanal (24,4 mg/kg) y octano (20,7 mg/kg).

En el caso de la menta fueron identificados y cuantificados 27 compuestos aromáticos, siendo carvona, limoneno, *cis*-carveol, *trans*-sabineno, *trans*-cariofileno, mirceno, santeno y *trans*-β-ocimeno los compuestos mayoritarios. La concentración total de estos compuestos se encontró en mayor cantidad en el tiempo de recolección

M1, en comparación con el tiempo de recolección M2. En el caso del compuesto mayoritario, carvona, se observó este mismo comportamiento variando la concentración desde 2462 mg/kg en M1 hasta 1854 mg/kg en M2 (reducción de un 24,7 %). Por el contrario, en algunos compuestos como, por ejemplo, limoneno, se incrementó su concentración (incremento de un 24,6 %) en el segundo de los tiempos de recolección (M2). De acuerdo a Shiwakoti *et al*. (2015) y Zheljazkov *et al*. (2013) el tiempo de cultivo y el de recolección pueden afectar a las concentraciones de la composición volátil de *Menta canadensis*, mientras que según Brar *et al*., (2014), este contenido no se ve afectado por el tiempo de plantación aunque sí por el tiempo de recolección, viéndose reducida la concentración significativamente cuando se retrasa la cosecha.

Finalmente, se determinaron los perfiles sensoriales de las muestras bajo estudio, mediante el estudio de los atributos herbal (característico de la especie), vegetal (césped verde), cítrico, especiado, pino, terroso y amaderado. Sólo se evaluaron las muestras con una mayor cantidad de compuestos volátiles para así disponer de un modelo de perfil sensorial óptimo. La menta obtuvo las mayores intensidades de los atributos vegetal, cítrico y especiado, mientras que, el cilantro se caracterizó por tener gran intensidad de olor herbal, cítrico y especiado.

4.1.3. Conclusiones

Tras el análisis de los resultados obtenidos en este estudio podemos concluir que, de acuerdo a la concentración de aceites esenciales y a la calidad sensorial de las muestras bajo estudio, la fecha de recolección óptima para las hierbas aromáticas estudiadas es la 2ª cosecha comercial en el caso del eneldo (4ª semana de febrero aproximadamente), la 1ª cosecha para el perejil (3ª semana de noviembre aproximadamente), la 2ª cosecha para el cilantro (1ª semana de enero, aproximadamente), y la 1ª cosecha para la menta (2ª semana de diciembre).

4.2. Publicación 2

Irrigation dose and plant density affect the essential oil content and sensory quality of parsley (*Petroselinum sativum*)

Hussein El-Zaeddi, Ángel Calín-Sánchez, Juan Martínez-Tomé, Luis Noguera-Artiaga, Francisco Burló, Ángel A. Carbonell-Barrachina

Scientia Horticulturae 2016; 206, 1–6

Este estudio tuvo como **objetivo** evaluar la influencia que la dosis de riego y la densidad de plantación tienen sobre la producción, calidad morfológica, aromática y sensorial de perejil.

4.2.1. Resumen de materiales y métodos

Material vegetal. Las semillas de perejil (*Petroselinum sativum* L.), cultivar "Gigante Italiano Darkness" (tipo plano) se sembraron el 19 de septiembre de 2014 en bandejas de poliestireno expandido (EPS) (41 cm × 65 cm, con 260 células) y se colocaron en un invernadero ubicado en Santomera (Murcia, España) hasta el 17 de octubre. Luego, las plántulas se trasplantaron a un huerto comercial de perejil ubicado en Sucina (Murcia, España) con una superficie total de 2,5 ha. Las plantas de perejil se cultivaron utilizando un sistema de riego por goteo de alta frecuencia.

Diseño experimental. El riego se llevó a cabo de acuerdo con 3 dosis de riego que consistieron en las siguientes cantidades totales de agua:

(i) tratamiento de control, ID0, condiciones normales de riego, con 1300 m^3/ha;

(ii) tratamiento1, ID1, con condiciones de riego inferiores a lo normal, 861 m^3/ha; y,

(iii) tratamiento 2, ID2, con condiciones de riego superiores a lo normal, 1788 m^3/ha.

La densidad de plantación, de las plantas empleadas en los 3 tratamientos de dosis de riego, se alcanzó usando líneas de plantas separadas por 0,9 m y una distancia entre plantas en una misma línea de 0,20 m; lo que condujo a una densidad de plantación de 5,56 plantas/m^2 (es decir ~ 56 plantas en una superficie de 10 m^2). El volumen total de agua se ajustó según el tiempo de riego. El agua de riego se aportó empleando tubos de polietileno de 16 mm de diámetro. En condiciones de riego ID0, se utilizaron tubos de 16 mm, separados por 0,9 m, con una distancia entre dos goteros consecutivos de 0,32 m; el flujo fue de 1,6 L/h para cada emisor con una superficie de riego de 0,29 m^2; el número total de goteros por hectárea fue 34722, con un volumen total de agua de 53,87 m^3/ha (~ 24 h de riego). ID1 fue la dosis de riego más baja y en estas condiciones, se utilizaron tubos de 16 mm, separados por 0,9 m, con una distancia del emisor de 0,50 m; el flujo fue de 1,6 L/h para cada emisor con una superficie de riego de 0,45 m^2; el número total de goteros por hectárea fue 22222, con un volumen total de agua de 35,55 m^3/ha (~ 24 h de riego). Finalmente, ID2 fue la dosis de riego más alta y en estas condiciones, se utilizaron tubos de 16 mm, separados por 0,9 m, con una distancia del emisor de 0,32 m; el flujo fue de 2,2 L/h para cada emisor con una superficie de riego de 0,29 m^2; el número total de goteros por hectárea fue 34722, con un volumen total de agua de 74,07 m^3/ha (~ 24 h de riego).

En un experimento paralelo, se trabajó con 3 tratamientos de densidad de plantación:

(i) tratamiento de control, PD0 (5,56 plantas/m^2), o densidad de plantación normal, con líneas de plantas separadas por 0,9 m y una distancia de 0,20 m entre plantas de la misma línea;

(ii) tratamiento 1, PD1, con una densidad de plantación inferior a la normal (4,44 plantas/m^2), con líneas de plantas separadas por 0,9 m y una distancia de 0,25 m entre las bolas de raíz; y,

(iii) tratamiento 2, PD2, con una densidad de plantación superior a la normal (7,41 plantas/m^2), con líneas separadas por 0,9 m y 0,15 m entre las bolas de raíz.

La contribución del agua se llevó a cabo utilizando tubos de polietileno de 16 mm de diámetro, con una distancia entre goteros de 0,33 m. El flujo fue de 1,6 L/ha por cada emisor, lo que arrojó un volumen total de agua de 1290 m^3/ha, según el tiempo de riego.

Treinta y seis plantas de cada tratamiento fueron analizadas en las siguientes superficies: PD0 requirió una superficie total de 6,48 m^2, la superficie PD1 fue 8,10 m^2 y finalmente PD2 necesitó una superficie total de 4,86 m^2.

El agua de riego fue de buena calidad, destacando su pH ligeramente básico (7,91) y una conductividad eléctrica de 1,26 mS/cm, que es adecuada para el cultivo de hierbas aromáticas.

El suelo presentaba una textura uniformemente franco-limosa, con un bajo contenido en materia orgánica (1,22 %), condiciones de salinidad media (3,35 mS/cm) y niveles apropiados de sulfatos (37,83 meq/L) para el desarrollo de perejil. A lo largo del desarrollo de plantas de perejil, la fertilización se llevó a cabo con una cantidad total de N de 130 kg/ha, P (P_2O_5) de 60 kg/ha y K (K_2O) de 160 kg/ha. Todos los tratamientos de campo se realizaron por triplicado.

Extracción del aceite esencial o la fracción volátil. Las fracciones volátiles (o aceites esenciales) de las muestras de perejil empleadas en este estudio se realizaron por hidrodestilación con un aparato Deryng, tal y como se ha descrito en la publicación 1.

Análisis cromatográfico. La configuración del sistema cromatográfico (GC-MS) utilizada para la separación e identificación de los compuestos volátiles fue la reseñada en la publicación 1; sin embargo, la semicuantificación se realizó por el método que pasamos a describir GC-FID (**Figura 6**) y que consistió en:

- un cromatógrafo de gases Shimadzu 2010,
- un detector de ionización de llama (GC-FID), y

- una columna TRACSIL Meta.X5.

Las condiciones cromatográficas utilizadas para el proceso de semicuantificación fueron las descritas previamente para el proceso de identificación en la publicación 1.

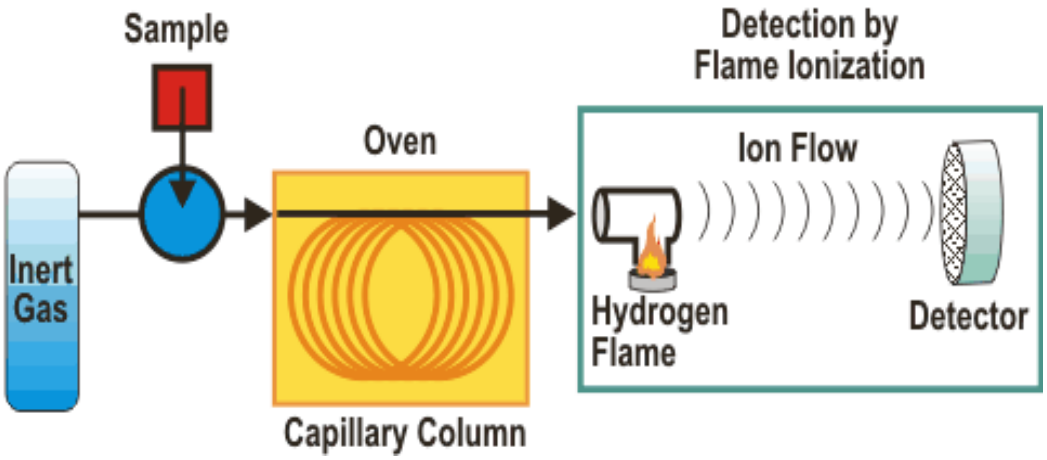


Figura 6. Representación esquemática del sistema cromatográfico empleado en este estudio (GC-FID, del inglés *gas chromatogramphy-flame ionization detector*) (Scientia, 2017).

Análisis sensorial. La evaluación sensorial de las muestras de perejil bajo estudio se realizó empleando el panel y la metodología anteriormente descrita en la publicación 1.

Tratamiento estadístico. Los resultados obtenidos (media de 3 repeticiones) se expresaron como el valor medio ± error estándar. Se emplearon el test de ANOVA y el de rangos múltiples de Tukey.

4.2.2. Resumen de resultados y discusión

Para la realización de este estudio se llevaron a cabo 3 tratamientos de riego, ID0 o control, ID1 e ID2 (1300, 861 y 1788 m^3/ha, respectivamente) y 3 densidades de plantación PD0 o control, PD1 y PD2 (5,56, 4,44 y 7,41 plantas/m^2, respectivamente).

La aplicación de la reducción del agua de riego del tratamiento ID1 no tuvo incidencia significativa sobre la producción total de perejil, la longitud de los tallos, ni sobre el peso de estos. Sin embargo, tras la aplicación de un incremento en la dosis de riego (ID2) se obtuvo un aumento de la producción (ID0 = ~ 7000 kg/ha; ID2 = ~ 9500 kg/ha), aunque, no se vio reflejado este incremento en la longitud, ni en el peso de los tallos de perejil. Este resultado está en concordancia con los obtenidos por Petropoulos *et al*. (2008) y Hassan & Ali (2014) que describieron el efecto que el agua de riego tiene sobre la calidad de diferentes hierbas aromáticas. En cuanto al efecto que la aplicación de diferentes densidades de producción tuvo sobre la producción y la morfología del perejil, no se observaron diferencias significativas entre los tratamientos estudiados.

Tras el estudio de la **composición volátil** de los aceites esenciales de perejil (obtenidos por hidrodestilación mediante el sistema Deryng) se aislaron, identificaron y cuantificaron 18 compuestos aromáticos de los que 8 fueron monoterpenos, 3 terpenos, 3 sesquiterpenos, 1 monoterpenoide, 1 alcohol monoterpénico, 1 éster, y 1 fenilpropanoide. Los compuestos mayoritarios presentes en las muestras de perejil fueron β-felandreno, 1,3,8-p-mentatrieno, miristicina, mirceno, terpinoleno, limoneno, α-pineno y α-felandreno. Estos mismos compuestos fueron encontrados por otros investigadores, aunque los niveles de concentración variaban en función de la procedencia de las muestras y de la técnica de extracción empleada (Petropoulos *et al*., 2004; Vokk *et al*., 2011).

En general, la aplicación de los tratamientos de riego sometidos a estudio tuvo incidencia significativa sobre la concentración de los compuestos aromáticos de perejil, siendo ID1 el que obtuvo mayores concentraciones en los compuestos claves y mayoritarios (β-felandreno, 1,3,8-p-mentatrieno, miristicina, limoneno, α-pineno y α-felandreno). Estos resultados coinciden con los obtenidos previamente en perejil y otras hierbas aromáticas por Simon *et al*. (1992), Baher *et al*. (2002), Petropoulos *et al*. (2004) y Khazaie *et al*. (2008).

En cuanto a cómo afecta la incidencia de la densidad de plantación a la composición del aceite esencial de perejil, la aplicación de los tratamientos bajo estudio (PD1 y PD2) provocó un descenso significativo de la concentración de los compuestos aromáticos totales (PD0= 409 mg/kg; PD1 = 277 mg/kg; PD2= 279 mg/kg), así como en los compuestos clave en este tipo de hierba aromática.

La aplicación de los tratamientos de riego y densidades de plantación aquí estudiados, tuvo incidencia directa en la **calidad sensorial** de las muestras, estudiada mediante un análisis sensorial descriptivo. Las muestras de perejil obtenidas bajo el tratamiento de riego ID1 y una densidad de plantación PD0 se caracterizaron por tener una intensidad de aroma característico de su variedad, aroma cítrico y vegetal, superiores a las del resto de tratamientos.

4.2.3. Conclusiones

Considerando todos los datos obtenidos tras el estudio de cómo el tratamiento de riego y la densidad de plantación influyen sobre la calidad de perejil, estamos en condiciones de afirmar que con la combinación de una dosis de riego de 861 m^3/ha y la aplicación de una densidad de plantación de 5,56 plantas/m^2, así como con la combinación de una dosis de riego de 1290 m^3/ha y la aplicación de una densidad de plantación de 5,56 plantas/m^2, se puede producir perejil con gran cantidad de compuestos aromáticos y una gran calidad sensorial. Por otra parte, si lo que se pretende es conseguir una gran producción, la mejor combinación de riego y densidad de plantación es de 1788 m^3/ha y 7,41 plantas/m^2, respectivamente.

4.3. Publicación 3

Irrigation dose and plant density affect the volatile composition and sensory quality of dill (*Anethum graveolens* L.)

Hussein El-Zaeddi, Juan Martínez-Tomé, Ángel Calín-Sánchez, Francisco Burló, Ángel A. Carbonell-Barrachina

Journal of the Science of Food and Agriculture 2016; 97, 427–433

Este estudio tuvo como **objetivo** evaluar la influencia que la dosis de riego y la densidad de plantación tienen sobre la producción, calidad morfológica, concentración de compuestos volátiles y calidad sensorial de eneldo.

4.3.1. Resumen de materiales y métodos

Material vegetal. Las semillas de eneldo (*Anethum Graveolens* L., cultivar "N3") se sembraron el 18 de septiembre de 2014 en bandejas de poliestireno expandido (EPS) (41 cm × 65 cm, con 260 células) y se colocaron en un invernadero ubicado en Santomera (Murcia, España) hasta el 20 de octubre de 2014. Las plántulas se trasplantaron a un huerto comercial de eneldo ubicado en Sucina (Murcia, España) con una superficie total de 2,5 ha. Las plantas de eneldo se cultivaron utilizando sistemas de riego por goteo de alta frecuencia. Las plantas se cosecharon el 21 de enero de 2015 para experimentos de riego y densidad de plantación.

Diseño experimental. Se realizaron dos experimentos independientes en este estudio:

- el primero evaluó los efectos de la dosis de riego sobre el perfil volátil y la calidad sensorial del eneldo, y
- el segundo evaluó los efectos de la densidad de plantación sobre los mismos parámetros.

El riego se llevó a cabo de acuerdo con tres dosis de riego consistentes en las siguientes cantidades totales de agua:

(i) tratamiento de control (ID0), condiciones normales de riego, con 1585 m^3/ha;

(ii) tratamiento 1 (ID1), con condiciones de riego inferiores a lo normal, 1015 m^3/ha; y,

(iii) tratamiento 2 (ID2), con condiciones de riego superiores a lo normal, 2180 m^3/ha.

La densidad de plantación, de las plantas empleadas en los 3 tratamientos de dosis de riego, se alcanzó usando líneas de plantas separadas por 0,9 m y una distancia entre plantas en una misma línea de 0,20 m; lo que condujo a una densidad de plantas de 5,56 plantas/m^2 (es decir ~ 56 plantas en una superficie de 10 m^2). El volumen total de agua se ajustó según el tiempo de riego. El agua de riego se aportó empleando tubos de polietileno de 16 mm de diámetro. En condiciones de riego ID0, se utilizaron tubos de 16 mm, separados por 0,9 m, con una distancia entre dos goteros consecutivos de 0,32 m; el flujo fue de 1,6 L/h para cada emisor con una superficie de riego de 0,29 m^2; el número total de goteros por hectárea fue 34722, con un volumen total de agua de 55,55 m^3/ha (~ 28.5 h de riego). ID1 fue la dosis de riego más baja y en estas condiciones, se utilizaron tubos de 16 mm, separados por 0,9 m, con una distancia del emisor de 0,50 m; el flujo fue de 1,6 L/h para cada emisor con una superficie de riego de 0,45 m^2; el número total de goteros por hectárea fue 22222, con un volumen total de agua de 35,55 m^3/ha (~ 28.5 h de riego). Finalmente, ID2 fue la dosis de riego más alta y en estas condiciones, se utilizaron tubos de 16 mm, separados por 0,9 m, con una distancia del emisor de 0,32 m; el flujo fue de 2,2 L/h para cada emisor con una superficie de riego de 0,29 m^2; el número total de goteros por hectárea fue 34722, con un volumen total de agua de 76,39 m^3/ha (~ 28.5 h de riego).

Para hacer posible la reproducibilidad de los tratamientos de riego en otros climas o regiones, la evapotranspiración de referencia (ET_0) se ha calculado de acuerdo con Allen *et al*. (1998) y se proporciona a continuación, junto con los

parámetros climáticos clave, para los cuatro meses del experimento, es decir, octubre, noviembre y diciembre de 2014 y enero de 2015:

- ET_0: 83,00, 55,05, 52,66 y 31,95 mm, respectivamente;
- radiación acumulada: 426, 283, 291 y 208 MJ/m^2, respectivamente;
- temperatura media: 20,7, 15,1, 11,3 y 10,4 ºC, respectivamente;
- velocidad del viento media: 1,33, 1,56, 1,83 y 1,33 m/s, respectivamente;
- número de horas de sol: 252, 212, 225 y 154 h, respectivamente; y
- déficit de presión de vapor: 1,08, 0,61, 0,59 y 0,63 kPa, respectivamente.

En un experimento paralelo, se trabajó con 3 tratamientos de densidad de plantación:

(iv) tratamiento de control, PD0 (5,56 plantas/m^2), o densidad de plantas normal, con líneas de plantas separadas por 0,9 m y una distancia de 0,20 m entre plantas de la misma línea;

(v) tratamiento 1, PD1, con una densidad de plantas inferior a la normal (4,44 plantas/m^2), con líneas de plantas separadas por 0,9 m y una distancia de 0,25 m entre las bolas de raíz; y,

(vi) tratamiento 2, PD2, con una densidad de plantas superior a la normal (7,41 plantas/m^2), con líneas separadas por 0,9 m y 0,15 m entre las bolas de raíz.

La contribución del agua se llevó a cabo utilizando tubos de polietileno de 16 mm de diámetro, con una distancia entre goteros de 0,33 m. El flujo fue de 1,6 L/ha por cada emisor, lo que arrojó un volumen total de agua de 1290 m^3/ha, según el tiempo de riego.

Treinta y seis plantas de cada tratamiento fueron analizadas en las siguientes superficies: PD0 requirió una superficie total de 6,48 m^2, la superficie PD1 fue 8,10 m^2 y finalmente PD2 necesitó una superficie total de 4,86 m^2.

El agua de riego fue de buena calidad, destacando su pH ligeramente básico (7,91) y una conductividad eléctrica de 1,26 mS/cm, que es adecuada para el cultivo de hierbas aromáticas.

El suelo presentaba una textura uniformemente franco limosa, con un bajo contenido en materia orgánica (1,22 %), condiciones de salinidad media (3,35 mS/cm) y niveles apropiados de sulfatos (37,83 meq/L) para el desarrollo de eneldo. A lo largo del desarrollo de plantas de eneldo, la fertilización se llevó a cabo con una cantidad total de N de 130 kg/ha, P (P_2O_5) de 60 kg/ha y K (K_2O) de 160 kg/ha. Todos los tratamientos de campo se realizaron por triplicado.

Extracción del aceite esencial o la fracción volátil. Las fracciones volátiles (o aceites esenciales) de las muestras de eneldo empleadas en este estudio se realizaron por hidrodestilación con un aparato Deryng, tal y como se ha descrito en las publicaciones 1 y 2.

Análisis cromatográfico. Tanto la identificación como la semicuantificación de los compuestos volátiles se llevaron a cabo empleando los equipos y condiciones descritas en la publicación 2, concretamente GC-MS y GC-FID, respectivamente.

Análisis sensorial. La evaluación sensorial de las muestras de eneldo bajo estudio se realizó empleando el panel y la metodología anteriormente descrita en las publicaciones 1 y 2.

Tratamiento estadístico. Los resultados obtenidos (media de 3 repeticiones) se expresaron como el valor medio ± error estándar. Se emplearon el test de ANOVA y el de rangos múltiples de Tukey.

4.3.2. Resumen de resultados y discusión

La aplicación de los diferentes tratamientos de riego llevados a cabo en este estudio provocó cambios estadísticamente significativos en la **producción** y la **morfología** de las muestras de eneldo. La aplicación de una reducción del aporte de agua durante el cultivo (tratamiento ID1) provocó un descenso de producción con respecto al control (ID0= 25,51 t/ha; ID1= 17,61 t/ha) y una reducción en el peso

de los tallos de eneldo (ID0= 1,62 g; ID1= 1,05 g). No se observaron diferencias significativas al comparar los resultados obtenidos tras la aplicación del tratamiento PD2 frente al control en ninguno de estos parámetros.

Teniendo en cuenta los diferentes tratamientos de densidad de plantación, no se observaron diferencias significativas entre PD1 y la muestra control a la hora de analizar la producción y la longitud de los tallos de eneldo obtenidos, aunque, sí se apreciaron diferencias en el peso de estos últimos, incrementándose desde 1,72 g (PD0) hasta 2,30 g (PD1). La aplicación de una densidad de plantación PD2 provocó un descenso en la producción, en la longitud de los tallos y en el peso de los mismos. Esta relación de una menor densidad de plantación y una mayor calidad morfológica de los tallos también fue descrita previamente por Callan *et al*. (2007) tras estudiar el efecto que la maduración y la densidad de plantación tienen sobre el aceite esencial de eneldo.

Dieciocho **compuestos aromáticos** fueron encontrados tras el análisis del aceite esencial de eneldo mediante cromatografía de gases, de los cuales la familia de los monoterpenos fue la mayoritaria con 11 compuestos, seguida de alcanos con 2 compuestos y de aldehídos, fenilpropanos, sesquiterpenos, cetonas monoterpénicas y éteres monoterpénicos con 1 compuesto cada una de ellas. De todos ellos, el compuesto mayoritario fue α-felandreno (~ 47 mg/100 g), seguido de éter de eneldo (~ 8 mg/100 g) y β-felandreno (~ 6 mg/100 g). Estos tres compuestos componen aproximadamente entre un 85 y un 90 % de la concentración total de la composición volátil del aceite esencial de eneldo. Similares resultados fueron obtenidos en estudios con muestras de eneldo obtenidas en diferentes localizaciones de Europa y África (Orhan *et al*., 2013; Huopalahti & Linko, 1983; Rãdulescu *et al*., 2010; Vokk *et al*, 2011). La aplicación de los tratamientos de riego bajo estudio tuvo incidencia significativa en la concentración total de compuestos volátiles, siendo el tratamiento ID2 el que obtuvo una mayor concentración de los mismos (ID0= 62,6 mg/100 g; ID1= 49,7 mg/100 g; ID2= 73,3 mg/100 g), así como en muchos de los compuestos clave y/o mayoritarios. Por el contrario, la aplicación del tratamiento ID1

causó un descenso en la cantidad de estos compuestos. Resultados similares se encontraron tras estudiar el efecto del riego en otras hierbas aromáticas como romero o anís (Zehtab-Salmasi *et al*., 2001).

Después de analizar los resultados obtenidos en el estudio de las diferentes densidades de plantación, la muestra PD2 (108 mg/100 g) obtuvo una mayor concentración de compuestos volátiles que las muestras PD0 y PD1 (57,3 y 68,4 mg/100 g respectivamente), además de ser, también, la muestra que obtuvo una mayor cantidad de los compuestos volátiles mayoritarios (α-felandreno, éter de eneldo y β-felandreno). Esta misma tendencia se observó en las muestras del tratamiento PD1, cuyos contenidos en compuestos volátiles fueron superiores a los del tratamiento control, PDO; de este modo la tendencia general para las concentraciones de los compuestos claves fue la siguiente: PD2 > PD1 > PD0. Estos resultados concuerdan con los obtenidos por Callan *et al*. (2007), quienes también constataron un incremento de compuestos volátiles en eneldo al incrementar la densidad de plantación.

En cuanto a los resultados obtenidos de **calidad sensorial** en función de los diferentes tratamientos de riego y las diferentes densidades de plantación planteadas en este estudio, las muestras ID2 y PD2 fueron las que obtuvieron intensidades más altas (estadísticamente significativas) en los atributos característicos de la variedad, como por ejemplo el aroma a eneldo, cítrico y vegetal. Al mismo tiempo, obtuvieron las intensidades más bajas en los atributos amaderado y terroso, relacionados con una menor calidad sensorial en este tipo de producto.

4.3.3. Conclusiones

Considerando los resultados obtenidos en este estudio, estamos en condiciones de recomendar que, si el objetivo final es producir muestras de eneldo con una gran cantidad de compuestos aromáticos y gran calidad sensorial, la combinación de una dosis de riego de 2180 m^3/ha y una densidad de plantación de 7,41 plantas/m^2 es la opción más recomendable. Por otra parte, si lo que se pretende es conseguir una

gran producción, la mejor combinación de riego y densidad de plantación es de 1585 m^3/ha y 5,56 plantas/m^2, respectivamente.

4.4. Publicación 4

Preharvest treatments with malic, oxalic and acetylsalicylic acids affect the phenolic composition and antioxidant capacity of coriander, dill and parsley

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En este estudio se analizó la influencia que la aplicación en pre-cosecha de ácido málico, ácido oxálico y ácido acetil salicílico, tienen sobre la bioactividad y la capacidad antioxidante de cilantro, eneldo y perejil.

4.4.1. Resumen de materiales y métodos

Material vegetal. Las muestras fueron cultivadas bajo las condiciones agronómicas típicas de la región mediterránea. El perejil (*Petroselinum crispum*, cultivar "Gigante Italiano Darkness") se plantó el día 25 de noviembre de 2015, en líneas con una separación de 0,5 m y una distancia entre plantas de 0,20 cm; el eneldo (*Anethum graveolens* L., cultivar "Ella") se plantó el 4 de diciembre de 2015 con una separación entre líneas de 0,50 m y una distancia entre plantas de 0,20 cm; y el cilantro (*Coriander sativum*, cultivar "Marino") se plantó el día 25 de diciembre de 2015 con una separación entre líneas de 1,5 m y una distancia entre plantas de 0,07 cm. La aplicación de los ácidos orgánicos estudiados en este trabajo, ácido málico (MA), ácido oxálico (OA) y ácido acetil salicílico (ASA) tuvo lugar, simultáneamente para los tres cultivos, el día 6 de marzo de 2016, empleando 3 concentraciones diferentes para cada uno de ellos (1, 2 y 3 mM), mediante pulverización foliar y realizando 4 repeticiones para cada una de las combinaciones. Finalmente, las hierbas aromáticas fueron recolectadas el día 13 de marzo de 2016.

Extracción de compuestos polifenólicos. La extracción de estos compuestos se realizó de acuerdo al método descrito por Wojdylo *et al.* (2008, 2013). La extracción comenzó mezclando aproximadamente 1 g de material vegetal liofilizado con 20 mL de una mezcla de metanol al 80 % y ácido clorhídrico al 1 %. Esta mezcla se sonicó y se dejó 24 h a temperatura ambiente (~ 20 °C) en oscuridad. El extracto se centrifugó y los sobrenadantes se guardaron a 4 ºC hasta su análisis antes de 24 h.

Análisis cromatográfico. El sistema cromatográfico empleado para la identificación de los compuestos polifenólicos consistió en:

- un sistema UPLC ACQUITY de cromatografía líquida de alto rendimiento, con un controlador binario de disolvente (Waters Corp., USA),
- un detector de espectrometría de masas Micromass Q-Tof Micro (Waters, Manchester, UK), con una fuente de ionización en electroespray (ESI), operando en modo negativo,
- una columna C18 UPLC BEH (1,7 µm, 2,1 x 100 mm, Waters Corp.; Milford, USA), operando a 30 ºC, y,
- un software de procesado, MassLynx 4.0 ChromaLynx.

Se trabajó con una velocidad de flujo de 0,45 mL/min y se inyectaron 10 µL de los extractos de las hierbas aromáticas. El programa de elución se completó en 15 min. La fase móvil estuvo constituida por 2 disolventes, ácido fórmico al 2,5 % (disolvente A) y acetonitrilo al 100% (disolvente B). El programa de elución fue el siguiente: 0-1 min, elución isocrática con 99 % de disolvente A; y después se usó un gradiente lineal hasta el minuto 12, bajando el disolvente A hasta 0 %. Del minuto 12,5 al 13,5, se volvió a la composición inicial de 99 % del disolvente A, y se mantuvo constante para el re-equilibrado de la columna.

El detector trabajó en modo escaneo (*scan*) en el rango 100 a 1500 m/z, y como patrón interno se usó encefalina leucina. El tiempo de retención y las masas moleculares exactas se usaron para la caracterización de cada uno de los fragmentos individuales, y cada compuesto fenólico se identificó de acuerdo a su masa molecular

$[M-H]^-$ en modo negativo antes y después de su fragmentación. Los compuestos fueron monitorizados a distintas longitudes de onda: 320 nm (hidroxicinamatos) y 360 nm (glucósidos de flavanol). El espectro del detector de fotodiodos (PDA) se midió en el rango 200-600 nm, y el tiempo de retención y espectros se compararon con los de los correspondientes estándares. Se prepararon curvas de calibrado en el rango 0,05 a 5,00 mg/mL.

Capacidad antioxidante. Los mismos extractos empleados para determinar el perfil polifenólico se emplearon para evaluar su capacidad antioxidante. Se trabajó con los métodos ORAC, ABTS, y FRAP de acuerdo a la metodología inicialmente desarrollada por Ou *et al*. (2002), Re *et al*. (1999) y Benzie & Strain (1996), respectivamente. Para el método ORAC se empleó un espectrofluorímetro RF5301 PC (Shimadzu, Kyoto, Japón), y para los otros dos métodos se usó un espectrofotómetro UV2401 PC del mismo fabricante. Los resultados se expresaron en mmol Trolox/100 g de materia seca.

4.4.2. Resumen de resultados y discusión

Se identificaron un total de 30 **compuestos fenólicos** en las hierbas aromáticas bajo estudio, de los cuales 16 fueron flavonoles, 4 flavonas y 10 derivados de ácidos fenólicos.

El hexósido de dimetoxicinamoilo y el quercetin-3-*O*-rutinósido fueron los principales compuestos fenólicos encontrados en las muestras de cilantro. Tras la aplicación de los tratamientos pre-cosecha estudiados, se incrementaron las concentraciones de los compuestos fenólicos, con respecto a la muestra control, alcanzándose el máximo valor cuando se aplicaron a una concentración 2 mM en el caso de las muestras tratadas con MA y ASA (7523 mg/kg y 10584 mg/kg, respectivamente), y con una concentración 3 mM cuando se aplicó OA (13290 mg/kg). Este resultado concuerda con los obtenidos previamente por Giménez *et al*. (2014) y Martínez-Esplá *et al*. (2014) tras la aplicación de tratamientos pre-cosecha similares en cereza.

El ácido neoclorogénico y el glucurónido de quercetina fueron los compuestos fenólicos principales detectados en las muestras de eneldo, mientras que el apigenin-7-apiosilglucósido (apiina) y el isorhamnetin-3-*O*-hexósido fueron los principales en el caso de las muestras de perejil. En ninguna de estas dos hierbas aromáticas, la aplicación de los tratamientos pre-cosecha aquí estudiados tuvo efecto positivo en la concentración de los compuestos fenólicos. Las muestras control presentaron los valores más altos, 11324 mg/kg en el caso de las muestras de eneldo y 2047 mg/kg en el caso del perejil.

Para medir la **capacidad antioxidante** de las muestras de cilantro, eneldo y perejil se emplearon tres métodos analíticos: ORAC, FRAP y ABTS. La aplicación de los tratamientos con ácidos orgánicos (ASA, OA y MA) incrementó la capacidad antioxidante en las muestras de cilantro, en cada una de las concentraciones estudiadas. En el caso del método ORAC se incrementó, por ejemplo, de 2,85 mmol Trolox/100 g en las muestras control hasta 9,34 mmol Trolox/100 g en las muestras tratadas con OA 3 mM; en el caso del método FRAP, para este mismo ácido orgánico y esta misma concentración, se incrementó desde 4,60 mmol Trolox/100 g en la muestra control hasta 10,59 mmol Trolox/100 g. El ácido oxálico fue el que causó un mayor incremento en la capacidad antioxidante de cilantro, seguido de ASA y MA. En el caso del ensayo ABTS para las muestras de cilantro, únicamente se observaron diferencias estadísticamente significativas en las muestras tratadas con OA, sin que este efecto dependiera de la concentración empleada.

En las muestras de eneldo se observaron diferencias significativas únicamente mediante el método FRAP para cada uno de los ácidos orgánicos y para cada una de las concentraciones estudiadas. Mediante el método ORAC no se obtuvieron diferencias significativas entre las muestras tratadas con ácidos orgánicos y las muestras control, y mediante el método ABTS únicamente en las muestras tratadas con MA 1 mM se observó un incremento de la capacidad antioxidante de esta hierba aromática.

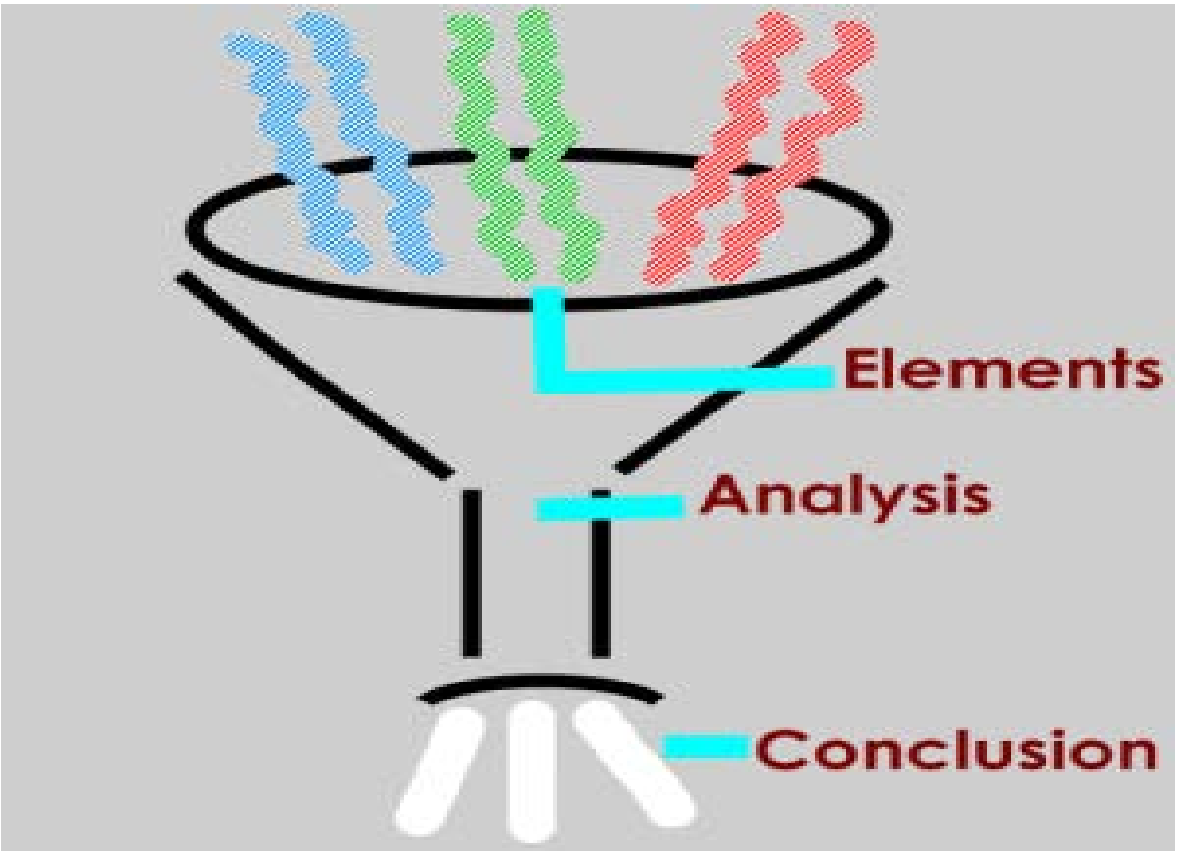
Por último, tras analizar los datos obtenidos sobre la capacidad antioxidante de las muestras de perejil se observó que la aplicación de los ácidos orgánicos MA, ASA y OA no tuvo ningún efecto en ninguna de las concentraciones estudiadas.

Como resumen, podríamos afirmar que las tres hierbas aromáticas estudiadas mostraron respuestas diferentes tras la aplicación pre-cosecha de los ácidos orgánicos. Mientras que esta tuvo efectos positivos en el caso del cilantro, sólo en el método FRAP se observaron mejoras en las muestras de eneldo y en ningún método se observaron diferencias en las muestras de perejil.

4.4.3. Conclusiones

La aplicación de ácidos orgánicos en pre-cosecha tuvo efecto positivo en cilantro, incrementando el contenido de compuestos fenólicos y su capacidad antioxidante; efectos intermedios en el caso del eneldo, puesto que únicamente la aplicación de ácido málico 1 mM tuvo incidencia positiva en los parámetros estudiados; y finalmente, no tuvo ningún efecto positivo tras su aplicación en perejil. Estos resultados han probado que el método más sensible a la hora de medir la capacidad antioxidante de este tipo de productos es el método FRAP.

Chapter 5.- Conclusions



5. CONCLUSIONS

The main purpose of this study was to investigate the effect of different agricultural practices (harvest date, irrigation dose, plant density, and preharvest treatments with organic acids) on production and quality of popular aromatic herbs from the Mediterranean region of Spain (dill, parsley, coriander, and mint). The global conclusions of the Ph.D. dissertation and this study can be concluded as follows:

1. The optimal harvest date for the highest essential oil content and high sensory quality is to harvest at the following dates: dill (2nd commercial harvest, approximately 4th week of February), parsley (1st commercial harvest, approx. 3rd week of November), coriander (2nd commercial harvest, approx. 1st week of January), and mint (1st commercial harvest, approx. 2nd week of December).

2. For parsley plants, the final recommendation was to use (i) irrigation dose of 861 m^3/ha at the applied plant density of 5.56 plants/m^2, and (ii) plant density of 5.56 plants/m^2 at the applied irrigation dose of 1290 m^3/ha, if the objective is to produce parsley samples with the highest aromatic and sensory quality. However, if the only objective was to produce high amounts of parsley, the best options were 1788 m^3/ha dose and 7.41 plants/m^2 of plant density.

3. For dill plants, the recommended farming conditions were (i) irrigation dose of 2180 m^3/ha at the applied plant density of 5.56 plants/m^2 and (ii) plant density of 5.56 plants/m^2 at the applied irrigation dose of 1290 m^3/ha, if the objective is to produce dill samples with the highest aromatic and sensory quality. However, if the only objective was to produce high amounts of dill, the best options were 1585 m^3/ha dose and 7.41 plants/m^2 of plant density.

4. The leaf spray of organic acids (malic, oxalic, or acetylsalicylic acids) was very positive in coriander plants, in which they increased the contents of most of the phenolic compounds and the antioxidant capacity. Intermediate improvements were observed in dill plants, where only the treatment with malic acid at 1 mM showed positive results, and finally no significant beneficial effects were observed in parsley plants.

All these recommendations are already under use by the companies supporting this PhD dissertation. Thus, the practical application of the results has been immediate and show the relevance of the conducted studies.

5.1. Conclusiones

El objetivo principal de esta Tesis Doctoral fue investigar el efecto de diferentes prácticas agronómicas (fecha de recolección, dosis de riego, densidad de plantación, y tratamientos precosecha con ácidos orgánicos) sobre la producción y calidad de hierbas aromáticas populares (eneldo, perejil, cilantro y menta) en la región Mediterránea de España. Las conclusiones globales de esta Tesis Doctoral se muestran a continuación:

1. Las fechas óptimas de recolección con el mayor contenido de aceites esenciales y elevada calidad sensorial son la siguientes: eneldo (2º recolección comercial, aproximadamente la 4º semana de febrero), perejil (1º recolección comercial, aproximadamente la 3º semana de noviembre), cilantro (2º recolección comercial, aproximadamente la 1º semana de enero), y menta (1º recolección comercial, aproximadamente la 2º semana de diciembre).

2. En cuanto a perejil, la recomendación final fue el uso de (i) una dosis de riego de 861 m^3/ha y una densidad de plantación de 5.56 plantas/m^2, y (ii) una densidad de plantación de 5.56 plantas/m^2 y una dosis de riego de 1290 m^3/ha, si el objetivo es producir perejil con la mayor calidad aromática y sensorial. Sin embargo, si el único objetivo es producir grandes cantidades de perejil, la mejor opción fue la de aplicar una dosis de riego de 1788 m^3/ha y una densidad de plantación de 7.41 plantas/m^2.

3. En eneldo, las prácticas agronómicas más recomendables fueron el uso de (i) una dosis de riego de 2180 m^3/ha y una densidad de plantación de 5.56 plantas/m^2, y (ii) una densidad de plantación de 5.56 plantas/m^2 y una dosis de riego de 1290 m^3/ha, si el objetivo es producir eneldo con la mayor calidad aromática y sensorial. Sin embargo, si el único objetivo es producir grandes

cantidades de perejil, la mejor opción fue la de aplicar una dosis de riego de 1585 m^3/ha y una densidad de plantación de 7.41 plantas/m^2.

4. El tratamiento foliar con ácidos orgánicos (málico, oxálico o acetilsalicílico) fue muy efectivo en cilantro, en cuyas plantas incrementaron el contenido de la mayoría de los compuestos fenólicos y la capacidad antioxidante. Se observaron mejoras intermedias en eneldo, donde tan sólo el tratamiento con ácido málico a 1 mM mostró resultados positivos, y finalmente, no se produjeron beneficios significativos en perejil.

Todas estas recomendaciones, están actualmente en uso por las empresas colaboradoras de esta Tesis Doctoral. Por lo tanto, la aplicación práctica de los resultados obtenidos ha sido inmediata y muestra la relevancia de los estudios realizados.

Chapter 6.- Future Developmente of the Research

6. POSSIBLE FUTURE DEVELOPMENTS OF THE RESEARCH

The research information produced during this PhD dissertation has been already implemented at the companies supporting the research presented:

- Rambla de los Molinos S.A., and
- Cooperativa Agrícola Católica de Orihuela Sdad. Coop.

The research included in this PhD dissertation had, from the very beginning, a full practical approach to help companies in deciding about the optimal conditions of different farming operations, such as irrigation dose, plant density, and harvest time. Thus, the companies were timely informed about the results obtained in the different experiments, and they immediately implemented these conditions into their farms in Spain.

The fact that the companies were able to obtain fully transferable information and recommendations made this PhD dissertation a model for the transfer of results to agricultural companies in the Mediterranean region of Spain. It is essential that private companies get practical and easy to implement data, information, and recommendations. If so, they are willing to fund research at universities, and the research group “Food Quality and Safety” is in conversation with these two companies to extend the cooperation.

Chapter 7.- References

7. REFERENCES

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