AN ABSTRACT OF THE THESIS OF

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Mina R. McDaniel

Lemon oil quality is affected by numerous factors including lemon variety, climate, soil type, extraction method, etc.. Therefore, quality largely depends upon the lemons' origin, and aroma profiles of oils obtained throughout the world have potential to vary considerably. This research was conducted to identify differences in the aroma profiles of lemon oil samples from a variety of sources (Argentina, Brazil, California coast, California desert, Spain, South Africa). Two sensory methods commonly used to identify such differences are descriptive analysis and gas chromatography/olfactometry (GCO).

A trained sensory panel identified significant differences ($p<0.05$) in the aroma profiles of the nine tested lemon oils in terms of overall intensity, peel, lime, orange, and sweet aromas. While descriptive analysis is useful in identifying perceived product differences, it does not provide information regarding the chemical components responsible for product differences.

GCO is an effective method for identifying a flavor system's important odorants and their odor quality, but there are numerous methods by which it may be performed. Two different GCO methods, Osme and aroma extract dilution analysis with flavor dilution (FD) factors, were used to analyze cold-pressed lemon oil, and results obtained from each method were compared. Conclusions drawn from each method regarding the most critical

odorants were slightly different, and better agreement among subjects was found to be present with Osme.

Upon concluding that Osme provides a less variable method of identifying critical odorants, the relationship between Osme and descriptive analysis data was examined to determine if samples were characterized similarly by both sensory methods. Considerable agreement between methods was evident. In addition, neral, geranial, limonene, linalool, and gamma-terpinene were identified as important base contributors to lemon oil aroma.

Gas Chromatography/Olfactometry and Descriptive Analysis of Cold-Pressed Lemon Oil Aroma

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APPROVED:

Major Professor, representing Food Science and Technology

Chair of Department of Food Science and Technology

Dean of Graduate School

I understand that my thesis will become part of the permanent collection of Oregon State University libraries. My signature below authorizes release of my thesis to any reader upon request.

Sheri L. Young, Author

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Jose Flores is responsible for the financial support provided by FMC Corp. for this research. Lisa Kane assisted by collecting lemon oils samples and chemical standards necessary to complete this research. Both are responsible for identifying the need for this research and for defining its objectives.

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INTRODUCTION

Lemon oil is the second most widely used citrus essential oil, behind sweet orange oil (Robbins, 1983). The extensive use of lemon oil is due to its wide variety of applications, including foods, beverages, flavors, cleansers, cosmetics, and pharmaceuticals.

The United States (mainly Arizona and California) accounts for approximately 40% of lemon oil produced throughout the world (Staroscik and Wilson, 1982), while Italy and Argentina are also significant contributors (Robbins, 1983). Smaller contributors include Australia, Brazil, Chile, Cyprus, Greece, Guinea, Indonesia, Israel, Ivory Coast, Peru, Spain, and Venezuela (Wright, 1995). Most citrus-producing regions of the world lie within the "citrus belt", which extends from approximately 35°N latitude to 35°S latitude (Burke, 1967) and encompasses a variety of climates.

Climate affects the chemical composition of citrus oils (Stanley and Vannier, 1959; Dellacassa et al., 1995; Staroscik and Wilson, 1982). However, fruits grown in similar climates are not guaranteed to provide oils of equal quality, as there are numerous additional factors which may influence product quality.

Lemon oil composition has been studied by numerous researchers, with the most recent reviews published by Lawrence (1989) and Shaw (1979). Terpene hydrocarbons are the most abundant compounds present in lemon oil (Temelli et al., 1988), but oxygenated compounds are responsible for the characteristic aroma and flavor of lemon oil. Aldehyde content, calculated as percent citral, is the measurement most commonly used to assess lemon oil quality and serves as an indicator of commercial value.

While chemical differences among lemon oils have been documented, little work has been done to determine if such chemical differences affect the sensory properties of lemon oil. Descriptive sensory analysis is a useful tool for defining sample differences but does not identify which chemical compounds contribute to sensory differences. Gas chromatography/olfactometry (GCO) combines gas chromatography with olfactory perception and response to serve as an effective method for identifying a flavor system's important odor-active compounds and their odor quality. GCO involves mixing the GC eluate with humidified air and directing it to a sniff port, where a subject sits and describes the odor quality of the perceived odorants upon elution. There are three main objectives of GCO (DaSilva et al., 1994): 1) to identify aroma-active compounds present in a flavor system, 2) to determine the odor quality of the aroma-active compounds, and 3) to quantify the odor significance of each aroma-active compound in the flavor system.

Several methods are available for conducting GCO, including extract dilution sniffing analysis (EDSA), aroma extract dilution analysis (AEDA), and Osme. AEDA and EDSA are based on odor thresholds and determine the significance of an odorant by calculating the ratio of the concentration of the odorant to its odor threshold. This ratio was originally referred to as the aroma value (Rothe and Thomas, 1963), but various researchers have used alternate terms to describe this ratio: odor unit number (Guadagni et al., 1966), odor intensity units (Teranishi et al., 1971), odor value (Mulders, 1973), threshold odor number (Hill and Barth, 1976), Charm value (Acree et al., 1984a,b), and flavor dilution (FD) factor (Ullrich and Grosch, 1987).

Threshold-based methods operate under two basic assumptions: 1) the relationship between concentration and perceived intensity of an odorant is linear and 2) the slope of the line representing this relationship is the same for all odorants. The use of thresholds to suggest the relative contribution of odorants in mixtures has been criticized. According to Steven's Law of psychophysics, a power function best describes the relationship

between concentration and perceived intensity (Stevens, 1957). And, researchers have shown the power function exponent to be different for different odorants (Cain, 1969), with no relationship to threshold values.

To overcome the problems associated with these assumptions, a method known as Osme has been developed (McDaniel et al., 1990; DaSilva et al., 1994). Osme is based on psychophysical laws of odor perception and allows for the direct measure of an odorant's perceived intensity. This method combines GCO and time-intensity techniques by allowing subjects to record their perceptions using a time-intensity device, resulting in collection of the following information for each odorant: retention index, duration of odor detection, odor intensity, and peak area of odor detection. In addition, the odor quality of each detected odorant is verbally expressed by the subject and recorded by the researcher. Piggot (1990) commented that Osme is more satisfactory and reliable than threshold-based methods since thresholds are often poorly defined.

The objective of this research is four-fold: 1) to identify aroma differences between lemon oils obtained from Argentina, Brazil, South Africa, Spain, the California coast, and the California desert using a descriptive analysis panel, 2) to compare the results obtained and conclusions drawn, regarding critical odorants, from analysis of lemon oil using both Osme and threshold-based GCO methods, 3) to examine the relationship between Osme and descriptive analysis data, and 4) to identify compounds responsible for each sample's characteristic aroma profile.

LITERATURE REVIEW

Cold-Pressed Lemon Oil

Consumption and applications

Lemon oil is the second most widely used citrus essential oil, behind sweet orange oil (Robbins, 1983). Cold-pressed lemon oil is defined by the Food Chemicals Codex (1981) and U.S. Pharmacopoeia (1970) as, "a volatile oil obtained by expression, without the aid of heat, from the fresh peel of the fruit *Citrus limon* L. Burmann filius (Fam. *Rutaceae*), with or without the previous separation of the pulp and the peel."

Lemon oil is Generally Recognized as Safe (GRAS), and has no limitations on its use as a flavoring (Wright, 1995). A fresher, more natural citrus flavor is said to be present in foods and beverages to which lemon oil has been added. These products commonly include concentrates (Kesterson and Braddock, 1975), isotonic drinks, dry or canned teas, dry or liquid lemonade (Lodge et al., 1984), confections, pie fillings, baked goods, liqueurs, and butterscotch, pineapple, and banana prepared flavors (Robbins, 1983; Wright, 1995).

In addition, lemon oil has been utilized in the following non-food applications: perfumes, cosmetics, pharmaceuticals, insecticides, shaving creams, lotions, room fresheners, detergents, polishes, soaps, rubber, textiles, paint, and as the solvent in pet shampoos and waterless hand cleansers (Matthews and Braddock, 1987; Robbins, 1983).

Processing

Citrus oils are most commonly obtained by expression, due to their superior odor characteristics as compared to distilled oils exposed to heat, creating off-flavors and off-

odors. The absence of off-flavors has resulted in cold-pressed oils being regarded as more pure than distilled oils (Robbins, 1983). Cold-pressed oils also retain natural antioxidants not recovered during distillation, making them more stable and resistant to oxidation (Wright, 1995).

Currently, FMC Inline Extraction (FMC Corp., Lakeland, FL) and Brown Oil Extraction (Brown International, Inc., Fort Lee, NJ) are the processes most commonly used to recover cold-pressed oils. Oil is expressed from the oil glands in the fruit's peel by pressure or rasping and washed with water to form an oil-in-water emulsion. Adequate amounts of water must be used to prevent the peel from readsorbing the oil.

The FMC Inline Extraction process recovers oil during the juicing process. Upper and lower extractor cups exert pressure on the fruit, causing oil sacs to rupture. The oil is captured in a water-in-oil emulsion by means of a spray-ring. Lastly, the emulsion is centrifuged in two stages to recover the oil.

The first stage of centrifugation, desludging, involves centrifuging at 7000 - 10,000xg to separate the emulsion into three phases: 1) aqueous phase, 2) solid (sludge) phase, and 3) oil rich emulsion which consists of 60 - 80% oil. The second stage, polishing, involves centrifuging at 8000 - 10,000xg. The centrifuge continuously discharges oil while intermittently discharging aqueous waste (Matthews and Braddock, 1987).

The Brown Oil Extractor recovers lemon oil from the whole fruit, before being processed for juice. Lemons roll across a series of rollers covered with needle-like projections which pierce the oil glands and release the oils under water to prevent oxidation. A stream of water flows countercurrent to the flow of fruit, causing the formation of an oil-in-water emulsion. The emulsion is screened to remove large peel particles and centrifuged, as described above, to separate the oil and water.

After centrifugation, winterization or dewaxing may be performed by cooling the product, allowing for the precipitation of the solid waxes that cause haziness or cloudiness. This is typically conducted as a batch process in tall, narrow, 1000 - 5000 gallon stainless steel tanks with a conical bottom to facilitate precipitation. Two time/temperature combinations are commonly used for winterization. At -29°C, the oil sits 3 - 5 days to separate, followed by centrifugation to remove the precipitated wax. Alternately, at -4°C, the product sits 4 - 5 weeks before the clear oil is decanted from the precipitate (Matthews and Braddock, 1987).

For flavoring applications, it is sometimes desirable to concentrate cold-pressed oils by means of distillation or solvent extraction (Robbins, 1983). Terpenes are present in the greatest quantity but contribute little to flavor and aroma. Therefore, a portion of the terpenes, mainly limonene, is removed to concentrate the aldehydes, which are responsible for aroma and flavor. In addition to improving sensory quality of the oil, concentration improves stability and water solubility.

Regions of production

The production of lemon oil has increased drastically from approximately 544,800 kg worldwide in 1961 (Burke, 1967) to an annual production of just over 2 million kg (Wright, 1995). The United States (Arizona and California) accounts for approximately 40% of this production (Staroscik and Wilson, 1982). Italy and Argentina also contribute largely to the world's lemon oil supply (Robbins, 1983), while smaller contributors include Australia, Brazil, Chile, Cyprus, Greece, Guinea, Indonesia, Israel, Ivory Coast, Peru, South Africa, Spain, and Venezuela (Wright, 1995).

Quality and purity standards

Specifications concerning the quality and purity of lemon oil have been established by the United States Pharmacopeial Convention, Inc. (USP, 1970) and the Food Chemicals Codex (1981). Aldehyde content, calculated as percent citral and typically determined using the hydroxylamine method, must be between 2.2 - 3.8% for California lemon oil and between 3.0 - 5.5% for Italian lemon oils. Aldehyde content is the measurement most commonly used to assess quality and serves as an indicator of commercial value.

In addition, lemon oil must legally possess the following characteristics: angular rotation, 57° - 65.6°; refractive index, 1.473 - 1.476 at 20°C; and specific gravity, 0.849 - 0.855. California oils must exhibit an ultraviolet absorbance >0.2, while that of Italian oils must be >0.49. Lastly, limits on impurities are as follows: <3ppm arsenic, <40ppm heavy metals, and <10ppm lead. Analytical tests for these physical and chemical specifications are described in more detail in the Food Chemicals Codex (1981).

Composition

The composition of lemon oil is quite complex. The most abundant component of lemon oil is limonene, while alpha-pinene is also present in relatively high concentrations. Hydrocarbons are the most abundant of all compound classes present in lemon oil, but they rarely contribute much to the odor quality of an essential oil (Kimball, 1991).

Oxygenated compounds, mainly aldehydes, are responsible for the aroma and flavor of lemon oil. The main aldehyde of interest is citral, composed of the isomers of geranial and neral in a ratio of 65:35, respectively (Kimura et al., 1982) In addition, octanal, nonanal, citronellal, linalyl acetate, and geranyl acetate also contribute to the characteristic sensory properties of lemon oil (Finnemore, 1926). Table 1 provides a more detailed list

Table 1. Volatiles previously identified in lemon oil*

Hydrocarbons	alpha-bergamotene	beta-pinene
	alpha-humulene	camphene
	alpha-phellandrene	caryophyllene
	alpha-pinene	gamma-terpinene
	alpha-terpinene	limonene
	alpha-thujene	myrcene
	beta-bisabolene	p-cymene
	beta-cubebine	sabinene
	beta-humulene	terpinolene
	beta-phellandrene	
Alcohols	alpha-terpineol	linalool
	citronellol	terpinen-4-ol
	geraniol	tetrahydrogeraniol
Esters	citronellyl acetate	linalyl acetate
	decyl acetate	neryl acetate
	geranyl acetate	octyl acetate
Aldehydes	citral	heptanal
	citronellal	neral
	decanal	nonanal
	dodecanal	octanal
	furfural	undecanal
	geranial	
Ketones	carvone	
	methylheptenone	
	nootkatone	

*Lawrence, 1989; Shaw, 1979; Chamblee et al., 1991; Zeigler, 1971

of volatiles identified in lemon oil (Lawrence, 1989: Shaw, 1979; Chamblee et al., 1991; Zeigler, 1971).

Most non-volatiles in lemon oil are coumarins and psoralens and act as natural antioxidants to stabilize the oil during storage (Shaw, 1979). Coumarins have been identified as limettin, 5-isopenteneoxy-7-methoxy- and 5-geranoxy-7-methoxy-coumarin. Psoralens include isoimperatorin, bergamottin, oxypeucedanin hydrate, imperatorin, phellopterin, byakongelicin, and 5-geranoxy-8-methoxy-psoralen (Stanley and Jurd, 1971).

Factors affecting composition

Most citrus-producing regions of the world lie within the "citrus belt", which extends from approximately 35° N latitude to 35° S latitude (Burke, 1967) and encompasses a variety of climates, both tropical and subtropical. Climate, in fact, is one of the most significant factors affecting the chemical composition of citrus oils. Dellacassa et al. (1995) reported that Uruguayan lemons grown in the south provide oils higher in oxygenated compounds and lower in hydrocarbons than those grown in the north. Studies comparing oils obtained from coastal and desert fruits confirm these findings (Staroscik and Wilson, 1982; Stanley and Vannier, 1959). Staroscik and Wilson (1982) reported that California coastal lemon oils are higher in beta-pinene and richer in oxygenated compounds than desert oils from Arizona, owing to their greater sensory impact. Desert oils, however, contain greater amounts of total hydrocarbons. Other chemical constituents exhibiting differences between desert and coastal oils include linalool, nonanol, and geranyl acetate. For these reasons, desert and coastal oils are often blended to reduce variation in their physical, chemical, and sensory properties. In addition, Argentinian oils, typically more abundant in citral, are often blended with Californian oils to improve their quality (Robbins, 1983). Harvesting season also

contributes to compositional differences. When comparing seasonal (early-, mid-, and late-season) variation, the citral content in both desert and coastal oils was similar, but decreased in desert oils from later seasons while increasing in coastal oils. Since quality is based on citral content, this difference can be of great importance.

While climate and season are the most distinct factors affecting lemon oil quality, fruits grown in similar climates are not guaranteed to produce oils of equal quality, as there are many additional factors involved, including soil type, tree age, rootstock, and lemon variety (Burke, 1967).

Principal lemon-growing regions of Argentina include the Delta region, as well as the Tucuman, Salta, and Jujuy provinces. One of the most common lemon varieties grown in Argentina is an Italian variety known as Genova. Typically, lemons are grown on sour orange rootstock in deep, light, and sandy loams. The primary harvesting periods are May through August with some additional harvesting in October and November (Burke, 1967).

Due to the less than ideal climate for growing lemons in Brazil, Brazilian lemon oils typically exhibit an intense green color which is not well-accepted and is associated with poor quality (Robbins, 1993). However, the quality has reportedly improved greatly over the years. Most of Brazil's lemon trees are grown in São Paulo and Rio de Janeiro on sour orange rootstock in deep, sandy loams. The most common variety is the Eureka, and May is the primary harvesting time (Burke, 1967).

California encompasses three climatic regions: 1) the Mediterranean-like coast, 2) the desert, and 3) the interior valley, allowing for year-round harvesting (Burke, 1967). The majority of lemons grown in California are Eureka and Lisbon varieties. While coastal fruit consists of both varieties, desert fruit is exclusively the Lisbon variety. Lemon trees

grow in soils ranging from fine sand to heavy clay, and the average age of coastal trees tends to be slightly greater than desert trees (Staroscik and Wilson, 1982). Most lemons are grown on sweet orange rootstock, but some are also grown on sour orange or rough lemon (Burke, 1967).

Spain's prominent lemon-producing areas are the Mediterranean coastal regions of Murcia and Alicante, where the Berna lemon is the principal variety. Sour orange serves as the primary rootstock, and soils range from sandy, light loams to heavy, brown loams. Harvesting occurs year-round, but tends to be more abundant from April to August (Burke, 1967).

Nelspruit serves as the principal lemon-growing region of South Africa. Most lemons are grown on rough lemon rootstock in soils which range from light, sandy loams to heavy clays. Harvesting is performed throughout the year, with slightly higher activity from April to June (Burke, 1967).

Descriptive Analysis

Descriptive analysis refers to, "... the detection and the description of both the qualitative and quantitative sensory aspects of a product by trained panels... (Meilgaard et al., 1991)." Qualitative differences refer to differences in the descriptors used to characterize product appearance, aroma, flavor, and/or texture, while rating intensities of specific product attributes to discriminate between samples provides quantitative differences. Qualitatively, it is crucial for all panel members to have a clear and concise understanding of the language being used to describe specific product attributes. Likewise, panelists must be familiar with the scale and be able to use it consistently in order to provide useful quantitative data.

Qualitative and quantitative panel agreement is achieved through extensive training. Rutledge and Hudson (1990) provide a detailed account of their method for training a descriptive analysis panel. At the start of training, the panel leader must work with panelists to identify attributes which are present in the product of interest. With knowledge of the test objective, the panel leader can then eliminate descriptors which are not important for the project. Once descriptors are established, references must be developed to illustrate each descriptor. In addition, a specific method for evaluating products should be developed to help control variation between panelists. Next, the panel must become familiar with the chosen scaling technique. This may be accomplished by presenting the panel with reference standards of varying intensities to illustrate the "steps" of the scale while also reinforcing that particular product attribute. Lastly, many sessions of practice, discussion, and feedback are required to achieve panel agreement and reproducibility. While this is the general approach to training a descriptive analysis panel, several methods have been developed which provide more specific details for training and testing procedures.

Flavor profile method

One of the first formal descriptive analysis methods was the Flavor Profile Method, developed by Arthur D. Little Inc. (Cambridge, MA) in the late 1940's (Cairncross and Sjostrom, 1950). This method uses four to eight panelists, who monadically and individually evaluate aroma and flavor of test samples. Panelists identify the product's aroma and flavor character notes, then rate their intensities using a four-point intensity scale with markings at each point for barely detectable (threshold), slight, moderate, and strong (ASTM, 1996). Panelists also rate the product's overall sensory impression using a three-point scale, where 3 = high, 2 = medium, and 1 = low. Both of these scales are often further divided into half-units. Lastly, panelists indicate the order of occurrence of the perceived character notes and describe any aftertaste which may be present. At the

completion of evaluation, the panel leader leads a discussion regarding the panelists' perceptions, and a consensus profile for each sample is developed.

While this consensus procedure is useful for determining qualitative sample differences, little quantitative information is obtained. In addition, there is potential for the consensus profile to be slanted toward the perceptions of panelists with dominant personalities. Lastly, the Flavor Profile Method has been criticized for using a relatively small scale, limiting the degree of difference which can be expressed between products.

Since the Flavor Profile Method is limited to evaluation of aroma and flavor, there was a need for a method to assess product texture. Thus, General Foods Corporation developed the Texture Profile Method to define textural characteristics of foods (Brandt et al., 1963; Szczesniak et al., 1963; Szczesniak, 1963).

Texture profile method

The basis of the Texture Profile Method is the assumption that all texture attributes can be divided into three basic categories: 1) mechanical, 2) geometrical, and 3) other, with the latter consisting mainly of fat and moisture effects (ASTM, 1996). Using this method, texture attributes are defined and measured using a scale which has defined reference standards for each attribute. Increased attention is given to developing a standard method for evaluating textural properties, i.e. which teeth to bite with, how many chews, etc. Samples are rated monadically and individually, as in the Flavor Profile Method. Likewise, a consensus profile is developed through discussion and final interpretation by the panel leader. However, more recently, researchers have begun to move toward individual evaluations without consensus (ASTM, 1996).

Quantitative descriptive analysis (QDA)

A more quantitative approach to descriptive analysis, quantitative descriptive analysis (QDA®) (Stone et al., 1974), was developed by Tragon Corporation. Training by the QDA® method involves learning to describe and quantify all sensory properties, and separate panels are trained for different products. Upon developing a list of product descriptors, the terms are grouped into sensory modalities and placed in their order of occurrence within each modality (ASTM, 1996). Finally, panelists must develop a standardized procedure for evaluating the products, then learn to consistently score samples using a 15 cm line scale. As with previously described methods, QDA® requires panelists to evaluate products monadically and in individual testing booths. However, four replications are recommended for most tests (ASTM, 1996).

A major difference from the above-mentioned profiling methods is that QDA® panels do not discuss results after each evaluation session. Instead, the data are entered into a computer and subjected to statistical analysis, often analysis of variance, to provide quantitative measures of product differences. Results are reported in either tabular form with numerical values or in graphical form.

All three previously mentioned methods are criticized for the way in which they use references. Although references are used to illustrate the character of a particular descriptor, no direction is provided in rating the intensity of references. Therefore, it is more difficult to achieve panelist agreement in terms of scale use, and a more structured approach to scaling and references may be useful.

Spectrum method

The Spectrum™ Method has been developed by Gail Civille at Sensory Spectrum (Chatham, NJ) to overcome some of the previously mentioned criticisms. The Spectrum™ Method relies heavily upon use of references to identify differences in attribute intensities with an absolute scale, rather than a relative scale. Thus, a universal scale has been developed, with two to five anchored points distributed along the scale. The universal scale remains unchanged regardless of product, allowing for more useful comparisons across products. Use of reference points also allows for more accurate comparisons with instrumental measurements and data obtained over time. Data are subjected to statistical analysis as with QDA® (Meilgaard et al. 1991).

While each individual method has definite procedures to follow for descriptive analysis training and testing, it is often more practical to use a combination of methods to fulfill the test and/or project objectives. Therefore, it is at the discretion of the panel leader to determine which procedures are most suitable for attaining the desired goals.

Olfaction and Odor Perception

Odor refers to the sensory perception of smells via the olfactory system. Olfaction is very different from hearing, sight, taste, and touch in that it is closely linked with the area of the brain associated with emotion and memory (Almagor, 1990), making it very powerful in our lives.

For a substance to be odorous, it must be volatile. Upon volatilization, molecules released by the substance enter the nasal passages, where they are adsorbed by the nasal passage walls. After a short period of time, the molecules are released, or desorbed. Although the exact mechanisms of adsorption and desorption are not fully understood, it

is believed that molecules of different sizes and shapes adsorb for different lengths of time before desorption occurs (Moncrieff, 1970).

Originally, animals used their sense of smell to distinguish between toxic and non-toxic plants (Goldstein, 1989). Although humans do not rely on their sense of smell for survival, human perception of odors is quite keen. Humans are capable of detecting odors at very low concentrations and can recognize more than 2000 odors if untrained and more than 10,000 if trained (Engen, 1982).

Although the human nose is extremely sensitive, numerous chemical, physical, and psychological factors affect the ability of humans to detect, classify, and discriminate between odors. The mechanisms behind many of these factors are not well understood, but researchers agree that they contribute significantly to differences in perception and odor thresholds among subjects.

One factor which greatly affects odor perception is familiarity with the odor and the context in which it is normally perceived. As a result of the link between odors and the emotional part of the brain, many odors recall memories of past experiences, both pleasant and unpleasant. This phenomenon is partly responsible for situations where a single odor is perceived as pleasant by one person and unpleasant by another.

Age is another factor affecting odor perception, with researchers finding that odor acuity and identification ability decrease with increasing age (Cain et al., 1990). Peak performance, in terms of odor identification, is believed to occur throughout the third, fourth, and fifth decades of life, while performance declines markedly after the seventh decade (Doty, 1989). Lawless (1985) reported that young children respond and react to pleasant and objectionable odors quite differently than adults. In addition, Stevens and

O'Connell (1991) observed that older subjects (over 35) possessed lower thresholds for pemenone than younger subjects.

The effect of gender on odor perception was addressed by a National Geographic survey of volunteers which demonstrated that females scored higher than males in both odor identification and odor recognition (Wysocki and Gilbert, 1989). However, the researchers believe that women are not necessarily more sensitive, but more capable of verbally expressing their odor perceptions.

While age and gender definitely appear to affect odor perception, there are numerous factors whose effects are less clearly defined. This is evident when considering that Marin et al. (1988) reported significant differences in odor detection thresholds between individuals of the same gender and age.

Additional sources of variation may include differences in personality types, smoking status (Moncrieff, 1970), menstrual status, pregnancy, hunger, and mood (Maruniak and Mackay-Sim, 1984). As a result of these individual differences, odor perception is unique to every individual, and it is virtually impossible to develop a "universal" understanding of odor.

In addition to subject variability, the method of odorant presentation affects odor perception. Different sniffing techniques affect the amount and flow rate of air entering the nasal passages. Altering sniffing rates by forcing air through the nostrils leads to variable stimulus intensity perception. Natural sniffing results in an air delivery volume of about 150 cm^3 (Laing, 1983) and relatively constant perceived intensities for constant stimuli, a phenomenon known as odor constancy (Engen, 1991). Weaker sniffs draw in a smaller volume of air, and the perceived stimulus intensity decreases. However, stronger

sniffs take in larger volumes of air, but perceived stimulus intensity does not drastically increase.

Lastly, physical parameters of odorants affect perception. Of great influence is substance concentration. Although odor quality remains constant at most concentrations, odor intensity may change drastically with slight alterations in concentration since small increases in concentration correspond to large increases in the number of volatilized odor molecules. Temperature and surface properties also affect volatility of substances, resulting in variations in perceived intensity (Meilgaard et al., 1991). Lastly, Theimer and McDaniel (1971) reported differences in odor quality and intensity for chemical isomers.

Gas Chromatography/Olfactometry

The most sensitive gas chromatograph can detect compounds in concentrations of 10^9 molecules/ml (Meilgaard et al., 1991). However, some substances can be detected by the nose in concentrations of 10^7 molecules/ml (Harper, 1972). Therefore, gas chromatography alone cannot predict the compounds of importance in flavor systems. To overcome this problem, gas chromatography has been combined with olfactory perception and response to yield a technique known as gas chromatography/olfactometry (GCO). Soon after the invention of the gas chromatograph in 1952, researchers began sniffing the GC effluent to determine the odor quality of chemicals of interest (Acree, 1997). Of course, this technique had several distinct disadvantages, including risk of burning oneself and detection of off odors due to high detector temperatures. Thus, researchers began designing more sophisticated sniff ports to overcome these problems and improve upon results obtained by GC sniffing. A more detailed review of sniff port evolution is discussed by Acree (1997). Today, GCO involves mixing the effluent with humidified air to decrease drying of nasal membranes.

Researchers initially involved with GC sniffing were mainly interested in describing the odor quality of eluting compounds. These studies provided useful information regarding odor-active compounds and their odor quality, but limited attention was given to intensity or duration of the compounds detected. As time elapsed, scientists became interested in collecting more information regarding the odor-active compounds' significance to the product's flavor system. As a result, the current purpose of GCO is three-fold (DaSilva et al., 1994): 1) to identify odor-active compounds in flavor systems, 2) to determine the odor quality of each odor-active compound, and 3) to quantify the odor significance of each odor-active compound in the flavor system. Therefore, a more quantitative approach was developed to predict odor importance and improve the usefulness of GCO.

GC sniffing was combined with traditional threshold determination methods, leading to the concept of the aroma value (Rothe and Thomas, 1963). An odorant's aroma value is the ratio of its concentration to its odor detection threshold. Alternate terms have been used to describe this ratio, including the odor unit number (Guadagni et al., 1966), odor intensity unit (Teranishi et al., 1971), odor value (Mulders, 1973), and threshold odor number (Hill and Barth, 1976). Use of these ratios requires time-consuming quantification and threshold determination for each odorant, detracting from the method.

A technique known as aroma extract dilution analysis (AEDA) was developed to make use of threshold data more efficient and valuable. This is achieved by determining the relative odor potency of each odorant within a sample. The odor potency is determined by conducting GCO on serial dilutions until no odor is perceived by the subject. This information is then incorporated into various methods of data analysis, including CharmAnalysis (Acree et al., 1984a,b) and flavor dilution (FD) factor analysis (Ullrich and Grosch, 1987).

Acree et al. (1984a) developed Charm, a bioassay to measure biological activity of odorants in a flavor system. Charm gets its name from the common meaning '... a feature in something or someone that attracts or delights people', but has also been reported to be an acronym for Combined Hedonic Response Measurement (Acree et al., 1984b). The length of time an odorant is detected and the its odor quality are recorded. Data are converted to Charm values, dimensionless measures of odor intensity, using the following equation:

$$c = d^{(n-1)}$$

where d = the dilution factor used in preparing subsequent dilutions and n = the number of dilutions in which the odorant of interest was detected. A series of hydrocarbons or n-paraffins are chromatographed to assist in determining retention indices.

Charm values are plotted against retention indices to obtain a Charm chromatogram. Charm values are inversely related to the panelist's odor detection threshold for that particular odorant in the gaseous state. Odorants with higher Charm values are assumed to be the most critical contributors to the sample's overall aroma character.

CharmAnalysis has been used successfully for numerous applications. Cunningham et al. (1986) characterized apple volatiles of 40 different apple cultivars, while Marin et al. (1992) used CharmAnalysis to determine the sample constituents contributing the most to the aroma of pasteurized, mechanically-squeezed and fresh, hand-squeezed orange juices. Acree and Cottrell (1985) utilized CharmAnalysis to aid in developing chemical indices for measurement of wine quality, while Moio et al. (1993) analyzed water buffalo and bovine mozzarella cheeses to determine which odorants are responsible for aroma and flavor similarities and/or differences between the two cheeses.

Ullrich and Grosch (1987) applied the concept of flavor dilution (FD) factors to express the significance of each odorant in the sample's overall aroma. FD factors are the

last dilution at which an odorant is detected at a specific retention index. Odorants which possess higher FD factors are considered to contribute more to the sample's aroma profile. This technique has been used to confirm flavor and aroma differences in lump and claw blue crab meats (Chung et al., 1995) and to characterize the most potent aroma volatiles of Cheddar cheese (Christensen and Reineccius, 1995) and a lemon oil/citric acid emulsion (Schieberle and Grosch, 1988).

Abbott et al. (1993) compared the use of Charm values and FD factors in interpreting GCO data for beer extracts. They concluded that it is difficult for subjects to consistently detect the end of an odor peak, possibly resulting in large errors in Charm peak areas. However, they also felt that CharmAnalysis may be more useful than FD factors in determining the importance of individual odorants to a product's overall aroma profile since it incorporates peak areas. Ultimately, it is recommended that data be treated both ways and compared (Abbott et al., 1993).

Although threshold-based methods of GCO have been used extensively for food and beverage research, they have been criticized due to the two assumptions on which they are based: 1) the relationship between an odorant's concentration and it's perceived intensity is linear and 2) the slope of the plot for perceived intensity vs. concentration is equal for all odorants. According to Steven's Law of psychophysics, a power function best describes the relationship between concentration and perceived intensity (Stevens, 1957). And, researchers have shown the power function exponent to be different for different odorants (Cain, 1969).

Osme, the Greek word meaning "odor", is a GCO method developed at Oregon State University and first reported by McDaniel et al., (1990). Osme is based on psychophysical laws and attempts to overcome the previously mentioned problems associated with threshold-based methods. Piggot (1990) commented that threshold

methods are somewhat unreliable due to poorly defined thresholds, and also stated that Osme is a more satisfactory GCO method.

Osme combines time-intensity and GCO to provide the analyst with the following information for each odorant: 1) retention index (in conjunction with a series of hydrocarbons chromatographed under identical analytical conditions), 2) duration of odor detection, 3) intensity of odor as determined through use of a time-intensity device labeled with a 16-point intensity scale, and 4) peak area. In addition, the odor quality of each perceived odorant is verbally described by the subject and recorded by the researcher. After obtaining this data, odor intensity or peak area is plotted against retention time to form an Osmegram.

DaSilva et al. (1994) used Osme to determine the relationship between a compound's concentration and it's sensory response. The author found that plotting odor intensity vs. stimulus concentration yields a power function while peak area vs. stimulus concentration yields a linear relationship. Osme has also been used to identify the most critical odorants in extracts of beers brewed with and without hops (Sanchez et al., 1992) and to characterize the aroma and flavor of a corn-based snack throughout storage (DaSilva et al., 1993). Bazemore (1995) and Plotto (1995) utilized Osme to obtain aroma/flavor profiles of aqueous orange essence and Gala apples, respectively.

An additional advantage of Osme is its use of more subjects and replications than that reported by most researchers using threshold-based GCO methods. While Osme typically uses three or four subjects with three or four replications of each sample, threshold methods often use only one or two subjects with very few, if any, replications. Two notable exceptions include the comparison of Charm and FD factor analysis (Abbott et al., 1993) in which six subjects completed two replications and the study by Chung et al. (1995) which incorporated two subjects and three replications.

Sensitivity varies considerably across individuals and across time within the same individual. Due to this inconsistency, use of several subjects is beneficial in obtaining a more general overview of a product's profile, whereas use of only one subject severely limits the inferences which can be drawn regarding the samples of interest.

Regardless of the method of choice, GCO is subject to a number of criticisms. In a review of aroma extract dilution analysis (AEDA), Grosch (1993) points out that the number of odorants detected in the GC effluent depends upon several factors. The odor threshold of each odorant, as well as the sensitivity of the subject performing the sniffing task, will determine the number of peaks identified. This was confirmed by Abbott et al. (1993), who observed that different subjects set different response criteria for reporting the detection of a peak. Some subjects are conservative and report fewer peaks than a subject with a more liberal response criteria. In addition, these authors observed that it is more difficult to detect the end of an odor peak than its beginning, and the sensitivity of individuals changes throughout a single day as well as over longer periods of time. Grosch (1993) also mentioned that the amount of food sampled, dilution of volatiles by the solvent, and the amount of sample injected are additional factors of influence in the number of peaks detected. For these reasons, Grosch concluded that GCO techniques can only be considered screening procedures.

DESCRIPTIVE ANALYSIS OF COLD-PRESSED LEMON OIL AROMA

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Abstract

Cold-pressed lemon oil is expressed from lemon peel, then incorporated into various products, improving their aroma and flavor. Lemon oil quality is affected by numerous factors including lemon variety, climate, soil type, extraction method, etc.. Therefore, quality largely depends upon the lemons' origin, and aroma profiles of oils obtained throughout the world have potential to vary considerably. This study was conducted to identify differences in the aroma profiles of lemon oil samples from a variety of sources (Argentina, Brazil, California coast, California desert, Spain, South Africa), as determined by a trained sensory panel. Samples were significantly different ($p<0.05$) in terms of overall intensity, peel, lime, orange, and sweet aromas.

Introduction

Lemon oil is the second most widely used citrus essential oil, behind sweet orange oil (Robbins, 1983). The extensive use of lemon oil is due to its wide variety of applications. In addition to numerous food and beverage applications, lemon oil is also incorporated into a variety of non-food items, including cleansers, insecticides, and pharmaceuticals.

The United States (mainly Arizona and California) accounts for approximately 40% of lemon oil produced throughout the world (Staroscik and Wilson, 1982). Italy and Argentina also contribute largely to the world's lemon oil supply (Robbins, 1983), while smaller contributors include Australia, Brazil, Chile, Cyprus, Greece, Guinea, Indonesia, Israel, Ivory Coast, Peru, Spain, and Venezuela (Wright, 1995). Most citrus-producing regions of the world lie within the "citrus belt", which extends from approximately 35°N latitude to 35°S latitude (Burke, 1967) and encompasses a variety of climates, both tropical and subtropical.

Climate has been shown to affect the chemical composition of citrus oils (Stanley and Vannier, 1959; Dellacassa et al., 1995; Staroscik and Wilson, 1982). Staroscik and Wilson (1982) determined that coastal oils obtained from California are higher in oxygenated compounds than desert oils obtained from Arizona. In the United States, desert and coastal fruit oils are often blended to reduce variation in their physical, chemical, and sensory properties (Staroscik and Wilson, 1982). In addition, Argentinian oils, typically more abundant in citral, are often blended with Californian oils to improve their quality (Robbins, 1983). While climate greatly affects lemon oil composition, fruits grown in similar climates are not guaranteed to provide oils of equal quality, as there are numerous additional factors which may influence product quality. Among these factors are fruit variety, rootstock, soil type, tree age, season of harvest, and method of extraction.

Lemon oil quality and purity are measured by analytical methods and standards set by the United States Pharmacopeial Convention, Inc. (USP, 1970) and the Food Chemicals Codex (1981), the latter being officially recognized by the Food and Drug Administration. These measures include assessment of aldehyde content calculated as percent citral, angular rotation, refractive index, specific gravity, ultraviolet absorbance, solubility, and arsenic, lead, heavy metal, and foreign oil contents. Aldehyde content is most commonly used to assess quality and serves as an indicator of commercial value.

Lemon oil composition has been studied by numerous researchers with the most recent reviews published by Lawrence (1989) and Shaw (1979). These studies have identified aldehydes, esters, terpenes, sesquiterpenes, alchohols, ketones, and several non-volatile compounds as constituents of lemon oil. Terpene hydrocarbons are the most abundant compounds present in lemon oil, with d-limonene accounting for more than 90% of the oil's composition. However, terpenes typically contribute little to lemon oil aroma (Kimball, 1991). Oxygenated compounds, mainly aldehydes, are responsible for the

characteristic aroma and flavor of lemon oil, with citral being the greatest contributor. Thus, oils rich in citral tend to exhibit a stronger lemon aroma and flavor than those rich in hydrocarbons.

Despite these compositional studies, few researchers have made comparisons between the aroma profiles of oils obtained from a variety of locations. This study involves descriptive analysis of lemon oil aroma by a trained sensory panel. Samples, all produced using an FMC Inline Extractor, were obtained from various locations around the world including Argentina, Brazil, South Africa, and two each from Spain, the California coast, and the California desert. The objective was to determine if any aroma differences exist between samples. This information could be useful to the food, pharmaceutical, cosmetic, and textile industries when choosing oils for their product formulations.

Materials and Methods

Quality and purity measurements

Refractive index, UV absorption, and aldehyde content determinations were conducted in triplicate for all lemon oil samples using procedures outlined by Kimball (1991) in accordance with the United States Pharmacopeia (1970) and Food Chemicals Codex (1981) standards (Table 2). Refractive index measurements were taken using an Auto Abbe Refractometer, Model 10500 (Leica Inc., Buffalo, NY). A Shimadzu UV 160U visible spectrophotometer (Shimadzu Corp.) with a cell pathlength of 1cm was used to measure UV absorption. For determining aldehyde content, hydroxylamine hydrochloride was reacted with lemon oil to form hydrochloric acid, which was then titrated to pH 3.5 with sodium hydroxide. Titrations were conducted with a Brinkmann Multi-Dosimat (model 655) equipped with a digital pH meter (model 605) and

Table 2. Cold-pressed lemon oil samples and their quality/purity measurements

Origin	Aldehyde content[a]	Refractive Index[b]	UV Absorption[c]	Color[d]
Argentina	3.56	1.47475	0.920	golden yellow, slight green/olive
Brazil	4.06	1.47481	0.900	dark greenish-yellow
California coastal A	3.14	1.47476	0.946	golden yellow, slight green/olive
California coastal B	3.21	1.47473	0.879	golden yellow, slight green/olive
California desert A	2.19	1.47476	0.976	golden yellow, slight green/brown
California desert B	2.56	1.47477	0.971	golden yellow, slight green
Spain A	2.51	1.47473	0.827	pale yellow
Spain B	2.85	1.47520	0.852	bright yellow, lemon yellow
South Africa	3.13	1.47455	0.594	pale/light yellow

Specifications for lemon oil (United States Pharmacopeia, 1970; Food Chemicals Codex, 1981)

[a] 2.2 - 3.8% for California, 3.0 - 5.5% for Italian - calculated as % citral

[b] 1.473 - 1.476 (at 20°C)

[c] $\geq$ 0.20 for California, $\geq$ 0.49 for Italian (at 315nm)

[d] visual assessment by four panelists

Impulsomat (model 614) (Metrohm Ltd., Herisau, Switzerland). Lastly, visual color assessments were collected from four panelists to document perceived color differences.

Sample origin and storage

Samples were supplied by FMC Corp. (Lakeland, FL). All lemon oils were obtained from the Fall 1996 lemon harvest and extracted by each individual processor using an FMC Inline Extractor. Samples were placed in 4.0ml amber glass vials, flushed with nitrogen gas and stored in darkness at 2°C to prevent oxidation.

Sample preparation

For evaluation, samples were prepared by immersing a fragrance testing filter (Orlandi, Inc., Farmingdale, NY) 1.3 cm into the oil. The lower 1.3 cm piece was cut off and placed into a 300ml amber glass, then covered with an aluminum lid.

Panel training

The trained panel consisted of eleven (eight female and three male) student volunteers from Oregon State University. Panelists were trained during 19 one-hour sessions, over a period of six weeks, to describe and rate aroma descriptors of various lemon oils, including those listed in Table 2.

The final ballot included the 13 descriptors listed in Table 3, which also includes standards and reference values for each descriptor. Samples were evaluated using a 16-point intensity scale to rate the intensity of each descriptor where 0 = none, 3 = slight, 7 = moderate, 11 = large, and 15 = extreme. Panelists were required to review all aroma standards prior to testing each day.

Table 3. Lemon oil descriptors, standards, and intensity scale values

Descriptor	Standard	Intensity Scale[a] Value
Overall Intensity	none	none[d]
Peel[b]	score peel with knife to release oils, cut into small (2mm x 4mm) pieces, combine 2.5g lemon peel, 1.5g lime peel, 1.0g orange peel, 1.0g grapefruit peel	none[d]
Lime[b]	1/4 fresh lime	none[d]
Lemon[b]	1/4 fresh lemon	none[d]
Lemon Grass	0.2 ul lemon grass oil on end of fragrance testing filter (Orlandi, Inc., Farmingdale, NY) in 300ml amber glass covered with aluminum lid	8
Orange[b]	1/8 fresh Valencia orange	none[d]
Grapefruit[b]	1/8 fresh Ruby grapefruit	none[d]
Sweetness[b]	1 piece of "Nobel" Super Lemon candy (Nobel Confectionery Co., Ltd., Japan), crushed	6
Oxidized[b]	1 tsp. ReaLemon lemon juice from concentrate (Borden, Inc., Columbus, OH) in 10ml distilled water	10
Grassy[c]	50ml of 25ppm cis-3-hexen-1-ol (Fluka, Ronkonkoma, NY) in distilled water	7
Floral[c]	50ml of 0.1ppm violet oil (Uncommon Scents, Eugene, OR) in distilled water	9
Musty[c]	50ml of 50ppm terpinen-4-ol (Aldrich, Milwaukee, WI) in distilled water	7
Metallic[c]	a variety of brass nuts and bolts	none

[a] 16-point intensity scale: 0 = none, 3 = slight, 7 = moderate, 11 = large, 15 = extreme
[b] presented in 240ml clear wine glass with aluminum lid
[c] presented in 120ml clear glass jar with teflon-lined lid
[d] no scale value due to inherent variation in fresh produce

Experimental design and sample presentation

Samples were coded with three-digit random numbers, served monadically, and evaluated in individual testing booths. A randomized, complete block design was used with four replications (four consecutive testing days). Samples for all four replications originated from the same batch. Each day, panelists evaluated each of the nine samples with a break of at least 15 minutes after every third sample. Serving order was randomized across trays and panelists.

A specific sample evaluation routine was adopted to allow for both sample and panelist recovery between sniffs. Each sample was required to sit for two minutes after preparation to allow for headspace accumulation. Then, panelists were permitted to take their first sniff and rate as many descriptors as possible. The sample was then covered for one minute before being evaluated the second time. The cycle was repeated until a maximum of three sniffs were performed. Only three sniffs were permitted to minimize sample oxidation from excess exposure to air.

Data analysis

Data were collected in the testing booths using Computerized Sensory Analysis (CompuSense Inc., Guelph, Ontario, Canada). Interaction plots between sample and panelist were constructed for each individual descriptor. From these plots, it was apparent that one panelist was rating many descriptors inconsistently. This poor performance was supported by many large standard deviations. As a result, the panelist's data were eliminated, leaving ten panelists' data for analysis.

Data were exported into SAS (SAS, Inc., Cary, NC) and analyzed per descriptor using analysis of variance (Proc MIXED) and pairwise comparisons with a Type I error of 0.05 to determine significant differences among treatments. Pairwise comparisons were

conducted using a series of t-tests. The analysis of variance model included panelists, replication, sample, and all possible interaction terms. Panelists (and all interaction terms including panelist) were treated as random effects, using the three-way interaction term as the error term, to increase the inference which could be drawn from the study.

Data were also analyzed by Principal Component Analysis (PCA) using SAS to determine which descriptors were responsible for the most variation between samples. Results were averaged across panelists for each sample replication, resulting in 36 data points on the PCA plot (4 reps x 9 samples). The separation of samples on each principal component was determined by conducting analysis of variance (Proc GLM) on these 36 data points.

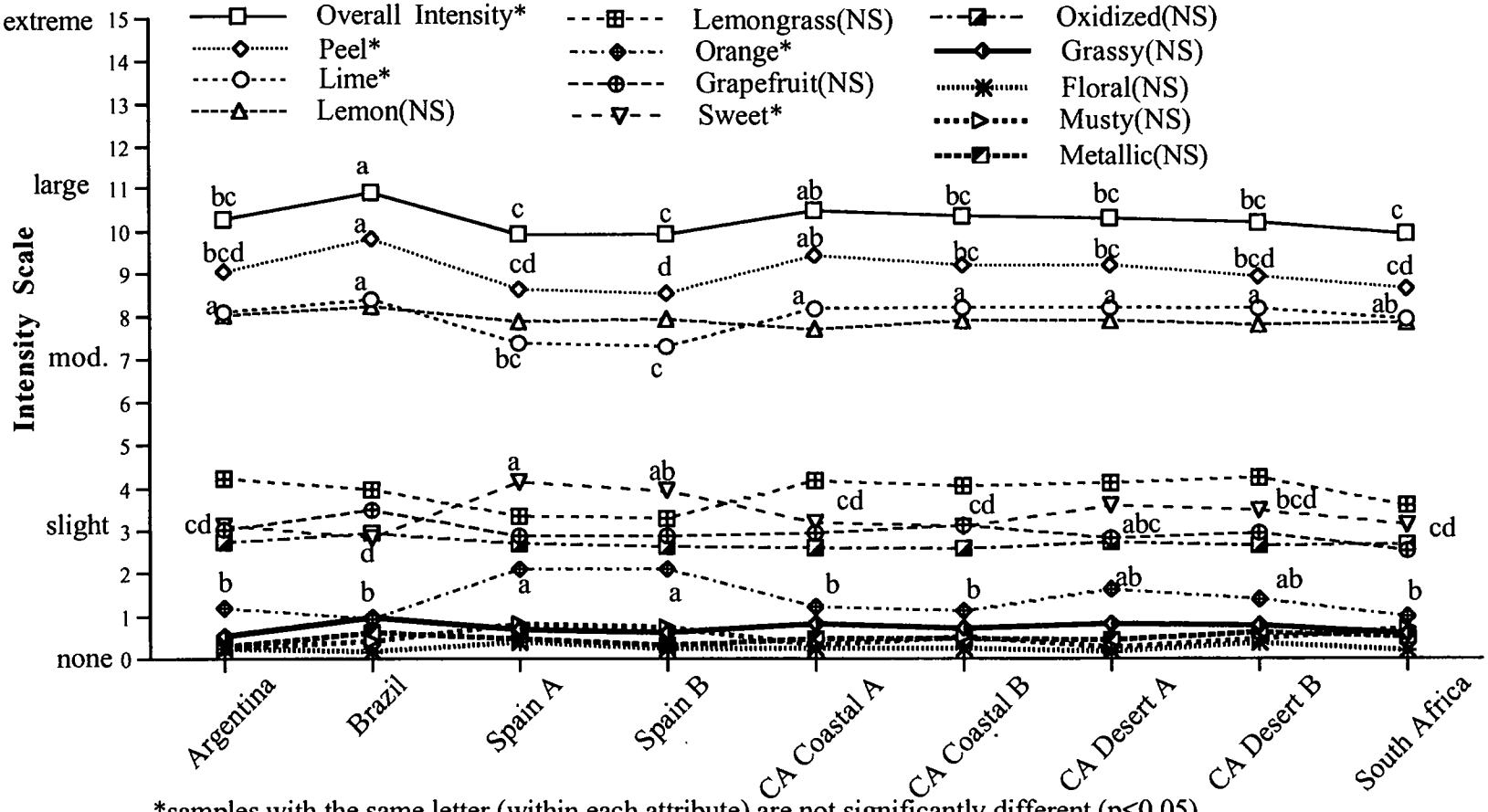
Results and Discussion

The results obtained for refractive index and UV absorption indicate that all samples fall within the accepted purity limits (Table 2). All samples also lie within the established aldehyde content range for Californian lemon oil except the Brazilian sample, which is considerably higher in aldehyde content. Since citral is often used as a predictor of quality, the higher aldehyde content of the Brazilian sample may suggest that it is of higher quality than all other samples analyzed in this study. Interestingly, the color of the Brazilian oil was much darker and more green than all other samples.

Results obtained from analysis of variance are illustrated in Figure 1. Significant sample differences were present for the following descriptors: overall aroma intensity ($p = 0.0013$), peel ($p = 0.0001$), lime ($p = 0.0007$), orange ($p = 0.0419$), and sweet ($p = 0.0024$) aromas.

In terms of overall aroma intensity and peel aroma, the Brazilian and California coastal A samples were rated significantly ($p<0.05$) more intense than samples obtained from

Figure 1. Intensity ratings for all lemon oil aroma descriptors



*samples with the same letter (within each attribute) are not significantly different ($p<0.05$)
(NS) = no significant sample differences
Each data point represents 40 observations

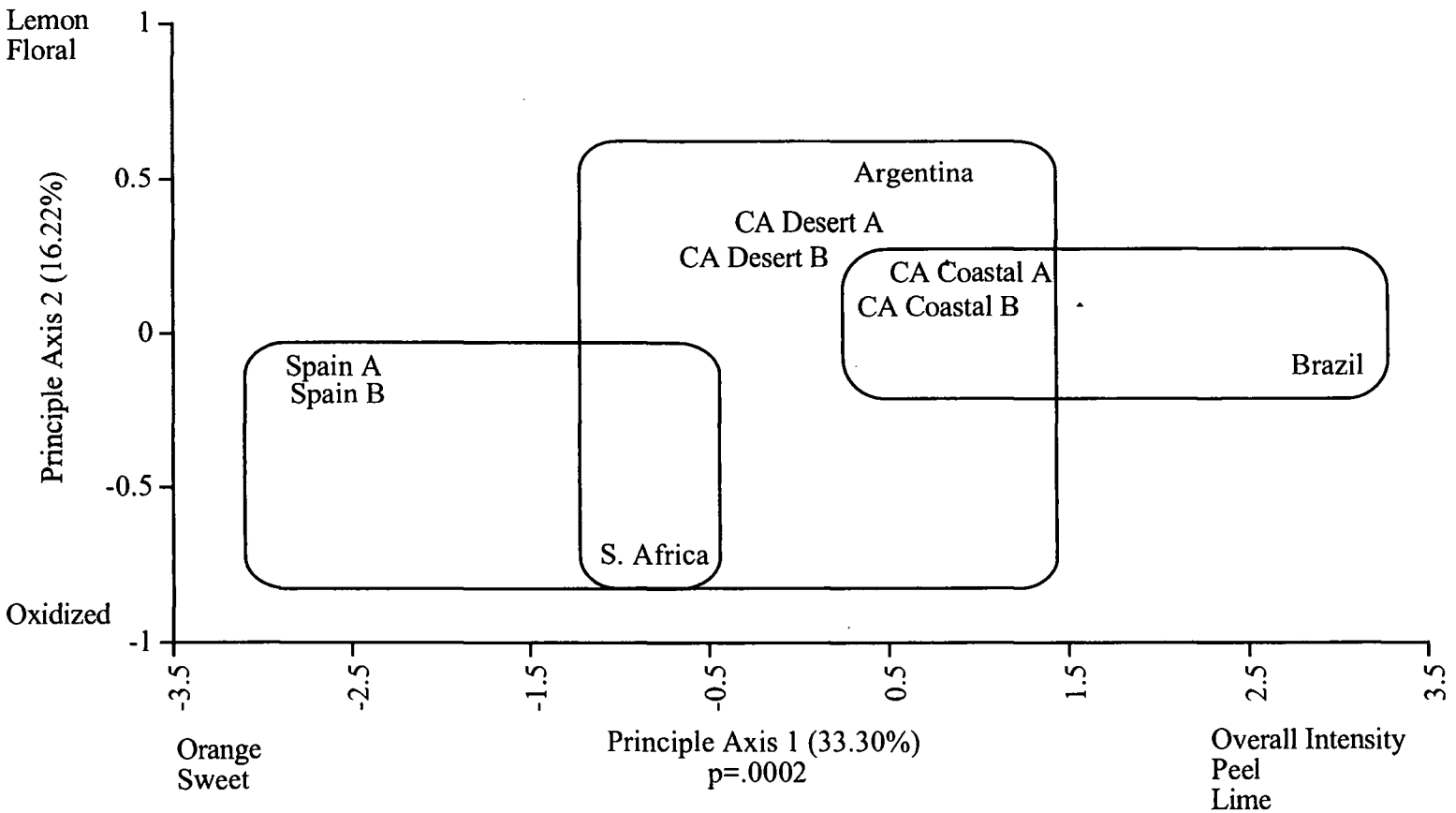
Spain and South Africa. The Brazilian sample exhibited the highest overall aroma intensity among all samples, and as mentioned earlier, also contained the most aldehydes. This is most likely due to the contribution of citral to the aroma and flavor quality of lemon oil, resulting in a greater sensory impact.

Samples from Argentina, Brazil, and California (all four) were significantly higher in lime aroma than both Spanish oils. Orange aroma was rated significantly higher for the Spanish samples, as compared to those obtained from South Africa, Brazil, Argentina, and the California coast (both A and B). The same trend was present for sweet aroma.

Principal Component Analysis (PCA) indicated that overall aroma intensity, peel, lime, orange, and sweet defined the first principal component, accounting for 33% of the variation between samples (Figure 2). Principal component 2 was defined by lemon, floral, and oxidized aromas, accounting for 16% of the variation between samples. The first principal component significantly separated samples with a p-value of 0.0002, but no significant differences existed on the second principal component. For simplicity, the average of four replications is shown for each sample.

The Brazilian sample was rated high in peel and lime aromas and low in orange and sweet aromas, while the Spanish samples exhibited the opposite trends. Samples from California, South Africa, and Argentina clustered in the center of the plot. Among Californian samples, those obtained from coastal regions were slightly higher in overall aroma intensity than those from desert regions. Coastal oils were also higher than desert oils in aldehyde content (Table 2). These results are in agreement with Staroscik and Wilson (1982) who compared the composition of oils obtained from both coastal and desert fruits of California and Arizona, respectively, using glass capillary gas chromatography. They found that coastal lemon oils are richer in oxygenated compounds, mainly citral, owing to their greater sensory impact. Desert oils, however,

Figure 2. Principal components plot of lemon oil samples



**samples within the same group are not significantly different ($p<0.05$) on Principal Axis 1
Each data point represents 40 observations

contain greater amounts of total hydrocarbons, including limonene and β-pinene, which contribute little to lemon oil aroma and flavor. Stanley and Vannier (1959) also reported finding higher citral contents for California coastal oils, as compared to oils obtained from inland areas.

The results obtained from this observational study are specific to lemon oils obtained using the FMC Inline Extractor, as different extractors are likely to result in oils of different composition and quality (Matthews and Braddock, 1987; Lawrence, 1989). Although oils obtained from the countries studied here may exhibit different characteristics each season due to factors discussed earlier, differences observed in this study were highly significant and similar trends are likely to be present from season to season. Lastly, there is evidence that citral content is directly related to the overall aroma intensity, and perhaps the peel aroma, associated with cold-pressed lemon oil.

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COMPARISON OF GAS CHROMATOGRAPHY/OLFACTOMETRY TECHNIQUES FOR THE ANALYSIS OF COLD-PRESSED LEMON OIL AROMA

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Abstract

Gas chromatography/olfactometry (GCO) is a method for identifying a flavor system's critical odorants and their odor quality. With the advent of GCO came numerous methods by which it may be performed. The objective of this study was to determine the differences in results obtained from analysis of cold-pressed lemon oil aroma using two different GCO methods: Osme and aroma extract dilution analysis with flavor dilution (FD) factors. Conclusions drawn from each method regarding the most critical odorants were slightly different. Better agreement between subjects was found to be present using Osme.

Introduction

Gas chromatography/olfactometry (GCO) involves combining gas chromatography with olfactory perception and response. The development, significance, and future of GCO has been reviewed by Acree (1997). Currently, the most commonly used GCO techniques involve mixing the GC eluate with humidified air and directing it to a sniff port, where a subject sits and describes the odor quality of the perceived odorants upon elution. There are three main objectives of GCO (Da Silva et al., 1994): 1) to identify aroma-active compounds present in a flavor system, 2) to determine the odor quality of aroma-active compounds, and 3) to quantify the odor significance of each aroma-active compound in the flavor system.

Several methods are available for conducting GCO, including extract dilution sniffing analysis (EDSA), aroma extract dilution analysis (AEDA), and Osme. Data obtained from AEDA or EDSA can be used to derive Charm values (Acree et al., 1984a,b) and flavor dilution (FD) factors (Ullrich and Grosch, 1987). Grosch (1993) reviewed the usefulness of AEDA in identifying the most critical odorants in various foods. In addition, Abbott et al. (1993) conducted a study using EDSA to compare data treated to

yield both Charm and FD factors. The authors concluded that CharmAnalysis may be more useful than FD factors in determining the significance of individual odorants to a product's overall aroma profile since it incorporates peak areas. However, they also concluded that there may be more variation in Charm values due to subjects' difficulty in detecting the end of odor peaks. Ultimately, Abbott et al. (1993) recommended treating data both ways and comparing results.

AEDA and EDSA are based on odor thresholds and determine the significance of an odorant by calculating the ratio of the concentration of an odorant to its odor threshold. This ratio was originally referred to as the aroma value (Rothe and Thomas, 1963), but various researchers have used alternate terms to describe this ratio: odor unit number (Guadagni et al., 1966), odor intensity unit (Teranishi et al., 1971), odor value (Mulders, 1973), threshold odor number (Hill and Barth, 1976), Charm value (Acree et al., 1984a,b), and flavor dilution (FD) factor (Ullrich and Grosch, 1987).

Threshold-based techniques are implemented with GCO chromatographic runs on serial dilutions until no odor is perceived by the subject. Odorants perceived in the most dilute samples are assumed to be most critical to the sample's aroma and flavor. Using these methods, the retention index of each odorant, the duration of odor detection, and the odorant's odor quality are obtained.

Threshold-based methods operate under two basic assumptions: 1) the relationship between concentration and perceived intensity of an odorant is linear and 2) the slope of the line representing this relationship is the same for all odorants. The use of odor thresholds to suggest the relative contribution of odorants in mixtures has been criticized. According to Steven's Law of psychophysics, a power function best describes the relationship between concentration and perceived intensity (Stevens, 1957). And,

researchers have shown the power function exponent to be different for different odorants (Cain, 1969), with no relationship to threshold values.

To overcome the problems associated with these assumptions, a method known as Osme has been developed (McDaniel et al., 1990; DaSilva et al., 1994). Osme, named after the Greek word meaning "odor", is based on psychophysical laws of odor perception. Unlike the previously mentioned threshold techniques, Osme allows for a direct measure of an odorant's perceived intensity. This method combines GCO and time-intensity techniques by sniffing only one dilution and allowing subjects to record their perceptions using a time-intensity device labeled with a sliding 16-point intensity scale, resulting in collection of the following information for each odorant: retention index, duration of odor detection, odor intensity, and peak area of odor detection. Additionally, the odor quality of each odorant is verbally described by the subject and recorded by the researcher. Sieffermann et al. (1996) reported the development of a GCO technique based on Osme, citing its advantage over Charm and aroma extract dilution analysis in terms of ability to evaluate odorant intensity. In addition, Piggot (1990) commented that Osme is more satisfactory and reliable than threshold-based methods since thresholds are often poorly defined.

Osme is a GCO technique still in its developmental stages. Therefore, a standard method of data analysis has not yet been adopted in terms of whether peak area or peak height is most useful in drawing conclusions. Most studies involving Osme have used peak height, a measure of maximum intensity, as the predictor of odorant importance (McDaniel et al., 1990; Sanchez et al., 1992; DaSilva et al., 1993; Bazemore, 1995). DaSilva et al. (1994) analyzed model solutions containing various concentrations of known compounds to determine the relationship between stimulus concentration and sensory perception. The authors determined that peak area is a more accurate predictor

of perception when related to concentration for some compounds, while peak height is better for others, concluding that the relationship is highly compound-dependent.

The objective of this study was to identify differences in results obtained from analysis of cold-pressed lemon oil using both Osme and threshold-based (flavor dilution factors) GCO techniques. Conclusions drawn from each method regarding critical odorants were compared.

Materials and Methods

Sample origin, preparation, and storage

All samples were obtained from the Fall 1996 lemon harvest and extracted using an FMC Inline Extractor (FMC Corp., Lakeland, FL) in their country of origin. Lemon oil samples from Brazil and Spain were chosen for this study based on results obtained from descriptive analysis of their aroma, which indicated the samples were significantly different from each other in several aroma descriptors (Young et al., 1997). In addition, a California coastal sample was analyzed based on its economic significance to the United States and its high citral content.

A series of four-fold serial dilutions in ethanol were prepared in 4.0 ml amber glass vials (Supelco Inc., Bellefonte, PA), flushed with nitrogen gas to minimize oxidation, capped with a teflon septum and screw top lid, then stored in darkness at 2°C. *Cis*-3-hexen-1-ol (Fluka Chemical Corp., Ronkonkoma, NY) was used as the internal standard.

Gas chromatographic parameters

Analyses were performed using a HP 5890 gas chromatograph (Hewlett Packard, Avondale, PA) equipped with a Rtx-5™ 0.53 mm x 30 m column coated (1 μm) with 95%

dimethyl-, 5% diphenylpolysiloxane (Restek Corp., Bellefonte, PA). The following operating parameters were used: 1) injection port temperature = 150°C, 2) detector (flame ionization) temperature = 250°C, 3) temperature program: initial temperature = 80°C, initial hold time = 4.0 min., initial rate = 3°C/min. to 120°C, rate A = 4°C/min. to 220°C, rate B = 60°C/min. to 300°C, final hold time = 15 min., 4) split ratio = 10:1, 5) carrier gas = helium with linear velocity of 30 cm/s, 6) injection volume = 1μl.

Gas chromatography/olfactometry (GCO)

A detailed description of the GCO set-up is provided by DaSilva et al. (1993). The sniff port was modified to have an internal diameter of 4 mm. The same GC parameters as described above were used for GCO. However, a splitless injection was used with a purge time of 0.2 min. The flow rate of humidified air from the sniff port was 4 liters/min.

GCO subjects

Screening

Seven graduate student volunteers from Oregon State University were screened by performing three replications on one sample using the Osme technique. Three subjects were chosen based upon their ability to consistently perceive and describe odors as they eluted from the sniff port. Subjects with poor sensitivity and/or inability to verbalize perceptions were not selected.

Training

Subjects not previously experienced with Osme required additional training in use of the time-intensity device. Training involved 4-5 additional sniffing trials to practice using the device labeled with a 16-point intensity scale (0 = none, 3 = slight, 7 = moderate, 11 =

large, 15 = extreme). The following standards were used as reference points on the scale: 3 = 30 ml safflower oil (Saffola Quality Foods, Los Angeles, CA), 7 = 20 ml Hi-C orange drink (The Coca-Cola Company, Houston, TX), 11 = 20 ml grape juice (Welch's, Westfield, NY), and 15 = 1 stick Big Red cinnamon chewing gum, unwrapped (W.M. Wrigley Jr. Co., Chicago, IL). Training was also used to determine how many dilutions were necessary for each subject to reach a point where no odors were perceived.

Experimental design

All dilutions were analyzed using the Osme method. Subjects rated the intensity of each odorant using the time-intensity device and also described the odor quality of each odorant. Only absence/presence data were utilized for calculating FD factors.

Osme must be conducted on a sample deemed suitable for sniffing, i.e. not too strong to cause subject fatigue and within the range of the scale to allow intensity differences to be detected. Dilution of the sample or extract is often required to attain such a sample. For this study, the most concentrated sample (dilution factor of 4) was suitable for sniffing, and the results obtained from this dilution were used for Osme data analysis.

The experimental design was based upon the number of dilutions necessary for each subject to reach a point where no odors were perceived. The number of dilutions ranged from 4 to 6, resulting in dilution factors of 256 and 4096, respectively. The order of sample presentation was randomized across subjects. Within each sample, dilutions and replications were also randomized. Each subject performed four replications of each dilution with two sample evaluations each day, four days/week. Each subject performed their evaluations at similar times each day to minimize variation in sensitivity at different times throughout the day. Subjects started sniffing two minutes after sample injection to allow the solvent to elute. Each session lasted approximately 30 minutes with at least 15 minutes between sessions.

Data analysis

Data collected for the most concentrated sample was used for Osme analysis, while all dilutions were used to obtain flavor dilution (FD) factors for the threshold-based method. For both GCO methods, an odorant was deemed significant if a subject detected it three times out of four replications.

Osme

For odorants detected at least three times, peak areas and Kovats indices were averaged over replications. However, if an odorant were detected only three times, zeroes were not used in the averaging process for the fourth replication. Odorants with the largest peak areas are considered to be most critical to lemon oil aroma.

Threshold-based/flavor dilution factor analysis

FD factors are the last dilution at which an odorant was detected at a specific retention index. Given the dilutions used in this study, FD factors could range from 0 to 1024. Odorants with the greatest FD factors are deemed most critical to lemon oil aroma.

Compound identification

The purpose of this study was not to identify specific compounds present in lemon oil. However, based upon compounds identified in previous studies on the composition of cold-pressed lemon oil, a variety of chemical standards were provided by FMC Corp. (Lakeland, FL). The standards were analyzed individually using the GC parameters stated above to determine their retention indices. Then, a series of mixtures representing compound classes (e.g. alcohols, esters, aldehydes, etc.) were prepared and analyzed by GCO, as stated above, to determine their odor quality. Of the 25 standards analyzed, 11 were tentatively identified based on the fact that their GC and GCO retention indices

matched, and the descriptors used for standards were the same as those used during sample evaluation. Six additional compounds had matching retention times, but their odor quality descriptors did not match, due most likely to the fact that some compounds' qualitative properties are dependent upon concentration. Of the remaining eight chemical standards, five were detected by the flame ionization detector but not perceived by GCO subjects, indicating they are either not present in these particular samples or not highly aroma-active.

Results and Discussion

The purpose of GCO is to identify the most critical odorants in a flavor system. Therefore, the main objective of this study was to determine if two different GCO methods, Osme and threshold-based, draw the same conclusions regarding the most critical odorants of cold-pressed lemon oils obtained from Brazil, Spain, and the California coast.

Selection of critical odorants

For the threshold-based GCO method, odorants assumed to be most critical to lemon oil aroma were selected based on the largest flavor dilution (FD) factors. Due to the stepwise nature of FD factors, the number of odorants identified for each subject for each sample varies. For example, there were 7, 9, and 7 odorants selected for Subjects 1, 2, and 3, respectively, for the Brazilian lemon oil (Table 4). Similar differences were present for the California coastal and Spanish lemon oils. However, a minimum of six odorants were selected. Table 4 includes the FD factors obtained for each odorant detected by each subject for all three lemon oil samples. Absence of an FD factor indicates that odorant was not detected.

Table 4. Critical odorants (shaded) for cold-pressed lemon oil aroma: determined by FD factors and Osme.

	Brazil						Califomia Coastal						Spain					
	Subject 1		Subject 2		Subject 3		Subject 1		Subject 2		Subject 3		Subject 1		Subject 2		Subject 3	
Kovat	FD[1]	Osme[2]	FD[1]	Osme[2]	FD[1]	Osme[2]	FD[1]	Osme[2]	FD[1]	Osme[2]	FD[1]	Osme[2]	FD[1]	Osme[2]	FD[1]	Osme[2]	FD[1]	Osme[2]
821							4	0.25										
847[4]					4	0.50	4	0.54	4	0.11	4	1.78			4	1.41	4	0.03
858	64	0.22							1024	0.25							64	0.77
915[4]					4	0.54												
945[3]	64	0.29	256	2.08			256	0.14	1024	1.78			16	0.32	1024	2.29		
984					4	0.45												
989	4	0.38	4	0.45	4	0.73	4	0.52									4	0.94
997	4	0.33																
1001[3]	64	0.73	16	1.00	64	3.84	64	1.47	64	1.36	64	3.11	64	1.45	64	1.63	64	3.58
1015[3]	64	1.01	16	1.88	64	2.39	64	0.62	64	1.78	16	1.85	64	0.94	64	2.01	16	1.25
1047[4]	64	3.91	64	5.33	64	6.25	256	3.64	256	5.60	64	9.04	64	2.77	64	8.51	64	6.85
1055							4	0.42	4	0.49	4	1.14						
1062					4	0.54							4	0.31				
1071													4	0.20				
1077[3]	16	1.31	16	2.54	64	4.28	16	1.52	16	2.66	64	3.68	16	1.89	64	4.26	64	4.37
1112[3]	64	1.13	64	3.19	64	2.00	256	0.92	64	4.21	64	4.21	64	1.52	64	3.73	4	2.56
1116	16	1.19			16		16	0.59					16	0.74			64	1.47
1134					4	2.13					4	0.58						
1168[4]	16	0.28	4	0.46	4	1.18	16	0.46	4	0.97	16	0.95	64	0.56	16	1.65		
1181[3]	4	0.57			4	2.37	4	1.15			4	1.09	4	0.97				
1186[3]	4	0.79	4	0.78			4	0.71			4	0.70			4	1.31		
1196	4	0.37	4	0.61	4	0.76	4	0.31					4	0.23				
1210[3]					4	1.18					4	1.00	4	0.30				
1221[4]	16	0.60	4	1.02	4	1.67	16	0.56	4	1.19	4	2.74	16	1.21	16	1.93	16	1.24
1242	4	0.51					4	0.38			4	0.64	4	0.80			16	1.54
1247[3]	4	0.95	16	1.74	4	3.54	4	0.24	4	4.15	16	1.56	4	0.81	4	3.45		
1251							4	0.59					4	0.27			4	1.58
1264[3]	64	1.75	64	3.47	16	3.92	64	1.82	16	4.90	64	3.53	64	0.85	64	3.71	16	2.20
1272	4	0.18	4	0.55	64	2.47					16	1.48	16	0.68			16	1.07
1280			4	1.16			4	0.34	4	2.28	4	1.77	4	0.41	4	2.90	64	1.20
1292[3]	16	1.21	64	6.99	64	5.81	64	1.79	64	5.37	64	5.64	64	1.24	64	5.03	16	3.59
1328	16	0.53	4	1.06	4	1.03	16	0.85	4	1.78	4	0.68	16	0.83	4	1.44		
1356													4	0.29	4	2.04	4	1.65
1421[4]	4	0.64			4	1.50					4	1.11						
1435													4	0.71				

[1]FD factor=last dilution of odorant detection, [2]Osme peak area, [3]matching Kovats and descriptors (945, alpha-pinene; 1001, myrcene; 1015, octanal; 1077, gamma-terpinene; 1112, linalool; 1181, terpinen-4-ol; 1186, alpha-terpineol; 1210, decanal; 1247, beta-citronellol; 1264, neral; 1292, geranial)
[4]matching Kovats only (847, furfural; 915, heptanal; 1047, limonene; 1168, citronellal; 1221, octyl acetate; 1421, decyl acetate)

For Osme, peak height is a more accurate predictor of perception for some odorants, while peak area is equally as effective in other cases (DaSilva et al., 1994). Initially, both peak parameters were considered to determine which was more appropriate for these samples. There was more agreement between subjects when using peak area. Thus, odorants with the largest peak areas were selected as those most critical to lemon oil aroma. The Osme measurement procedure allows for the selection of a discrete number of critical odorants. However, to facilitate comparisons with FD factors, the number of odorants selected was chosen to coincide with the number selected from FD factor analysis.

Odorants perceived by each subject in Brazilian, California coastal, and Spanish lemon oils, for both GCO methods, are listed in Table 4. Shaded boxes represent odorants deemed the most critical, based upon their respective parameter measurements (FD factor or Osme peak area), as described above.

Agreement between methods and subjects

Table 4 serves as a useful tool in comparing the critical odorants identified by each GCO method. It is evident that there is not full agreement between FD factors and Osme. A few odorants are selected by both GCO methods as the most critical constituents of lemon oil, but there are numerous instances where completely different odorants were identified.

A summary of the agreement between GCO methods in identifying the most critical odorants is provided in Table 5. For the Brazilian sample, both GCO methods identified four odorants that were common to all three subjects. However, the four odorants in agreement between subjects for FD factor analysis are different from those in agreement

Table 5. Agreement between GCO methods and subjects in identifying the most critical odorants for Brazilian, California coastal, and Spanish lemon oil aroma.

		Percentage of critical peaks in common between methods	Number of critical peaks in common between subjects within method	
Sample	Subject	FD + Osme	FD	Osme
Brazil	1	57		
	2	89	4	4
	3	71		
California Coastal	1	71		
	2	43	4	5
	3	100		
Spain	1	57		
	2	75	2	4
	3	50		

between subjects for Osme (Table 4). In fact, there was only one odorant (Kovat 1047, limonene) identified by both methods for all three subjects.

For the California coastal lemon oil, FD factors identified four odorants in common for all subjects while Osme identified five. Three odorants (Kovats 1047, limonene; 1112, linalool; and 1292, geranial) were in common between methods and across subjects (Table 4).

Lastly, for the Spanish sample, FD factors identified only two odorants in common for all subjects while Osme identified four. Similar to the Brazilian sample, limonene was the only odorant common to all subjects for both GCO methods. These results suggest little agreement between FD factor and Osme analyses in identifying odorants critical to lemon oil aroma.

There is considerable variation between subjects in the agreement between Osme and FD factor analysis. In fact, the percentage of critical odorants in common between methods ranges from just 43% to 100%, depending upon the subject (Table 5). There is also variation within each subject, with agreement between methods ranging from 57-71% for Subject 1, 43-89% for Subject 2, and 50-100% for Subject 3. Despite this variation within each subject, and based on the summary of subject agreement for each individual method, there is more agreement in identifying critical odorants for Osme than for FD factor analysis.

Subject variation

Lack of agreement for FD factor analysis is most likely due to the wide range of thresholds present in humans for various odorants. A subject with a very low threshold for an odorant will detect that odorant throughout numerous dilutions, resulting in a large

FD factor. On the other hand, another subject with a high threshold for the same odorant will detect it at only high concentrations, resulting in a small FD factor. Since the selection of critical odorants in FD factor analysis is based solely upon FD factors obtained for each subject, there is potential for disagreement between subjects. A prime example of this is alpha-pinene (Kovat = 945)(Table 4). For both California coastal and Spanish samples the FD factor for Subject 2 is 1024, but this compound is not even detected by Subject 3. Differences in subject sensitivities are also evident when evaluating the number of odorants detected by each subject. Subject 1 detected 33 odorants, while Subjects 2 and 3 detected 22 and 31 odorants, respectively.

Again, a low odor threshold for a specific odorant results in a high FD factor for that odorant. However, the subject will not necessarily perceive the odorant as high in intensity, resulting in a small Osme peak area. Likewise, an odorant with a high odor threshold and low FD factor will not always be perceived as low in intensity. This is due to the fact that an odorant's perceived intensity is not linear with its concentration (Stevens, 1957). Since threshold-type GCO methods are based on this assumption, some odorants are identified as critical by FD factors but not by Osme peak areas (Kovats 858), and vice versa (Kovats 1181). Since this occurrence is highly subject-dependent, there are few odorants for which these scenarios are present across all three subjects. However, several examples are present within each subject: Subject 1 - Kovats 858, 945, 1015, 1077, and 1181; Subject 2 - Kovats 858, 1001, 1015, 1247, and 1280; Subject 3 - Kovats 858 and 1264.

Subject variation in FD factors could also be related to each subject's response criteria for reporting the presence of an odorant. With increased dilutions, an odorant becomes more and more difficult to detect. Some subjects are more conservative and require a more intense stimulus in order to record its presence (Goldstein, 1989). On the other hand, some subjects are less conservative and will report perception of an odorant upon the

faintest detection. This variation in response criteria contributes to additional variation between subjects' FD factors. On the contrary, Osme analyzes a more concentrated sample, and such variation is likely to be present only for those odorants which are present at near sub-threshold concentrations and are most likely not significant contributors to the flavor system.

Analysis time

In terms of ease of implementation, Osme is less time-consuming than threshold-based methods. For this study, two hours of sniffing time were required by each subject for each sample in Osme analysis. However, FD factor analysis required each subject to sniff between 8 and 12 hours for each sample, depending on the number of dilutions required to reach the point of no perception. This time requirement is based on four replications of each dilution. However, few researchers use four replications when conducting CharmAnalysis or FD factor analysis, resulting in an analysis time similar to Osme.

Replications

To account for within-subject variation, sources of error associated with injection procedures, etc., Osme researchers believe a minimum of four replications are necessary. Grosch (1993) also stated that one GCO run is usually not sufficient to establish the difference between critical and less significant odorants in a sample. Our work is based on four replications for both Osme and FD factor analysis, with the requirement that each odorant be detected at least three times to be considered for analysis.

For the sake of comparison, data obtained for each individual replication of FD factor analysis were inspected to determine if identical FD factors were obtained for all four replications. Only 18%, 16%, and 41% of detected peaks for Subjects 1, 2, and 3,

respectively, resulted in identical FD factors for all four replications. Within a single odorant, FD factors spanned a range from 16 - 256 across replications for a single subject, indicating that conclusions could vary significantly if only one replication is performed. Chung et al, (1995) acknowledged this issue by log transforming FD factors and averaging the values across three replications.

Conclusion

Results obtained and conclusions drawn from the analysis of cold-pressed lemon oil by threshold-based and Osme methods of GCO are different in terms of the groups of odorants identified as most critical to lemon oil aroma. In addition, the agreement between subjects in identifying these odorants is different for each technique but generally greater for Osme. However, these results are limited to the subjects and samples used in this study. Lastly, if researchers wish to conduct several replications, Osme is considerably more efficient in terms of analysis time and, therefore, more practical.

While there is more subject agreeability with Osme than with FD factor analysis, there is still considerable subject variability using Osme analysis. Use of several subjects is crucial to obtaining representative data and an understanding of inter-subject variabilty. The use of several subjects and/or replications is not commonly reported by users of threshold-based GCO methods. Therefore, Osme is more likely to account for variation within and between subjects. Regardless of the method used, subject variation is an important factor to consider when interpreting GCO data.

Despite the apparent advantages of Osme in terms of efficiency and subject agreement, further investigation is necessary to determine which conclusions result in the most accurate predictor of lemon oil aroma. Future researchers must identify and quantify the odorants in each lemon oil sample. Model solutions containing the critical

odorants identified by each GCO method should be prepared in concentrations determined by quantification. Such solutions should then be subjected to descriptive analysis, in conjunction with the actual lemon oil samples, to determine which solution is more similar to its authentic counterpart.

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OSME ANALYSIS OF COLD-PRESSED LEMON OIL AROMA: RELATIONSHIP TO SENSORY DESCRIPTIVE PROFILE

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Abstract

Lemon oils significantly different ($p<0.05$) in their aroma profiles, as determined by a traditional descriptive analysis panel, were analyzed by Osme, a gas chromatography/olfactometry (GCO) technique. Descriptive analysis and Osme data were examined to determine if samples were characterized similarly by both sensory methods. Considerable agreement between methods was evident. In addition, citral (neral and geranial), linalool, limonene, and gamma-terpinene were identified as important contributors to basic lemon oil aroma.

Introduction

The chemical composition of lemon oil is dependent upon numerous factors, including the climate in which the lemons are grown, harvesting season, soil type, tree age, rootstock, and lemon variety (Burke, 1967). Climate, however, is perhaps the most influential factor. Dellacassa et al. (1995) reported that Uruguayan lemons grown in the south provide oils higher in oxygenated compounds and lower in hydrocarbons than those grown in the north. Studies comparing oils obtained from desert and coastal fruits confirm these findings (Staroscik and Wilson, 1982; Stanley and Vannier, 1959). Staroscik and Wilson (1982) reported that California coastal lemon oils are higher in beta-pinene and richer in oxygenated compounds than desert oils from Arizona, owing to their greater sensory impact. Desert oils, however, contain greater amounts of total hydrocarbons.

While these chemical differences have been documented, little work has been done to determine if such chemical differences affect the sensory properties of lemon oil. Recently, Young et al. (1997) conducted a descriptive analysis study comparing the aroma of lemon oils obtained from various locations throughout the world, including Argentina, Brazil, Spain, South Africa, and California (both coastal and desert sources).

All samples met the USP (1970) and Food Chemicals Codex (1981) requirements for ultraviolet absorption, refractive index, and aldehyde content. However, significant differences in overall aroma intensity and peel, lime, orange, and sweet aromas were present.

A useful method for determining the sensory significance of individual odorants is gas chromatography/olfactometry (GCO). There are several methods available for conducting GCO analysis, including aroma extract dilution analysis (AEDA), extract dilution sniffing analysis (EDSA), and Osme (McDaniel et al., 1990; DaSilva et al., 1994).

AEDA, a method based on odor thresholds, involves conducting GCO on a series of dilutions until no odors are perceived by the subject. The data can then be used to calculate either Charm values (Acree et al., 1984 a,b) or flavor dilution (FD) factors (Ullrich and Grosch, 1987). Odorants with large Charm values or FD factors are assumed to be critical to the sample's aroma/flavor. Schieberle and Grosch (1988) used AEDA with FD factors to identify the most potent flavor compounds in fresh and stored emulsions of lemon oil and citric acid.

Osme is based on psychophysical laws and provides a direct measure of perceived intensity for each odorant as it elutes from the GC column. Subjects' perceptions are recorded using a time-intensity device labeled with a 16-point intensity scale, providing the following information for each perceived odorant: 1) retention index, 2) duration of odor detection (peak width), 3) intensity of odor (peak height), and 4) peak area. In addition, the odor quality of each detected odorant is verbally expressed by the subject and recorded by the researcher. Odorants with the largest peak areas are assumed to be most critical to the samples' aroma.

Young and McDaniel (1997) conducted a study comparing results obtained from both Osme and FD factor analysis of cold-pressed lemon oils. The authors concluded that there are slight differences between the two methods in terms of the odorants identified as most critical to lemon oil aroma. In addition, more agreement between subjects was present with Osme analysis.

One disadvantage of GCO techniques is their inability to account for the effects of odor mixtures and/or interactions between volatiles and non-volatiles. Odor mixtures may be either homogeneous or heterogeneous (Berglund et al., 1976). The former involves mixing two odors to form a completely new odor, while the latter refers to a mixture of two odorants which remain capable of being perceived individually. In both situations, the resulting mixture intensity may be perceived as weaker, equal to, or stronger than the sum of the intensities of each component. Thus, relating perceived intensities of individual odorants (as obtained from GCO methods) to perceived intensities of mixtures (as obtained from descriptive analysis) is quite difficult and challenging.

Although non-volatiles do not contribute directly to the aroma profile of a food sample, interactions between non-volatiles and volatiles affect the perception of the sample's odor quality and intensity (Solms et al., 1973). GCO methods cannot take these interactions into account, resulting in perceptions which may not be entirely accurate. One way to determine if these interactions and/or odor mixture effects are meaningful is to examine the relationship between GC sniffing and descriptive analysis data.

This particular study involved Osme analysis of lemon oil samples known to be significantly different, as determined by descriptive analysis. The study's objective was four-fold: 1) to identify odor-active regions of the chromatogram which are responsible for the characteristic aroma of lemon oil by comparing FID chromatograms to Osmegrams, 2) to identify which odorants are critical to the aroma profiles of each

sample, 3) to identify Osme differences and/or similarities between lemon oil samples, and 4) to examine the relationship between Osme and descriptive analysis in characterizing lemon oil aroma.

Materials and Methods

Sample origin and storage

Lemon oils from Argentina, Brazil, California coast, California desert, Spain, and South Africa were supplied by FMC Corp. (Lakeland, FL). All samples were obtained from the Fall 1996 lemon harvest and extracted by each individual processor using an FMC Inline Extractor. Samples were placed in 4.0ml amber glass vials, flushed with nitrogen gas, and stored in darkness at 2°C to prevent oxidation.

Descriptive analysis panel

The trained panel consisted of eleven (eight female and three male) student volunteers from Oregon State University. Panelists were trained during 19 one-hour sessions, over a period of six weeks, to describe and rate aroma descriptors (Table 3) of various lemon oils, including those listed above. For more detailed information regarding training methods, reference standards, experimental design, and sample evaluation technique, see Young et al. (1997).

Osme sample selection and preparation

Lemon oils from Brazil and Spain were chosen for this study based on results obtained from descriptive analysis of their aroma, which indicated the samples were significantly different from each other in several aroma descriptors (Young et al., 1997).

In addition, a California coastal sample was analyzed based on its economic significance in the United States and its high citral content.

Dilutions of each sample (1 part lemon oil to 3 parts ethanol) were prepared in 4.0 ml amber glass vials (Supelco Inc., Bellefonte, PA), flushed with nitrogen gas to minimize oxidation, capped with a teflon septum and screw top lid, then stored in darkness at 2°C. *Cis*-3-hexen-1-ol (Fluka Chemical Corp., Ronkonkoma, NY) was used as the internal standard.

Gas chromatographic parameters

Analyses were performed using a HP 5890 gas chromatograph (Hewlett Packard, Avondale, PA) equipped with a Rtx-5™ 0.53 mm x 30 m column coated (1 μm) with 95% dimethyl-, 5% diphenylpolysiloxane (Restek Corp., Bellefonte, PA). The following operating parameters were used: 1) injection port temperature = 150°C, 2) detector (flame ionization) temperature = 250°C, 3) temperature program: initial temperature = 80°C, initial hold time = 4.0 min., initial rate = 3°C/min. to 120°C, rate A = 4°C/min. to 220°C, rate B = 60°C/min. to 300°C, final hold time = 15 min., 4) split ratio = 10:1, 5) carrier gas = helium with linear velocity of 30 cm/s, 6) injection volume = 1μl.

Gas chromatography/olfactometry (GCO)

A detailed description of the GCO set-up is provided by DaSilva et al. (1993). The sniff port internal diameter was modified from 1 cm to 4 mm. The same GC parameters as described above were used for GCO. However, a splitless injection was used with a purge time of 0.2 min. The flow rate of the humidified air from the sniff port was 4 liters/min.

Osme subjects

Screening

Seven graduate student volunteers from Oregon State University were screened by performing three replications on one sample using the Osme technique. Three subjects were chosen based upon their ability to consistently perceive and describe odors as they eluted from the sniff port. Subjects with poor sensitivity and/or inability to verbalize perceptions were not selected.

Training

Subjects not previously experienced with Osme required additional training in use of the time-intensity device. Training involved 4-5 additional sniffing trials to practice using the device labeled with a 16-point intensity scale (0 = none, 3 = slight, 7 = moderate, 11 = large, 15 = extreme). The following standards were used as reference points: 3 = 30 ml safflower oil (Saffola Quality Foods, Los Angeles, CA), 7 = 20 ml Hi-C orange drink (The Coca-Cola Company, Houston, TX), 11 = 20 ml grape juice (Welch's, Westfield, NY), and 15 = 1 stick Big Red cinnamon chewing gum, unwrapped (W.M. Wrigley Jr. Co., Chicago, IL).

Experimental design

Sample presentation order was randomized across subjects. Each subject performed four replications for each sample. Subjects performed two sample evaluations at similar times each day to minimize variation in sensitivity at different times throughout the day. Sniffing started two minutes after each injection to allow the solvent to elute first. Each session lasted approximately 30 minutes with at least 15 minutes between sessions.

Data analysis

An odorant was deemed significant if a subject detected it three times out of four replications. For odorants detected at least three times, peak areas and Kovats indices were averaged over replications. However, if an odorant were detected only three times, zeroes were not used for the fourth replication in the averaging process. Odorants with the largest peak areas were considered to be most critical to lemon oil aroma.

Peak areas were analyzed by analysis of variance (SAS, Inc., Cary, NC) to determine significant differences ($p<0.05$) among samples for each individual subject. The analysis of variance model included replication and sample. Data were also analyzed by Principal Component Analysis (PCA) using SAS to determine which odorants were responsible for the most variation between samples. Significant treatment differences on each principal component were determined by analysis of variance (Proc GLM).

Compound identification

Based upon compounds identified in previous studies on the composition of cold-pressed lemon oil, a variety of 25 different chemical standards were provided by FMC Corp. (Lakeland, FL). The standards were analyzed individually using the GC parameters stated above to determine their retention indices. Then, a series of mixtures representing compound classes (e.g. alcohols, esters, aldehydes, etc.) were prepared and analyzed by GCO as stated above to determine their odor quality. A peak was tentatively identified if the GC and GCO retention indices matched and the descriptors used for the standards were the same as those used during sample evaluation.

Results and Discussion

The main objectives of this research involved identifying aroma profile and/or chemical composition differences between three lemon oil samples. However, it is important to understand that a large amount of between-panelist variation is involved in obtaining product differences. For sensory analysis, each panelist is essentially a separate instrument. In descriptive analysis, sensory analysts attempt to minimize panelist to panelist variation through extensive training sessions. For GCO analysis, however, results are dependent upon individual subjects' sensitivity and response criterion, making "consensus" more difficult. Thus, when interpreting Osme data, it is crucial to evaluate subject differences as well as product differences.

Odorants detected by each subject for each sample are listed in Table 6. The table is divided into three segments, one for each subject, with each segment containing the odorants perceived in each of the three lemon oil samples. Shaded boxes indicate the odorants deemed most critical to each sample's aroma, as determined by the largest Osme peak areas. With this information, variation and/or disagreement between subjects can be evaluated. There appears to be variation in the sensitivities of subjects, with Subjects 1, 2, and 3 perceiving 33, 22, and 31 odorants, respectively. However, there is agreement between subjects in terms of the odorants selected as most critical to the aroma of each sample, as at least three critical odorants were common among all three subjects. Such agreement is not surprising since the critical odorants are most likely present at fairly low threshold levels. However, subject sensitivity becomes a factor when considering the odorants present at near sub-threshold levels.

The most critical lemon oil odorants to be compared across samples within each subject are highlighted in Table 6. Subject 1, for example, had three critical odorants in common between all samples (Kovats: 1047, limonene; 1077, gamma-terpinene; 1292,

Table 6. Critical lemon oil odorants (shaded) as determined by Osme peak area
(nd = not detected within subject, blank = not detected in any sample within subject)

Peak	Kovats	Tentative ID	Subject 1			Subject 2			Subject 3		
			Brazil	CA Coastal Descriptor	Spain	Brazil	CA Coastal Descriptor	Spain	Brazil	CA Coastal Descriptor	Spain
1	821		nd[b]	green, grass[a]	nd[b]						
2	847	furfural*	nd[b]	green, grass[a]	nd[b]	nd	apple, grass	apples, grass	grass, solvent	grass, solvent	grass, solvent
3	858		green, grass[a]	nd[b]	nd[b]	nd[b]	apple, grass[a]	nd[b]	nd[b]	nd[b]	grass[a]
4	915	heptanal*							grass[a]	nd[b]	nd[b]
5	945	alpha-pinene	pine needles[a]	woody[b]	pine needles[a]	new furniture	new furniture	new furniture			
6	984								metallic[a]	nd[b]	nd[b]
7	989		sweet, fruity[a]	sweet, fruity[a]	nd[b]	unpleasant[a]	nd[b]	nd[b]	metallic	nd	metallic
8	997		myrcene[a]	nd[b]	nd[b]						
9	1001	myrcene	myrcene	myrcene	myrcene	lemon	lemon	lemon	myrcene	myrcene	myrcene
10	1015	octanal	grapefruit	grapefruit	grapefruit	light lemon	lemon	lemon oil	citr., greasy[a]	citrus, flor.[ab]	citr., greasy[b]
11	1047	limonene*	mint	mint	mint	mint	mint	mint	mint	mint	mint
12	1055		nd	mint	nd	nd	eucalyptus	nd	nd[b]	menthol[a]	nd[b]
13	1062		nd[b]	nd[b]	cucumbers[a]				met., mush.[a]	nd[b]	nd[b]
14	1071		nd[b]	nd[b]	cucumbers[a]						
15	1077	gamma-terpinene	kerosene	kerosene	kerosene	clay[b]	clay[b]	clay[a]	ointment	ointment	ointment
16	1112	linalool	linalool	linalool	linalool	lemon	lemon, citral	lemon, citral	linalool[b]	linalool[a]	linalool[b]
17	1116		linalool	linalool	peel, linalool				nd	nd	citrus, greasy
18	1134								citrus, greasy	citrus	nd
19	1168	citronellal*	lemongrass	lemongrass	lemongrass	lemon[b]	lemon[b]	lemon oil[a]	lemon, greasy	lemon	nd
20	1181	terpinen-4-ol	dusty	dusty	metal., dusty				musty[a]	musty[b]	nd[c]
21	1186	alpha-terpineol	dusty, musty	musty	nd	earthy[ab]	nd[b]	stale bread[a]	nd[b]	metallic[a]	nd[b]
22	1196		linalool	linalool	green, grass	lemon[a]	nd[b]	nd[b]	greasy[a]	nd[b]	nd[b]
23	1210	decanal	nd[b]	nd[b]	grass, peel[a]				lemongrass[a]	citrus[a]	nd[b]
24	1221	octyl acetate*	linalool	linalool	linalool	lemon	lemon	sharp lemon	greasy	citrus, greasy	greasy
25	1242		waxy	waxy	myrcene, wax				nd	metallic	motor oil
26	1247	beta-citronellol	oily, floral	guava	floral, citrus	incense, oily	vegetable oil	incense	lemon[a]	lemon[ab]	nd[b]
27	1251		nd	lemongrass	lemongrass				nd	nd	lemongrass
28	1264	citral(neral)	lemongrass	lemongrass	lemongrass	citral	citral	citral	lgrass, lemon	lemon	lemon
29	1272		cucumbers	nd	sl. floral	oil, jasmine	nd	nd	lemongrass	lemongrass	lemongrass
30	1280		nd	cucumbers	cucum., flor.	oil, jasmine	flor., jasmine	floral, lemon	nd[c]	lemongrass[a]	lemongrass[b]
31	1292	citral(geranial)	lemongrass	lemongrass	lemongrass	citral	citral	citral, lemon	lgrass, lemon	lemon	lemon
32	1328		linalool	linalool	linalool	unpleasant	unpleasant	biting	floral[a]	citrus[ab]	nd[b]
33	1356		nd[b]	nd[b]	dusty, mush.[a]	nd[b]	nd[b]	lemon, oily[a]	nd[b]	nd[b]	metallic[a]
34	1421	decyl acctate*	metallic[a]	nd[b]	nd[b]				greasy, nutty	nutty, greasy	nd
35	1435		nd[b]	nd[b]	lemon peel[a]						

[a,b,c] Peaks with the same or no letter are not significantly different ($p<0.05$) within subject - a=largest mean.

*indicates odorants whose retention times matched with standards, but had different aroma descriptors

geranial). In addition, odorants determined to be significantly different ($p<0.05$) between samples within each subject are indicated. Subject 1 detected 33 odorants, 12 of which had significantly different ($p<0.05$) peak areas between samples. Of the 22 odorants detected by Subject 2, seven were significantly different in peak area, while 15 of 31 were significantly different for Subject 3.

One purpose of GCO is to identify the aroma-active compounds among numerous sample volatiles, many of which are not aroma-active. Figure 3 contains the FID chromatogram and an Osmegram from Subject 1 for California coastal lemon oil. Each peak is numbered to coincide with the peak assignments in Table 6. There are several regions of the chromatogram that contain compounds not detected by the subject. In fact, 77 peaks are present on the FID chromatogram while only 23 are present on the Osmegram. Similar results were obtained for Brazilian and Spanish lemon oils, whose FID chromatograms contained 65 and 79 peaks, respectively. However, less than half were detected by Osme subjects. These examples demonstrate the usefulness of GCO techniques in narrowing the scope of compounds which contribute to lemon oil aroma.

Some compounds present in high concentrations were not perceived by the subject, particularly the compounds eluting after 24 minutes. Other odorants detected by the subject were present in low concentrations (peaks 1, 27, 30, and 32). Lastly, several odorants were detected by the subject but no signal was recorded on the FID chromatogram (peaks 22, 24, and 25). This indicates that concentration is not always an accurate indicator of odor significance and supports evidence that the human nose is considerably more sensitive than any current gas chromatograph (Harper, 1972).

A main focus of this study was the identification of sensory differences between Brazilian, California coastal, and Spanish cold-pressed lemon oils. The Osmegrams from Subject 1 for all three lemon oil samples are provided in Figure 4. Sample differences are

Figure 3. FID chromatogram and Osmegram (Subject 1) for California coastal lemon oil

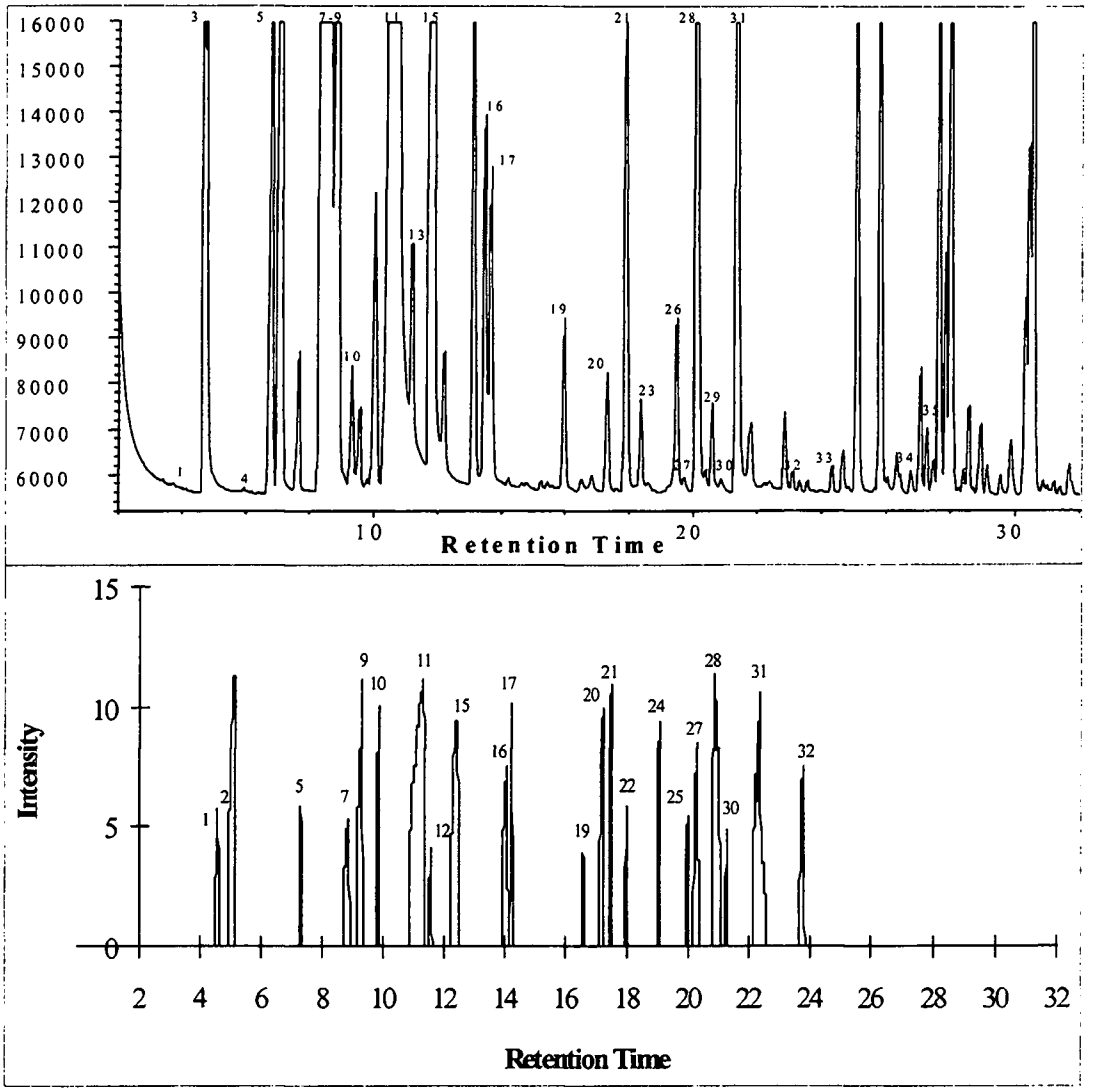
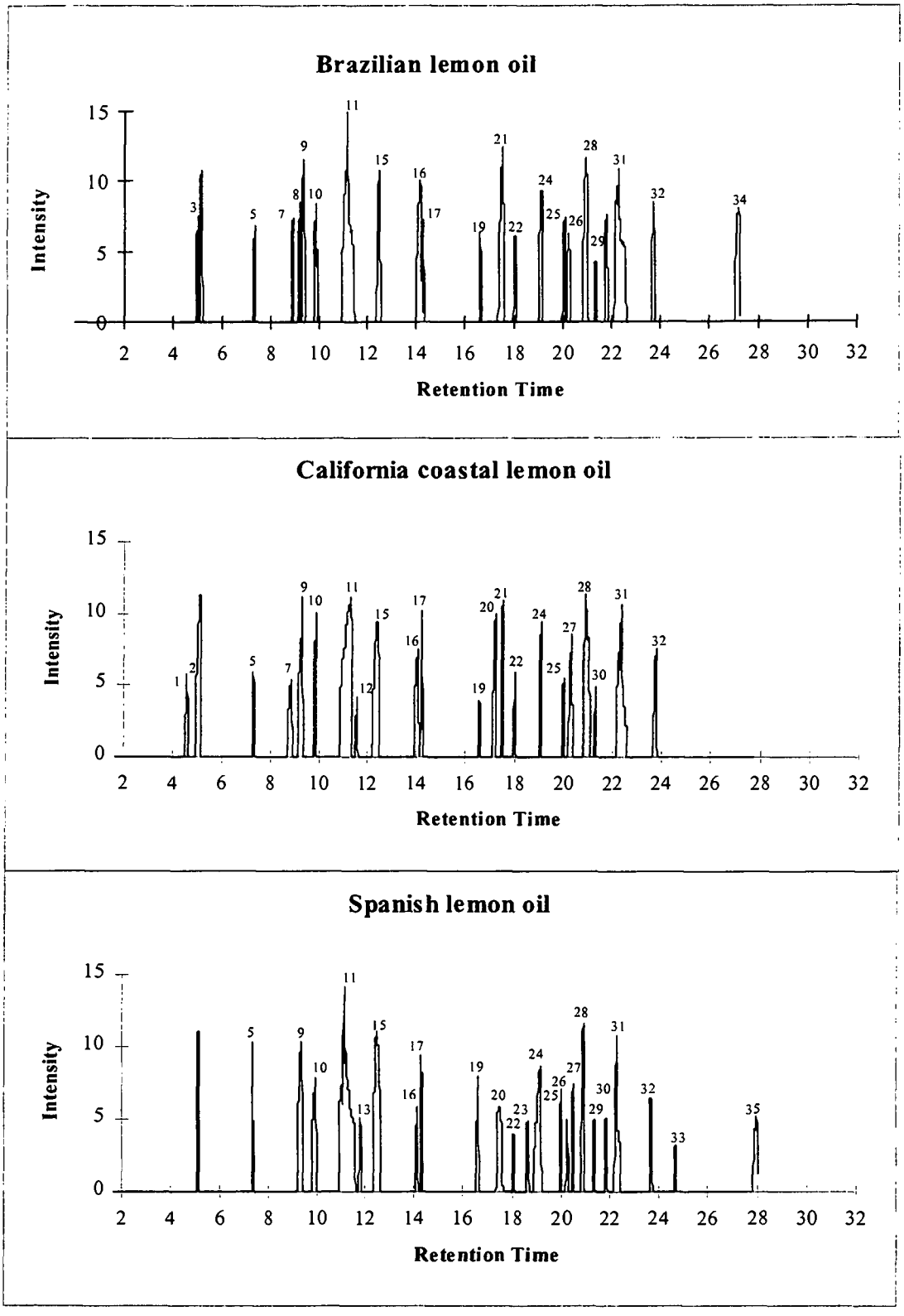


Figure 4. Osmegrams for Subject 1



evident, especially when comparing the Spanish lemon oil to the Brazilian and California coastal samples. The Spanish sample is lacking several peaks in the early portions of the Osmegram (peaks 1, 2, 3, 7, and 8), but contains several additional peaks between 18 and 28 minutes (peaks 23, 33, and 35). These Osmegrams represent only the perception of Subject 1 and do not reflect qualitative sensory responses in terms of each odorant's aroma descriptors. Refer to Table 6 for odor quality descriptors and significant peak area differences ($p<0.05$) between samples for each subject. Note that most of the significant differences between samples are due to a presence/absence effect, the result of some odorants being present in one sample and absent in the other two, and vice versa.

Additional and absent peaks detected by each subject for all three lemon oils are listed in Table 7, along with their aroma descriptors. There is one peak (Kovat 1055) which all three subjects detected in the California coastal sample but not in the Brazilian or Spanish oils. This peak was described as minty, menthol, and eucalyptus oil. Likewise, all three subjects detected an additional peak (Kovat 1356) in the Spanish lemon oil which was described as dusty/mushroom, oily/lemon, and metallic. The Brazilian sample was missing a peak (Kovat 1280), according to two subjects, which was described as cucumbers, floral, and lemongrass in the other samples.

Another means by which sample differences may be assessed is by comparing the odorants deemed most critical to each sample's aroma. These odorants are those with the largest Osme peak areas for each subject and have been identified in Table 6. Neral and limonene were identified as critical compounds for all three samples and all three subjects. Table 8 contains the number of critical odorants in common between samples. Several critical odorants are similar for all three lemon oil samples within each subject. This suggests that these compounds (Kovats: 1047; limonene; 1077, gamma-terpinene; 1112, linalool; 1264, neral; and 1292, geranial) represent the base components of lemon oil

Table 7. Additional[1] and missing[2] peaks from each subject for cold-pressed lemon oil

	Brazil				California Coastal				Spain			
	Additional Peaks[1]		Missing Peaks[2]		Additional Peaks[1]		Missing Peaks[2]		Additional Peaks[1]		Missing Peaks[2]	
Subject	Kovat	Descriptor	Kovat	Descriptor	Kovat	Descriptor	Kovat	Descriptor	Kovat	Descriptor	Kovat	Descriptor
1	858	green, grass	1251	lemongrass	821	green, grass	1272	cucum., floral	1062	cucumbers	989	sweet, fruity
	997	myrcene	1280	cucum., floral	847*	green, grassy			1071	cucumbers	1186*	dusty, musty
	1421*	metallic			1055	mint			1210*	grassy, peel		
									1356	dusty, mush.		
									1435	lemon peel		
2	989	unpleasant	847*	apple, grass	858	apple, grass	1186*	earthy, stale	1356	lemon, oily		
	1196	lemon			1055	eucalyptus						
	1272	oil, jasmine										
3	915*	grassy	1242	motor oil	1055	menthol	989	metallic	858	grassy	1134	citrus
	984	metallic	1280	lemongrass	1186*	metallic			1116	citrus, greasy	1168*	lemon
	1062	metal., mush.							1251	lemongrass	1181*	musty
	1196	greasy							1356	metallic	1210*	lemon
											1328	floral, citrus
											1421*	nutty, greasy

[1]indicates peaks that were detected in that sample, but not the other two

[2]indicated peaks that were not detected in that sample, but were detected in the other two

*847 = furfural; 915 = heptanal; 1168, citronellal; 1181 = terpinen-4-ol; 1186 = alpha-terpineol; 1210 = decanal; 1421 = decyl acetate

Table 8. Sample similarities in terms of number of most critical peaks in common between samples, as determined by largest Osme peak areas.

	Sample Comparisons			
Subject	Brazil vs. CA Coastal	Brazil vs. Spain	CA Coastal vs. Spain	All Samples
1	4	3	4	3
2	4	5	4	4
3	4	4	4	3

aroma. The latter two compounds are structural isomers of citral, and have traditionally been used to determine the quality and market value of lemon oil.

These results are in partial agreement with those obtained by Schieberle and Grosch (1988), who conducted FD factor analysis on fresh and stored aqueous emulsions of lemon oil and citric acid. Similar to Osme, FD factor analysis identified geranial, neral, and linalool as the most intense volatile constituents of lemon oil flavor. In addition, they found lower FD factors for myrcene, limonene, gamma-terpinene, octanal, nonanal, citronellal, and decanal. While Osme results are in agreement with this conclusion for octanal, citronellal, and decanal, Osme identified limonene, gamma-terpinene, and to a lesser extent, myrcene as compounds critical for lemon oil aroma.

Of the 25 chemical standards analyzed in an attempt to identify some of the important lemon oil constituents, 11 had matching retention times and similar odor quality descriptors. Six additional compounds had matching retention times but not matching descriptors. The concentration of each standard was not always identical to its concentration in the actual lemon oil samples. Since the odor quality of some compounds changes with concentration, the odors may have been perceived differently, leading to non-matching descriptors. Of the remaining 8 standards, five were detected by the flame ionization detector, but not perceived by GCO subjects, suggesting they may not be highly aroma-active. Standards were selected based solely on the fact that they were previously identified in lemon oil. Some of these compounds are the result of lemon oil deterioration. Therefore, inability to identify some of these compounds, such as p-cymene and carvone, may merely indicate that the oils did not undergo degradative reactions.

A final objective of this research was to examine the relationship between Osme and descriptive analysis data. Table 9 contains results obtained from the descriptive analysis

Table 9. Mean ratings of lemon oil aroma descriptors for Brazilian, California coastal, and Spanish lemon oils, as determined by descriptive analysis panel.

Descriptor	Sample		
	Brazil	CA Coastal	Spain
Overall Aroma Intensity	10.9[a]	10.5[a]	9.9[b]
Peel	9.8[a]	9.4[a]	8.6[b]
Lime	8.4[a]	8.2[a]	7.4[b]
Lemon	8.2	7.7	7.9
Lemongrass	4.0	4.2	3.4
Orange	1.0[b]	1.2[b]	2.1[a]
Grapefruit	3.5	3.0	2.9
Sweet	2.9[b]	3.2[b]	4.2[a]
Oxidized	3.0	2.6	2.7
Grassy	1.0	0.8	0.7
Floral	0.2	0.3	0.4
Musty	0.4	0.3	0.8
Metallic	0.6	0.5	0.5

[a,b] samples with the same or no letter are not significantly different ($p<0.05$)

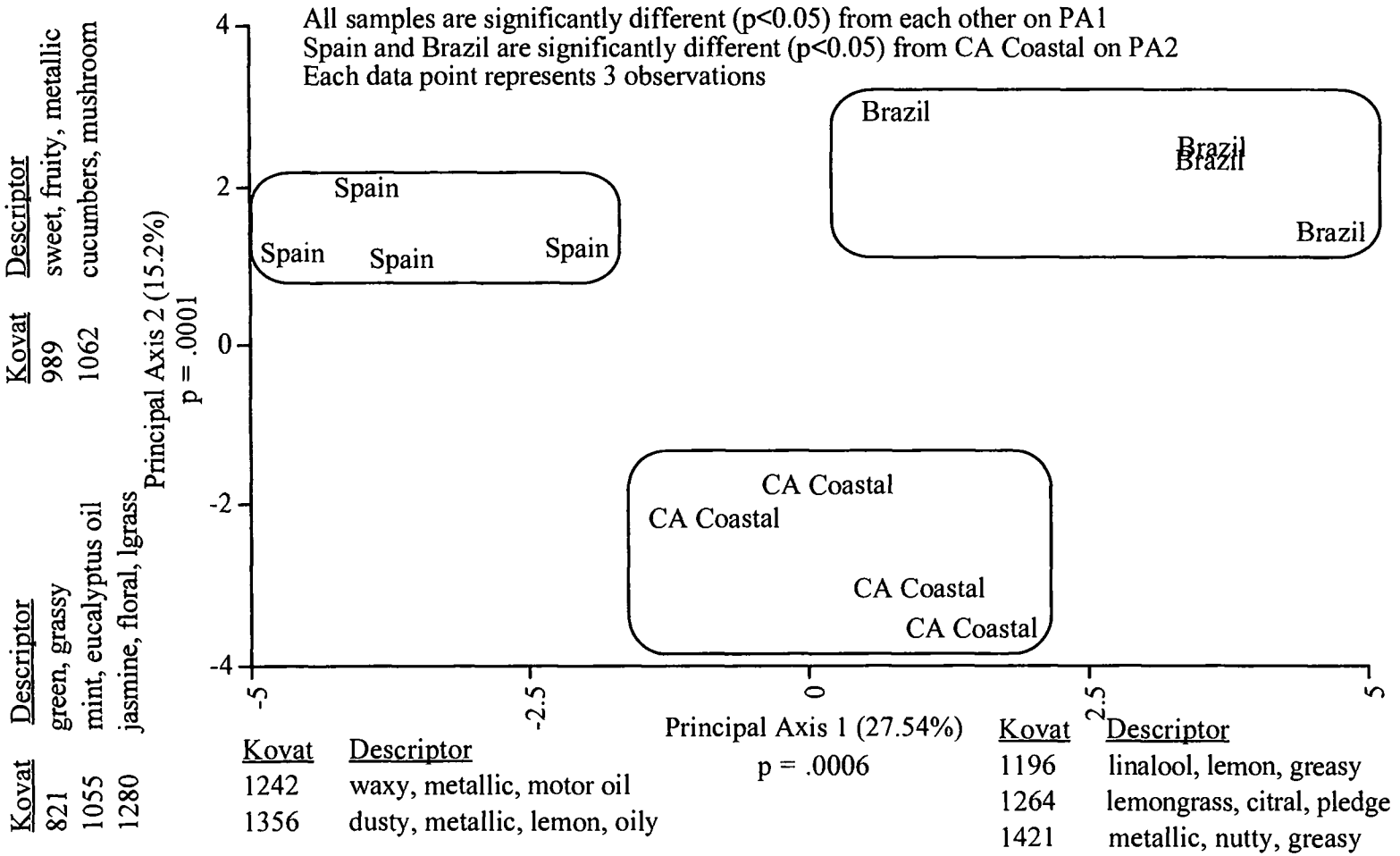
study conducted by Young et al. (1997). Panelists rated thirteen descriptors, and samples were determined to be significantly different ($p<0.05$) from each other in terms of overall aroma intensity and peel, lime, orange, and sweet aromas. The Brazilian and California coastal samples were significantly higher in overall aroma intensity, peel aroma, and lime aroma than the Spanish oil, but significantly lower in orange and sweet aromas. Similar sample differences were found using PCA, which indicated that the variation between samples was due mainly to the five previously-mentioned aroma descriptors (Young et al., 1997).

Overall, the most intense aromas for all samples were those described as peel, lime, and lemon. Since geranial, neral, linalool, limonene, and gamma-terpinene were identified as the most critical compounds for basic lemon oil aroma, it may be these compounds that, in combination, result in the peel, lime, and lemon aromas described by the descriptive analysis panel.

While not all odorants can be directly related to the descriptive sensory data, several conforming trends are evident. For example, there are several odorants described as grassy and metallic which are present in the Brazilian lemon oil, but not in the California coastal and Spanish samples (Table 7). The descriptive analysis data supports this observation (Table 9): the Brazilian sample received slightly higher ratings for grassy and metallic aromas.

The PCA plot of Osme peak areas (Figure 5) illustrates differences in the three lemon oil samples and indicates the peaks responsible for the most variation between samples. Samples were separated significantly on both Principal Axis 1 (PA1) and PA2. PA1 separated samples into three distinct groups, while PA2 yielded two groupings: 1) Brazil and Spain and 2) California coastal. PA1 is defined on the positive end by several odorants described as lemon-like (linalool, lemon, lemongrass), suggesting that

Figure 5. Principal components analysis plot of Osme peak areas



Brazilian lemon oil is higher in these qualities than the Spanish sample located at the opposite end of the axis. This is confirmed by the descriptive analysis data (Table 9). In addition, one of the peaks positively defining PA1 has been identified as citral. Aldehyde content (calculated as percent citral) determinations conducted by Young et al. (1997) indicated that the Brazilian lemon oil contained more citral than any other sample.

PA2 is positively defined by peaks with sweet aroma and negatively defined by peaks with grassy odors, suggesting the California coastal sample should be low in sweet aroma but higher in grassy aroma. The sensory data indicate that the coastal sample is significantly lower in sweet aroma than the Spanish sample.

Conclusion

There is considerable agreement between Osme and descriptive analysis data in characterizing the aroma of lemon oil samples. This study identified compounds responsible for the base aroma characteristics of lemon oil (geranial, neral, linalool, limonene, and gamma-terpinene). However, combinations of less obvious odorants are most likely responsible for the perceived sensory differences between samples. To identify these odorants, it is necessary to prepare model solutions with various combinations of odorants determined to be important by Osme analysis. These solutions must then be subjected to descriptive analysis and compared to authentic lemon oil samples.

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SUMMARY

Lemon oil quality and composition are affected by numerous factors, including climate, harvesting season, lemon variety, soil type, etc. Thus, lemon oils produced throughout the world have the potential to vary considerably in their aroma characteristics. A descriptive analysis panel characterized the aroma profiles of cold-pressed lemon oils from Argentina, Brazil, Spain, South Africa, the California coast, and the California desert. Samples were significantly different ($p<0.05$) in overall aroma intensity and peel, lime, orange, and sweet aromas.

Additional work was necessary to identify the significance of individual odorants and their contribution to sensory differences. Gas chromatography/olfactometry (GCO) is an effective method for obtaining this information, but there are several methods available for conducting GCO. Two GCO methods, Osme and threshold-based flavor dilution (FD) factor analysis, were conducted. The results obtained and conclusions drawn from each method were compared and determined to be different in terms of the odorants identified as most critical to lemon oil aroma. Agreement between subjects in identifying these odorants is different for each technique but generally greater for Osme. Lastly, if researchers wish to conduct several replications, Osme is considerably more efficient in terms of analysis time and, therefore, more practical.

While there is more subject agreeability with Osme than with FD factor analysis, there is still considerable subject variability using Osme analysis. Use of several subjects is crucial to obtaining representative data and an understanding of inter-subject variability. The use of several subjects and/or replications is not commonly reported by users of threshold-based GCO methods. Therefore, Osme is more likely to account for variation within and between subjects. Regardless of the method used, subject variation is an important factor to consider when interpreting GCO data.

Upon concluding that Osme provides a less variable method of identifying critical aroma-active compounds, it was desirable to examine the relationship between Osme and descriptive analysis data. There is considerable agreement between Osme and descriptive analysis data in characterizing the aroma of lemon oil samples. Geranial, neral, linalool, limonene, and gamma-terpinene were identified as the compounds responsible for the base aroma characteristics of lemon oil. However, combinations of less obvious odorants are most likely responsible for the perceived sensory differences between samples.

Future researchers must identify and quantify the odorants of each lemon oil sample. Model solutions containing the critical odorants identified by each GCO method should be prepared in concentrations determined by quantification. Such solutions should then be subjected to descriptive analysis, in conjunction with the actual lemon oil samples, to determine which solution is more similar to its authentic counterpart.

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