J. Agric. Sci. Mansoura Univ., 28(10): 7491 - 7500, 2003

IDENTIFICATION AND QUANTIFICATION OF THE MAJOR WATER-SOLUBLE AROMA COMPONENTS LOST FROM SOME ESSENTIAL OILS TO THE WASTE WATER DURING DISTILLATION.

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ABSTRACT

The identity and the absolute quantity (mg/L), of the major water-soluble aroma components in the waste water of distillation of 7 aromatic plants were investigated. These plants included sweet basil, spearmint, peppermint, sour orange petitgrain, lemongrass, eucalyptus, and clove essential oils. The major water-soluble aroma components being quantified were alcohols, ketones, aldehydes and epoxides. Eugenol from clove oil was found to be the most highly partitioned component into the water phase among all investigated components (854 mg/L), followed by d- carvone from spearmint oil (242 mg/L) then linalool from both sweet basil (171 mg/L) and petitgrain oil (128 mg/L). The investigation indicated that the solubility of certain aroma component in distillation waste water depends on two main factors: first, the partition coefficient of the aroma component between the parent oil phase and water, which in turn depends on the chemical structure of the aroma component and its interaction with water on one hand and with the oil phase on the other hand. Second, the abundance of aroma component present in the parent oil.

Keywords: Aroma soluble fraction, waste water, distillation, essential oils, partition coefficient , hydrophilicity, hydrophobicity.

INTRODUCTION

Water is the most common solvent for ionic compounds. The unique character of water as a solvent originates from its high dielectric constant (polarity). Thus when an ionic component come in contact with water, its ions get solvate (surrounded by the molecules of water through an electrostatic interaction between water molecule and the ionic compound (Csaky 1979). When an organic compound comes in contact with water, no ionic interaction will occur, but instead, part of the organic phase get partitioned into water due to another type of intermolecular interaction known as hydrogen bond (Vinogradov and Linnell 1971). The amount of individual organic compound partitioned into the water phase depend on its chemical structure and hence, ability to form hydrogen bonds with water.

Distillation of essential oil bearing plants requires a continuous contact between water and the essential oil for prolonged time. According to the chemical composition of the essential oil, part of its components will partition into the water phase and get solubilised in water through hydrogen bond formation. For example, citrus essential oil containing about 98% monoterpene and sesqueterpene hydrocarbons. These hydrocarbons are known for their hydrophobic nature, i.e. can not partition into water, so the loss of citrus oil hydrocarbon components during distillation is low. The same observation could be made for the distillation of American origin turpentine oil which contains about 90%pinene (SBP Boards of Consultants and Engineers

1979) which is a hydrophobic component, so it is poorly partitioned into distillation water. On the other hand, other essential oils contain high percentages of polar oxygenated compounds e.g. eugenol (80.8% of clove oil) (Deyama and Horiguchi 1971), rhodinol and geraniol (40%, 14% of rose oil, respectively) (Sood *et al* 1992), menthol and menthone (27%, 38% of pepper mint oil) (Chialva and Ariozzi 1992), in such type of oils, the loss of aroma components to the water phase during distillation is high. It was reported that the ratio of water-soluble aroma components to its parent essential oil could be 1:1 in case of basil essential oil (eugenol type) (Zukov *et al.* 1979), 1:2 in case of dittany oil (Dictamnus gymnostylis Stev.) (Kozhin *et al.* 1972), and 1:3 as in caraway oil (Fleisher and Fleisher 1988).

Depletion of the polar oxygenated components from essential oils to the water phase during distillation results in an unbalanced aroma and flavor profile of the oil compared with the original plant, which leads to an inferior oil quality as well as losing a fortune due to dumping the distillation water rich in expensive aroma flavoring (Fleisher 1991). For this reason some techniques had been developed in order to regain the water soluble aroma components and refortify their parent oils. One of the oldest technique being used is called "cohopation" or redistillation of the waste water which is fully described elsewhere (Fleished 1991). The disadvantage of this technique is that it leads to only a partial and disproportional recovery of oil constituents (Zukov *et al.* 1979). A more advanced technique used for recovery of water soluble aromatic components from waste water of distillation is known as "Poroplast" extraction technique (Fleisher 1990) which proved both technical and economic efficiency. The technique is based mainly on the concept of liquid-liquid distribution. The waste water containing the soluble aroma components is passed through a column filled with organic stationary phase of low polarity non-specifically held on a hydrophobic surface of a porous inert support. Depending on the selected systems of liquid phases, desirable components can be transfer from the aqueous phase into the organic one, or vice versa, providing an efficient recovery of soluble aroma compounds.

On the other hand, there are other investigators who consider the water soluble aromatic components in the waste water as an advantage rather than liability. When essential oils are used as antimicrobial agents, only their water soluble aroma fractions are the antimicrobial active principle because they perform their biological activity in the water phase where the microorganism lives and proliferates (Brocklehurst *et al.*, 1995). So waste water from aromatic plant distillation containing already soluble aroma component represent a natural antimicrobial material instead of emulsifying essential oil in water. Rose (2000 and 2001) claimed numerous uses and advantages of the water soluble aromatic fraction of essential oils (usually called hydrosols). Hydrosols are used in a type of holistic medicine called aromatherapy, being used in massage (Kenderdine 1999), inhaled (Yamada 1996), used internally as a tonic drink, or applied externally in skin are products for stress reliving, inflammations healing, skin cleansing and antiseption, or other healing purposes (Rose 1999 and Catty 2000). Water soluble aromatic fraction is also used to flavor some household products e.g. rose water (Baser 1992).

Despite of the wealth of water solubility data of many individual pure organic compounds of interest to environment, very few data are available for the solubility of individual flavor and fragrance compounds in water (Miller and Hawthorne 2000). To the best of our knowledge, there is no literature found about the absolute quantization of these aroma soluble components in the waste water of aromatic plants distillation. So, the author dedicate this investigation for that subject and to investigate the factors which control the water solubility of a mixture of aroma components like those in essential oils.

MATERIALS AND METHODS

The waste waters of distillation and their parent essential oils were donated from Horticultural Research Institute, Medicinal and Aromatic Plant Research Section, El Kanater El Khairya, Egypt. The oils were obtained by steam distillation of the fresh plant parts of sweet basil (*Ocimum basilicum L.*), peppermint (*Mentha piperita*), spearmint (*Mentha spicata* L.), sour orange petitgrain (*Citrus aurantium* L.), lemongrass (*Cimombogon citratus* L.), eucalypts (*Eucalyptus citrudora*). Clove essential oil was prepared at our department by hydro-distillation of the dry buds of (*Eugenia caryophyllata*), followed by centrifugation and separation to remove the fine oil droplets from the waste water.

No trace of essential oil droplets were detected in the waste waters as revealed by the light microscope.

Extraction of aroma components from the waste water:

Two hundred ml of the waste water of distillation of each aromatic plant were extracted 3 times with a sum of 400 ml methylene chloride (CH_2Cl_2) in a separating funnel (3 extractions X ca. 133 ml for each one). These extractions proved to be enough for collecting all the aroma volatiles dissolved in the waste water, as confirmed by GC analysis. The solvent was dried over anhydrous sodium sulfate, evaporated using rotary evaporator to ca. 5 ml and transferred into a vial immersed in an ice bath, then the remaining solvent was slowly evaporated under nitrogen stream to a volume of 1 ml extract exactly. Three µl of 3-nonanon (Aldrich Chemical Co.) was added to each vial as an internal standard to give a final concentration of 2.398 mg/ml solvent/200 ml waste water. The samples were then quickly caped and then analyzed by GC and GC-MS.

Gas chromatographic (GC) analysis of the aroma volatile:

GC analysis of the aroma components was conducted using HP model 5985 gas chromatograph equipped with flame ionization detector. A 60 m x 0.32 mm i.d. fused silica capillary column coated with DB-5 was used. The oven temperature was programmed from 50°C to 200°C at a rate of 3°C/min. The injector and detector temperatures were 250°C. Helium was used as a carrier gas at a flow rate of 1.0 mL/min. Values reported were an average of two analysis. The retention indices (Kovats index) of the volatile components were calculated with hydrocarbons (C_8-C_{23}, Aldrich Chemical

Co.) as references. Authentic samples of some compounds were injected for more confirmation of the unknowns.

Gas chromatographic-mass spectroscopic analysis (GC-MS) of the aroma volatile:

GC-MS analysis was conducted on HP 5985 gas chromatogram coupled with MS instrument system. The ionization voltage was 70 eV and the ion source temperature was 200°C. Other parameters are as in the GC conditions.

Components were identified using the (NBS) MS library or other published mass spectra (Stenhagen 1974) and also by comparing their retention index with published data (Sadtler 1986).Authentic samples were used to confirm the presence of some components. Only the major components in the oil or the waste water were reported.

RESULTS

Table (1) shows the relative area (%) of the major water-soluble aroma components detected in the distillation waste water of some aromatic plants and their relative area percentage in the parent oil. From the table it is evident that the area% of the water-soluble aroma components in the waste water is higher than that in the parent oil.

Table (1): Identification of the major aroma soluble components in the waste water of distillation of some essential oil in comparison with their parent oils.

Source of waste water	Component	RI*	Component concentration (relative area %)	
			Waste water	Parent oil
Sweet basil *Ocimum basilicum* L.	1-8 Cineol*	1036	6.9±0.4	5.1±0.4
	Linalool*	1107	59.2±0.8	65.9±0.8
	Eugenol*	1375	26.1±0.5	7.0±0.4
Peppermint *Mentha piperita*	1,8-Cineol*	1036	5.3±0.4	6.7±0.4
	Menthone*	1165	20.2±0.5	13.6±0.5
	Menthofuran	1171	nd	1.0±0.04
	Isomenthone	1176	9.8±0.6	5.7±0.4
	Menthol*	1186	48.6±0.8	22.1±0.5
	Linaly acetate*	1261	1.0±0.04	18.9±0.4
Spearmint *Mentha spicata* L.	1-8 Cineol*	1036	3.1±0.3	6.7±0.4
	Carvon*	1260	75.2±0.8	56.2±0.7
Petitgrain (sour orange) *Citrus aurantium* L.	Linalool*	1107	52.8±0.7	32.7±0.6
	α-Terpineol**	1204	24.7±0.7	nd
	Linalyl acetate*	1261	9.5±0.4	46.7±0.6
Lemongrass *Cimombogon citratus* L.	Linalool*	1107	9.3±0.5	4.0±0.3
	Neral*	1253	34.5±0.6	31.9±0.5
	Geranial*	1283	36.9±0.7	43.8±0.6
Eucaleptus *Eucaleptus citrudora*	Citronellal*	1160	34.3±0.6	76.1±0.8
	Citronellol*	1233	8.9±0.4	9.9±0.4
Clove *Eugenia caryophyllata*	Eugenol*	1375	96.6±1.1	84.4±1.0

Values are the means of triplicate analysis ± SD

*** Authentic sample were injected for confirming the compound beside MS and Kovats index.**

**** Artifact, not present in the parent oil**

nd: Not detected

Note: Response factor was not calculated

For example the area percent of eugenol in basil waste water is 3.7 times higher than in its parent oil. The same trend was clear for some other aroma components e.g. menthon, , isomenthon and menthol in peppermint waste water, carvon in spearmint waste water, linalool in petitgrain waste water, eugenol in clove waste water. These results did not mean that the absolute amounts of these aroma components in the waste water are higher than that in the parent oil but it rather means that the number of soluble aroma components in distillation water is less than the total components in their parent oil. This conclusion comes in accordance with the fact that the aroma components of the essential oil have different affinity for partitioning into water phase depending on their hydrophilicity. No monotrpene or sesquiterpine hydrocarbons were detected for any of the investigated waste waters which comes in accordance with the hydrophobic nature of these straight carbon/hydrogen containing compounds.

Table (1) also shows that there is a high concentration of some aroma components present in the waste water which are not detected in their parent oil, e.g. α-terpineol in the waste water of petitgrain (24.7%). This component is not detected in petitgrain essential oil in this study or in any other studies, so it is considered to be an artifact. The origin of α-terpineol could be justified as a degradation product from the hydrolysis of linalyl acetate as a result of heating the later with water during extraction of petitgrain oil. This assumption based on the fact that α-terpineol is formed as a by-product in the synthesis of linalyl acetate from linalool and acetic acid due to isomerization and cyclization ((West 1949 *et al.*)., so the reverse reaction (hydrolysis of linalyl acetate), may proceed via the reverse route.

Table (2) shows the absolute amounts of the water-soluble aroma components in the distillation waste water of the 7 aromatic plants. From the table it is evident that eugenol from clove oil had the highest concentration in the distillation waste water (854.2 mg/L). Carvon from spearmint oil was the second highest concentration among the investigated components (242.0 mg/L), followed by linalool from sweet basil water (171.8 mg/L), then linalool from petitgrain water (128.3 mg/L). Aldehydes like citronellal from eucalyptus water, neral and geranial from lemongrass water are the third highest concentrations in the waste waters. 1,8-Cineol, an epoxy compound, showed the lowest water solubility among the investigated components. From table (2) it is evident that the solubility behavior of the same component in waste water differs according to the source of the parent essential oil. This observation was clear from the solubility of eugenol from the clove buds compared to sweet basil, linalool from basil compared to petitgrain and lemongrass and 1,8-cineol from different plant sources. From the same table it is also evident that the polarity of certain aroma component was not a decisive factor for its extent of solubility. Citronellal (less polar) showed a higher solubility than citronellol (more polar), Also, most terpenic alcohols in this study (e.g. linalool) showed less water solubility compared to carvon (less polar). This indicates that there are other factors, beside polarity, that contribute to the overall water solubility behavior of a mixture of aroma components when brought in contact with water during distillation process.

Table (2): Absolute concentration (mg/L), of the major aroma components detected in the waste water of distillation of some aromatic plants.

Source of waste water	Component	RI	Absolute concentration of aroma components (mg/L) in the waste water
Sweet basil *Ocimum basilicum* L.	1,8-Cineol	1036	19.0±0.4
	Linalool	1107	171.8±1.0
	Eugenol	1375	89.8±0.8
Peppermint *Mentha piperita*	1,8-Cineol	1036	6.3±0.3
	Menthone	1165	24.4±0.3
	Menthol	1186	69.6±0.5
	Linalyl acetate	1261	11.7±0.2
Spearmint *Mentha spicata* L.	1,8-Cineol	1036	7.7±0.2
	Carvon	1260	242.0±1.6
Petitgrain (sour orange) *Citrus aurantium* L.	Linalool	1107	128.3±1.7
	α-Terpineol*	1204	87.7±0.4
	Linalyl acetate	1261	39.9±0.3
Lemongrass *Cimombogon citratus* L.	Linalool	1107	18.8±0.4
	Neral	1253	82.6±0.7
	Geranial	1283	96.7±0.5
Eucaleptus *Eucaleptus citriudora*	Citronellal	1160	117.5±0.8
	Citronellol	1233	24.4±0.2
Clove *Eugenia caryophyllata*	Eugenol	1375	854.2±1.8

Values are the means of triplicate analysis ± SD

***: Not present in the parent oil.**

3-Nonanone (98%) was used as an internal standard at concentration of 3 µl/ml solvent, 2.398 mg/ ml solvent).

The response factor (RF) and the response factor relative to that of the internal standard (RRF) was calculated for each component in the table.

DISCUSSION

The high concentration of soluble eugenol in the waste water of distillation of clove buds (854.2 mg/L) was expected. This solubility behavior was expected, since eugenol (a phenol), has a high affinity to water due to its ability to form multiple hydrogen bonds with water through the hydroxylic group (-OH) or through the by-electrons of the aromatic nucleus and the side chain which contains a double bond, this structure is known to increase the solubility of organic components in water (Verschueren 1983). Surprisingly, the concentration of eugenol in the waste water of sweet basil was only (89.8 mg/L) which is much less than the concentration of the same component from clove oil. In order to understand this contradiction of solubility behavior between the same components under the same conditions, let us consider the factors that affect the partitioning of organic components in water. Polarity (expressed in term of dielectric constant) is the major factor affecting component partitioning. Two factors determine the polarity of a component:

first: presence of hetero atom in its chemical structure (specially oxygen), second: presence of unsaturated double bonds (Vinogradov and Linnell 1971). Returning back to eugenol example, we found that eugenol form sweet basil and clove fulfill the same polarity characteristics. This indicates that the amount of water-soluble eugenol depends on another factor rather than its polarity. This factor could be the effect or influence of component abundance in the parent oil on its solubility in the waste water. This self-evident factor was based on the observation that eugenol peak area percent in basil oil is (7.05%) and in clove oil (84.4%) (table 1). Correlating these values to the absolute amount of eugenol in basil water (89.8 mg/L), and in clove waste water (854.2 mg/L) (table 2), we notice a straight forward correlation indicating that the abundance of the component in the parent oil plays a role in the extent of its solubility in the waste water of distillation.

The results in table (2) provide different examples which could reinforce our previous assumption. Comparing the concentration of water-soluble linalool in the distillation waste water of basil, petitgrain and lemongrass one can detect higher concentrations of linalool in basil water (171.8 mg/L) than petitgrain water (128.3 mg/L) then lemongrass water (18.8 mg/L) (table 2). These results are straightforward correlated with the abundance of linalool in each of the three parent oils which decreases in the same order, (65.9% for basil oil, (32.7%) for petitgrain oil than (7.09%) for lemongrass oil (table 1). The same result was observed for linalyl acetate from petitgrain (39.9 mg/L) and from peppermint oil (11.7 mg/L), which correlate with their abundance in their parent oil (46.7%, 18.9%, respectively) (Table 1).

The influence of components abundance in the parent oil on its partitioning into the waste water seems to exceed the influence of components polarity. This assumption was inferred from the solubility data of citronellol and citronellal in eucalyptus oil (table 2). According to the polarity concept, the concentration of citronellol (more hydrophilic), was expected to be more than citronellal (less hydrophilic). Experimentally, citronellal concentration (117.5 mg/L) is higher than citronellol concentration (24.4 mg/L), which is at the same order of abundance of the two components in their parent oils (76.1%, 9.9%, respectively), (table 1). The same observation was detected for the more hydrophilic isomer neral (82.6 mg/L) and the less hydrophilic isomer geranial (96.7 mg/L), in lemongrass oil. However, 1,8-cineol represents an exception. Its concentration in the waste water of basil (19.0 mg/L) is highly significant than its concentration in waste water of peppermint (6.33 mg/L) or spearmint (7.7 mg/L) (table 2). This result does not correlate with the abundance of 1,8-cineol in its parent oils, basil (5.11%), peppermint (6.7%), and spearmint oils (6.7%) (table 1). This could be explained on the basis of a third factor which could contribute to the factors affecting the solubility of a mixture of aroma components in water. This factor could be the intermolecular interactions that exist among different aroma components in the parent oil. This interactions seems to hinder the solubility of some components regardless to their abundance in the parent oil or their polarity. Carvon from the waste water of spearmint oil was the second highest water-soluble aroma component (242.0 mg/L). Its solubility is a clear example to show the influence of intermolecular interactions on the solubility of certain aroma components in distillation waste water. Carvon (a ketone) is less hydrophilic than some alcohols, e.g. linalool, at the same time, carvon abundance in spearmint oil (56.2%) is less than linalool in basil oil (65.9%) (table 1). So, according to abundance and polarity factors, we expect a high amount of linalool in basil waste water than the amount of carvon in spearmint waste water. Experimentally, table (2) shows that the amount of soluble linalool in basil waste water (171.8 mg/L is less than the amount of soluble carvon in spearmint waste water (242.0 mg/L).

In conclusion, the concentration of water-soluble aroma components resulting from distillation of aromatic plants depend on different factors. Unlike the water solubility of single pure aroma component which depends mainly on its polarity, a mixture of aroma components, as in essential oils, behave differently. Partitioning of aroma components mixture into water phase is a resultant of different factors like component polarity, component abundance in the essential oil and the type of interaction between different aroma components in the oil, which is complex and needs to be studied. As a result, the solubility of the same aroma component in the distillation waste water differ from one aromatic plant to another depending on the composition of each parent essential oil. In this study, the minimum concentration of aroma soluble component was 7.7 ppm for 1,8-cienol from spearmint oil and the maximum concentration detected was 854 ppm for eugenol from clove oil. One may think that these figures are small to be taken in consideration for recovery but we should take in consideration 3 main factors: First: These oxygenated aroma chemicals have a very low threshold values, i.e. small quantities of these components are enough to impart flavor to a large volume of product or should be returned back to their parent oil to enhance its quality. Second: The presence of a ready soluble aroma components in water will save the use of organic solvents to dissolve them or save the cost of surfactant needed for the solubilization or emulsification of these components to make preparations for food or pharmaceutical industry. Third: in a continuous distillation process, these aroma components could be concentrated by accumulation over an appropriate solid phase adsorbent which could be connected on-line with the exhaust of waste water in the distillation unit. The adsorbed aroma components could be then recovered from the adsorbent in a yield depending on the optimization of the process (Edris *et al* 2003).

ACKNOWLEDGEMENT

The author is indebte tó Dr. Said Gabr and Dr. Ebrahim Haridi at the Horticultural Research Institute, Medicinal and Aromatic Plant Research Section, El Kanater El Khairya, Egypt for donating the waste water necessary for this study.

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التقدير الكمي والكيفي للمركبات العطرية الرئيسية التي تفقد من بعض الزيوت العطرية في المياه الناتجة أثناء عملية الاستخلاص بالتقطير البخاري.

عمرو عبيدى ادريس
قسم مكسبات الطعم والرائحة المركز القومى للبحوث

تم في هذا البحث التعرف على نوعية بعض المركبات الأساسية المكونة للزيوت العطرية والتي تفقد منها أثناء عملية الاستخلاص بالتقطير البخاري كما تم أيضا تحديد تركيز هذه المركبات (مللى جرام / لتر) وقد أجريت الدراسة على ناتج ماء التقطير البخاري لسبع نباتات عطرية هي : الريحان الحلو - النعناع الفلفلي - النعناع البلدي - لابلوب لارنج - حشيشة الليمون - الكافور الليموني - القرنفل.

وقد وجد من نتائج البحث أن مادة Eugenol من زيت القرنفل هي أكثر المواد المفقودة بالمقارنة بباقي المواد المكونة للزيوت العطرية الاخري حيث بلغ تركيزها في الماء الناتج من تقطير القرنفل ٨٥٤ مللي جرام / لتر يليها مادة D- Carvon من زيت النعناع البلدي وبلغ تركيزها فى ماء التقطير ٢٤٢ مللي جرام / لتر ثم مادة Linalool من زيت الريحان الحلو (١٧١ مللي جرام / لتر) ونفس المادة من زيت لابلوب النارنج (١٢٨ مللي جرام / لتر).

وقد أثبتت هذه الدراسة أن ذوبان بعض المركبات العطرية فى ماء التقطير أثناء عملية الاستخلاص تعتمد على عده عوامل من أهمها قطبية هذه المواد، نسبة وجودها فى الزيت العطري، التداخل بين هذه المواد بعضها مع بعض فى داخل الزيت العطري واظهرت الدراسة أيضا عيوب ومميزات فقد كمية من المواد الطيارة المكونة للزيت العطري فى الماء الناتج من عملية الاستخلاص بالتقطير البخاري.