SOLVENT-FREE MICROWAVE EXTRACTION AND MICROWAVE-ASSISTED HYDRODISTILLATION OF ESSENTIAL OILS FROM SPICES

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ABSTRACT

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The undesirable effects of conventional methods generated the need for economical and safe techniques in the extraction of essential oils. Microwave-assisted hydrodistillation (MAHD) and solvent-free microwave extraction (SFME) are recently developed techniques, which are thought to overcome this problem.

Oregano *(Origanum vulgare* L.), laurel (*Laurus nobilis* L.) and rosemary (*Rosmarinus officinalis* L.) were chosen in this study since they have high antimicrobial and antioxidant effects and are widely grown and consumed in Turkey.

The objectives of this study were to examine the applicability of SFME in the extraction of essential oils from oregano and laurel, and MAHD in the extraction of rosemary essential oil. The effects of microwave power and extraction time on the yield, composition, and other quality parameters of

the extracts were also investigated. Hydrodistillation was performed as control.

SFME offered significantly higher essential oil yields (0.054 mL oil/g oregano) from oregano as compared to hydrodistillation (0.048 mL oil/g oregano). Conventional process time was reduced by 80%. Main aroma compound was thymol (650-750 mg thymol/mL oil).

For laurel, no significant differences were obtained in yields (about 0.022 mL oil/g laurel) obtained by SFME and hydrodistillation. Process time was reduced by 55-60%. Main aroma compound was 1,8-cineole (630-730 mg 1,8-cineole/mL oil).

In the case of rosemary, no significant differences were obtained in yields (about 0.026 mL oil/g rosemary) obtained by MAHD at 622 W and hydrodistillation. The process time was reduced by about 65%. Main aroma compounds were 1,8-cineole (430-500 mg 1,8-cineole/mL oil) and camphor (150-210 mg camphor/mL oil).

Keywords: Solvent-free microwave extraction (SFME), Microwave-assisted hydrodistillation (MAHD), *Origanum vulgare* L., *Laurus nobilis* L., *Rosmarinus officinalis* L.

ÖZ

ÇÖZÜCÜSÜZ MİKRODALGA EKSTRAKSİYONU VE MİKRODALGA YARDIMLI HİDRODİSTİLASYON YÖNTEMLERİ İLE BİTKİLERDEN ESANSİYEL YAĞLARIN ELDE EDİLMESİ

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Bitkilerden esansiyel yağ eldesi için kullanılan geleneksel yöntemlerin birtakım dezavantajlarının olması, araştırmacıları, bitkilerden esansiyel yağ eldesinde daha güvenilir ve ekonomik yöntemlerin arayışına yönlendirmiştir. Mikrodalga yardımlı hidrodistilasyon (MYH) ve çözücüsüz mikrodalga ekstraksiyonu (ÇME), bu dezavantajların etkisini büyük oranda azaltacağı tahmin edilen, son yıllarda geliştirilmiş yöntemlerdir.

Bu çalışmada, oldukça yüksek antimikrobiyal ve antioksidan özelliklere sahip olmaları, Türkiye'de çokça yetişmeleri ve tüketilmeleri nedeniyle dağ kekiği (*Origanum vulgare* L.), defne (*Laurus nobilis* L.) ve biberiye (*Rosmarinus officinalis* L.) seçilmiştir.

Bu çalışmanın amacı, ÇME yönteminin kekik ve defne uçucu yağları eldesinde, MYH yönteminin ise, biberiye uçucu yağı eldesinde kullanılabilirliğinin araştırılmasıdır. Aynı zamanda, mikrodalga gücünün ve işlem süresinin uçucu yağ verimi ve kalitesi üzerindeki etkisinin

incelenmesi amaçlanmaktadır. Hidrodistilasyon yöntemi kontrol amaçlı kullanılmıştır.

Kekikte, ÇME yöntemi (0.054 mL yağ/g kekik) ile hidrodistilasyona (0.048 mL yağ/g kekik) nazaran istatistiksel olarak belirgin derecede fazla uçucu yağı verimi elde edilmiştir. Hidrodistilasyon ile ulaşılan işlem süresi % 80 oranında düşürülmüştür. Kekiğin ana maddesinin timol (650-750 mg timol/mL yağ) olduğu görülmüştür.

Defnede, ÇME ve hidrodistilasyon yöntemleri ile elde edilen verimlerde (yaklaşık olarak 0.022 mL yağ/g defne) istatistiksel olarak önemli bir fark gözlenmemiştir. İşlem süresi % 55-60 oranında kısalmıştır. Defne yağının oldukça büyük bir kısmının 1,8-sineol'den (630-730 mg 1,8-sineol/mL yağ) oluştuğu anlaşılmıştır.

Biberiyede ise, MYH yöntemiyle ulaşılan verim ile hidrodistilasyonda elde edilen verim (yaklaşık olarak 0.026 mL yağ/g biberiye) arasında istatistiksel olarak önemli bir fark gözlenmemiştir. İşlem süresinin % 65 oranında azaldığı görülmüştür. Biberiyenin ana bileşenlerinin 1,8-sineol (430-500 mg 1,8-sineol/mL yağ) ve kamfor (150-210 mg kamfor/mL yağ) olduğu belirlenmiştir.

Anahtar kelimeler: Çözücüsüz mikrodalga ekstraksiyonu (ÇME), Mikrodalga yardımlı hidrodistilasyon (MYH), *Origanum vulgare* L., *Laurus nobilis* L., *Rosmarinus officinalis* L.

To My Family

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TABLE OF CONTENTS

ABSTRACT.....iv
ÖZ.....vi
ACKNOWLEDGMENTS.....ix
TABLE OF CONTENTS.....x
LIST OF TABLES.....xiii
LIST OF FIGURES.....xvi
CHAPTER
1. INTRODUCTION.....1
1.1. Essential Oils.....1
1.2. Oregano (*Origanum vulgare* L.) 9
1.3. Laurel (*Laurus nobilis* L.).....11
1.4. Rosemary (*Rosmarinus officinalis* L.) 14
1.5. Conventional Methods for the Extraction of Essential Oils.....17
1.6. Novel Technologies and Extraction of Essential Oils Using Microwaves.....19
1.7. Quality Parameters of Essential Oils.....25
1.7.1. Yield.....25
1.7.2. Composition.....25
1.7.3. Specific Gravity.....26
1.7.4. Refractive Index.....26
1.7.5. Solubility in Alcohol.....27
1.7.6. Optical Rotation.....27
1.8. Objectives of the Study.....28
2. MATERIALS AND METHODS.....30
2.1. Materials.....30
2.2. Methods.....31
2.2.1. Solvent-free Microwave Extraction (SFME).....31
2.2.2. Microwave-assisted Hydrodistillation (MAHD).....33
2.2.3. Hydrodistillation.....33
2.2.4. Analysis.....34

2.2.4.1. Particle Size Distribution.....34
2.2.4.2. IMPI-2L Test.....35
2.2.4.3. GC/GC-MS Analysis.....36
2.2.4.4. Specific Gravity Measurements.....40
2.2.4.5. Refractive Index Measurements.....40
2.2.4.6. Measurements of Solubility in Alcohol.....40
2.2.5. Statistical Analysis.....41
3. RESULTS AND DISCUSSION.....42
3.1. Oregano (*Origanum vulgare* L.) 42
3.1.1. Particle Size Distribution of Oregano Leaves.....42
3.1.2. Effect of extraction on quality parameters of oregano essential oil.....43
3.1.2.1. Yield.....43
3.1.2.2. Composition.....45
3.1.2.3. Other parameters.....55
3.1.2.3.1. Specific gravity.....55
3.1.2.3.2. Refractive index.....57
3.1.2.3.3. Solubility in ethanol.....59
3.2. Laurel (*Lauris nobilis* L.) 60
3.2.1. Particle Size Distribution of laurel leaves.....60
3.2.2. Effect of extraction on quality parameters of laurel essential oil.....61
3.2.2.1. Yield.....61
3.2.2.2. Composition.....63
3.2.2.3. Other parameters.....73
3.2.2.3.1. Specific gravity.....73
3.2.2.3.2. Refractive index.....75
3.3. Rosemary (*Rosmarinus officinalis* L.) 77
3.3.1. Particle Size Distribution of Rosemary Leaves.....77
3.3.2. Effect of extraction on quality parameters of rosemary essential oil.....78
3.3.2.1. Yield.....78

3.3.2.2. Composition.....80
3.3.2.3. Other parameters.....91
3.3.2.3.1. Specific gravity.....91
3.3.2.3.2. Refractive index.....93
4. CONCLUSION.....96
REFERENCES.....98
APPENDICES
A. ANOVA AND TUKEY'S PAIRWISE COMPARISON TEST TABLES.....124

LIST OF TABLES

TABLES

Table 1 Compounds present in the essential oil of *Origanum vulgare* L. obtained by different methods and their concentrations.....47

Table 2 Compounds present in the essential oil of *Laurus nobilis* L. obtained by different methods and their concentrations.....66

Table 3 Compounds present in the essential oil of *Rosmarinus officinalis* L. obtained by different methods and their concentrations.....82

Table A.1 ANOVA results for yield values obtained for *Origanum vulgare* L. (Box-Cox transformed).....124

Table A.2 Tukey's pairwise comparison test results for yield values obtained in the extraction of *Origanum vulgare* L. 124

Table A.3 ANOVA results for monoterpene hydrocarbon concentrations obtained in the extraction of *Origanum vulgare* L. 124

Table A.4 ANOVA results for oxygenated compound concentrations obtained in the extraction of *Origanum vulgare* L. 125

Table A.5 ANOVA results for sesquiterpene concentrations obtained in the extraction of *Origanum vulgare* L. 125

Table A.6 ANOVA results for specific gravity values obtained in the extraction of *Origanum vulgare* L. 125

Table A.7 ANOVA results for refractive index values obtained in the extraction of *Origanum vulgare* L. 125

Table A.8 ANOVA results for solubility in ethanol values obtained in the extraction of *Origanum vulgare* L. 126

Table A.9 ANOVA results for yield values obtained for *Laurus nobilis* L. 126

Table A.10 ANOVA results for monoterpene hydrocarbon concentrations obtained in the extraction of *Laurus nobilis* L. 126

Table A.11 ANOVA results for oxygenated compound concentrations obtained in the extraction of *Laurus nobilis* L. 126

Table A.12 ANOVA results for sesquiterpene concentrations obtained in the extraction of *Laurus nobilis* L. 127

Table A.13 Tukey's pairwise comparison test results for sesquiterpene concentrations obtained in the extraction of *Laurus nobilis* L. 127

Table A.14 ANOVA results for specific gravity values obtained in the extraction of *Laurus nobilis* L. 127

Table A.15 ANOVA results for refractive index values obtained in the extraction of *Laurus nobilis* L. 127

Table A.16 ANOVA results for yield values obtained for *Rosmarinus officinalis* L. 128

Table A.17 Tukey's pairwise comparison test results for yield values obtained in the extraction of *Rosmarinus officinalis* L. 128

Table A.18 ANOVA results for monoterpene hydrocarbon concentrations obtained in the extraction of *Rosmarinus officinalis* L. 128

Table A.19 Tukey's pairwise comparison test results for monoterpene hydrocarbon concentrations obtained in the extraction of *Rosmarinus officinalis* L. 128

Table A.20 ANOVA results for oxygenated compound concentrations obtained in the extraction of *Rosmarinus officinalis* L. 129

Table A.21 Tukey's pairwise comparison test results for oxygenated compound concentrations obtained in the extraction of *Rosmarinus officinalis* L. 129

Table A.22 ANOVA results for sesquiterpene concentrations obtained in the extraction of *Rosmarinus officinalis* L. 129

Table A.23 Tukey's pairwise comparison test results for sesquiterpene concentrations obtained in the extraction of *Rosmarinus officinalis* L. ...129

Table A.24 ANOVA results for specific gravity values obtained in the extraction of *Rosmarinus officinalis* L. 130

Table A.25 ANOVA results for refractive index values obtained in the extraction of *Rosmarinus officinalis* L. 130

LIST OF FIGURES

FIGURES

Figure 1 Developmental pattern of essential oil glands.....3

Figure 2 Major constituents of essential oils.....5

Figure 3 Structures of the major components of *Origanum vulgare* L. essential oil.....11

Figure 4 Structures of the major components of *Laurus nobilis* L. essential oil.....13

Figure 5 Structures of the major compounds of *Rosmarinus officinalis* L. essential oil.....16

Figure 6 Schematic diagram of a SFE system.....21

Figure 7 Schematic diagram of the SFME & MAHD processes.....32

Figure 8 Particle size distribution of *Origanum vulgare* L. leaves.....43

Figure 9 Variation of essential oil yield of *Origanum vulgare* L. during hydrodistillation and solvent-free microwave extraction (SFME) at different power levels.....45

Figure 10 Total ion chromatogram (obtained by GC-MS analysis) of the *Origanum vulgare* L. essential oil extracted by SFME at 100% power level.....46

Figure 11 Variation of component classes with time in the essential oil of *Origanum vulgare* L. obtained by hydrodistillation.....49

Figure 12 Variation of component classes with time in the essential oil of *Origanum vulgare* L. obtained by SFME at 100% power level.....50

Figure 13 Variation of component classes with time in the essential oil of *Origanum vulgare* L. obtained by SFME at 80% power level.....51

Figure 14 Variation of component classes with time in the essential oil of *Origanum vulgare* L. obtained by SFME at 60% power level.....52

Figure 15 Variation of component classes with time in the essential oil of *Origanum vulgare* L. obtained by SFME at 40% power level.....53

Figure 16 Variation of compound classes in the essential oil of *Origanum vulgare* L. with respect to different extraction methods.....55

Figure 17 Variation of specific gravity of the *Origanum vulgare* L. essential oil with time obtained by different methods.....56

Figure 18 Specific gravity values of *Origanum vulgare* L. essential oil extracted by different methods.....57

Figure 19 Variation of refractive index of the *Origanum vulgare* L. essential oil with time obtained by different methods.....58

Figure 20 Refractive index values of *Origanum vulgare* L. essential oil extracted by different methods.....59

Figure 21 Solubility in ethanol values of *Origanum vulgare* L. essential oil extracted by different methods.....60

Figure 22 Particle size distribution of *Laurus nobilis* L. leaves.....61

Figure 23 Variation of essential oil yield of *Laurus nobilis* L. during hydrodistillation and solvent-free microwave extraction (SFME) at different power levels.....62

Figure 24 Total ion chromatogram (obtained by GC-MS analysis) of the *Laurus nobilis* L. essential oil extracted by SFME at 100% power level.....65

Figure 25 Variation of component classes with time in the essential oil of *Laurus nobilis* L. obtained hydrodistillation.....68

Figure 26 Variation of component classes with time in the essential oil of *Laurus nobilis* L. obtained SFME-100% power level.....69

Figure 27 Variation of component classes with time in the essential oil of *Laurus nobilis* L. obtained SFME-40% power level.....70

Figure 28 Variation of monoterpene hydrocarbons and oxygenated compounds in the essential oil of *Laurus nobilis* L. with respect to different extraction methods.....72

Figure 29 Variation of sesquiterpenes in the essential oil of *Laurus nobilis* L. with respect to different extraction methods.....73

Figure 30 Variation of specific gravity of the *Laurus nobilis* L. essential oil with time obtained by different methods.....74

Figure 31 Specific gravity values of *Laurus nobilis* L. essential oil extracted by different methods.....75

Figure 32 Variation of refractive index of the *Laurus nobilis* L. essential oil with time obtained by different methods.....76

Figure 33 Refractive index values of *Laurus nobilis* L. essential oil extracted by different methods.....77

Figure 34 Particle size distribution of *Rosmarinus officinalis* L. leaves.....78

Figure 35 Variation of essential oil yield of *Rosmarinus officinalis* L. during hydrodistillation and microwave-assisted hydrodistillation (MAHD) at different power levels.....79

Figure 36 Total ion chromatogram (obtained by GC-MS analysis) of the *Rosmarinus officinalis* L. essential oil extracted by MAHD at 100% power level.....81

Figure 37 Variation of component classes with time in the essential oil of *Rosmarinus officinalis* L. obtained MAHD-100% power level.....86

Figure 38 Variation of component classes with time in the essential oil of *Rosmarinus officinalis* L. obtained MAHD-40% power level.....87

Figure 39 Variation of component classes with time in the essential oil of *Rosmarinus officinalis* L. obtained hydrodistillation.....88

Figure 40 Variation of compound classes in the essential oil of *Rosmarinus officinalis* L. with respect to different extraction methods.....89

Figure 41 Variation of specifiv gravity of the *Rosmarinus officinalis* L. essential oil with time obtained by different methods.....92

Figure 42 Specific gravity values of *Rosmarinus officinalis* L. essential oil extracted by different methods.....93

Figure 43 Variation of refractive index of the *Rosmarinus officinalis* L. essential oil with time obtained by different methods.....94

Figure 44 Refractive index values of *Rosmarinus officinalis* L. essential oil extracted by different methods.....95

CHAPTER 1

INTRODUCTION

1.1. Essential Oils

Essential oils are defined as “aromatic oily liquids” which can be obtained by a number of techniques from the leaves, flowers, buds, seeds, twigs, barks, herbs, woods, fruits and roots of the plant materials (Burt, 2004). Guenther (1948) also called essential oils as “volatile or ethereal oils” and stated that the effective component of a drug, Quinta essentia, which was named by Paracelsus von Hohenheim (Swiss reformer of medicine in the 16th century) is thought to be the source of the term “essential oil”.

Spices have been used for their perfume, flavour and preservative properties since antiquity (Bauer et al., 2001). However, only oil of turpentine among the known essential oils was mentioned by Greek and Roman historians (Guenther, 1948). Distillation was first used as a method of producing essential oils in Egypt, India and Persia (Guenther, 1948) more than 2000 years ago and it was improved in the 9th century by the Arabs (Bauer et al., 2001). The first authentic description of the distillation of real essential oils is generally ascribed to the Catalan physician, Arnald de Villanova, who may be said to have introduced the art of distillation into recognized European therapy (Guenther, 1948). Bauer et al. (2001) states that by the 13th century, essential oils were being made by pharmacies and their pharmacological effects were described in pharmacopoeias. However, their use does not appear to have been widespread in Europe until the 16th century (Crosthwaite, 1998). Guenther (1948) gave the names of two Strassburg physicians, Brunschwig and Reiff, who published separately in that century on the distillation and use of essential oils and mentioned the oils of turpentine, juniper wood, rosemary, spike (lavender),

clove, mace, nutmeg, anise and cinnamon. As the French physician, Joseph Du Chesne stated, the preparation of essential oils was well known in the 17th century and pharmacies generally stocked 15–20 different oils (Guenther, 1948). The use of tea tree oil for medical purposes has been documented since the colonisation of Australia at the end of the 18th century, although it is likely to have been used by the native Australians before that (Burt, 2004). The first experimental measurement of the bactericidal properties of the vapours of essential oils is said to have been carried out by De la Croix in 1881 (Burt, 2004). However, Guenther (1948) stated that in the 19th and 20th centuries the use of essential oils in medicine gradually became secondary to their use for flavour and aroma.

Today, essential oils are used in a wide variety of consumer goods such as detergents, soaps, toilet products, cosmetics, pharmaceuticals, perfumes, confectionary food products, soft and hard drinks, and also in insecticides. The world consumption of essential oil and perfumes is increasing very fast (Singh,1993). Van de Braak and Leijten (1999) claimed an estimated 3000 essential oils are known, of which about 300 are commercially important adding that the greatest use of essential oils in the European Union (EU) is in food (as flavourings), perfumes (fragrances and aftershaves) and pharmaceuticals (for their functional properties). Individual components of essential oils are also used as food flavourings, either extracted from plant material or synthetically manufactured. Aromatherapy is known to constitute slightly more than 2% of the total market (Burt, 2004).

The essential oils in aromatic herbs are known to be largely located within the glandular structures that develop on the surface of leaves and other organs of the plants (Gaspar and Leeke, 2004). Bosabilidis and Tsekos (1984) stated that the peltate hairs appear to contain most of the oil and is henceforth called ‘the glands’. Fig.1 illustrates the development of these

glands. The pattern is as follows: Each gland originates from a single protodermal cell that undergoes division and derives two unequally sized cells. The lower cell corresponds to the foot cell (FC) while the upper daughter cell re-divides to yield the stalk cell (SC) and the mother head cell (MHC). The foot and stalk cell remain unicellular throughout the subsequent development of the gland while the mother cell of the head further divides to give rise to 8 or 12 head cells (HC) by the end of the development (Gaspar and Leeke, 2004). Jerkovic et al. (2001) claimed that climatic factors, rates of plant metabolism, differentiation and secretory activity of glandular hairs affect synthesis and secretion of essentail oils.

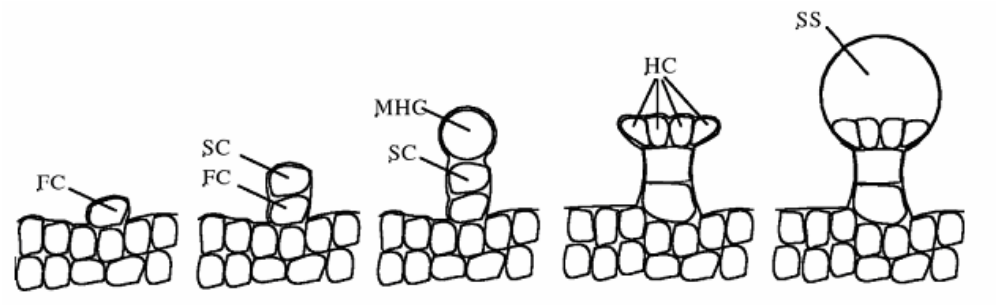


Fig 1. Developmental pattern of essential oil glands (Gaspar and Leeke, 2004).

Major constituents of most of the essential oils are hydrocarbons (terpenes, sesquiterpenes, etc.), esters, lactones, phenols, aldehydes, acids, alcohols, ketones, ethers,etc (Singh, 1993) as shown in Fig 2. Among these, the oxygenated compounds (alcohols, esters, aldehydes, ketones, lactones, phenols, etc.) are the principal odor carriers, although the terpenes and sesquiterpenes, too, contribute in some degree to the total odor and flavor of the oil (Guenther, 1948). The oxygenated compounds are more stable against oxidizing and more soluble in dilute alcohol as compared to other constituents. On the other side, unsaturated

constituents like monoterpenes and sesquiterpenes have tendency to oxidize or resinify easily under the influence of air and light (Singh, 1993) or under improper storing conditions which means spoilage of odor and flavor, and lowering of the solubility in alcohol (Guenther, 1948). The knowledge of individual constituents and their physical characteristics such as boiling point, vapour-pressure-temperature relationship, thermal stability are of paramount importance in technology development of oxygenated compounds (Singh; 1993).

Essential oil composition within many species of a plant can significantly differ depending on developmental and environmental factors (Johnson et al., 2004). For example, UV-B radiation has been shown to affect content and composition in *Ocimum basilicum* (Johnson et al., 1999; Ioannidis et al., 2002), *Mentha spicata* (Karousou et al., 1998) and *Mentha piperita* (Maffei and Scannerini, 2000), and visible radiation, acting through phytochrome photoreceptors, exerts similar effects in *Thymus vulgaris* (Tanaka et al., 1989) and *Satureja douglasii* (Peer and Langenheim, 1998). There are also several reports in the literature indicating that the essential oil composition of Origanum species varies with the season or day length (Putievsky et al., 1988, 1997; Circella et al., 1995; Kokkini et al., 1997). The strongest antimicrobial activity is generally attained in the essential oils produced from herbs harvested during or immediately after the flowering stage (McGimpsey et al., 1994; Marino et al., 1999). Lis-Balchin et al. (1999) showed that enantiomers of essential oil components exhibited antimicrobial activity to different extents. The composition of essential oils from different parts of the same plant can also differ widely (Burt, 2004).

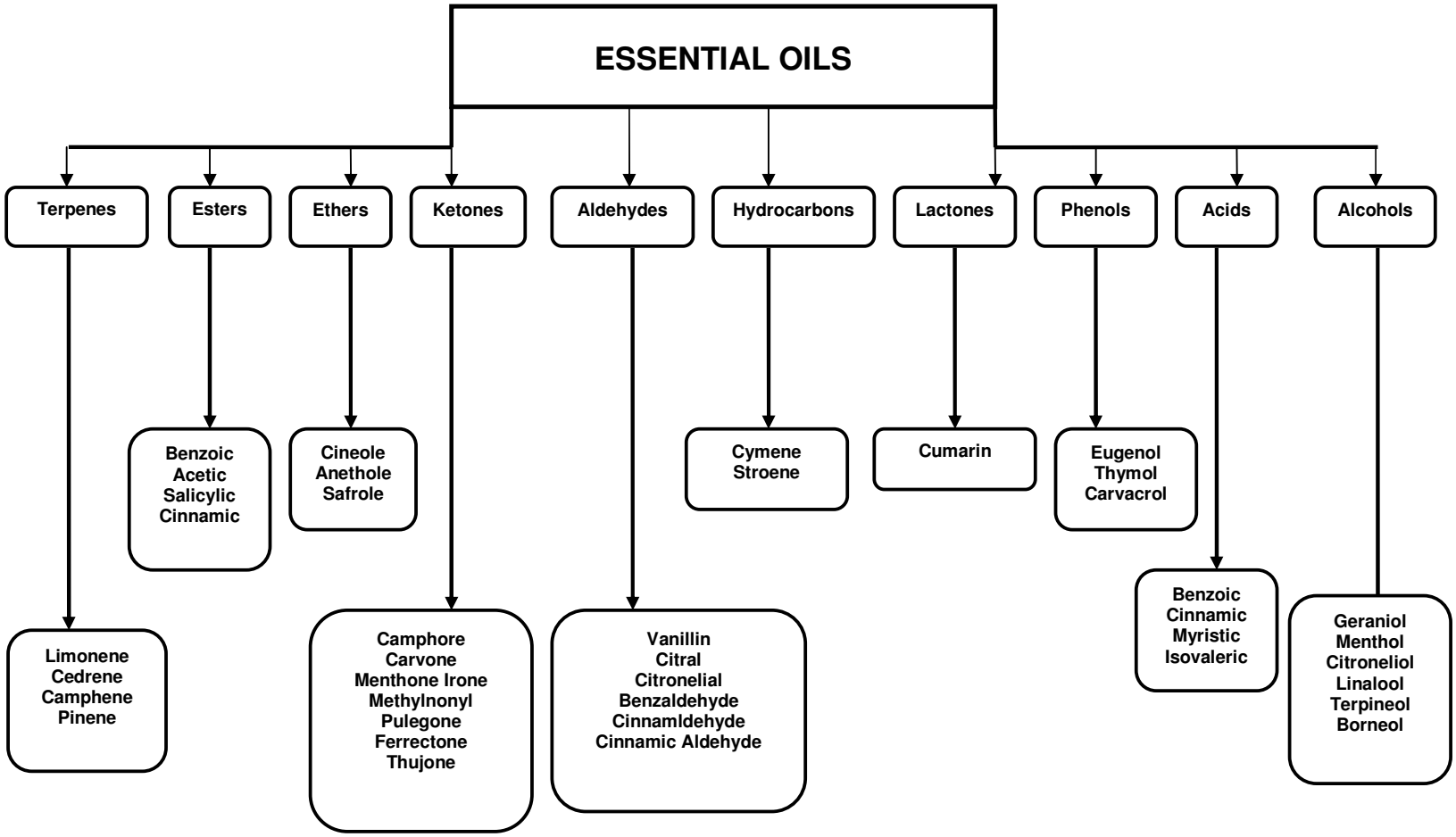


Fig.2. Major constituents of essential oils

Until the early 1990s, very few studies of the activity of essential oils in foods had been published (Board and Gould, 1991). Since then, a fair number of trials have been carried out with essential oils in foods (Burt, 2004). The antimicrobial activities of the essential oils of a variety of plant materials against many food borne pathogens have been tested up to now. For instance, the activity of rosemary oil against *Escherichia coli* was proved (Farag et al.,1989; Smith-Palmer et al.,1998; Hammer et al., 1999*;* Pintore et al., 2002*).* Rosemary essential oil has also been shown to possess antimicrobial activity against *Salmonella typhimurium* (Hammer et al., 1999), *Bacillus cereus* (Chaibi et al., 1997), *Staphylococcus aureus* (Pintore et al., 2002; Hammer et al., 1999; Smith-Palmer et al., 1998; Farag et al., 1989) and *Listeria monocytogenes* (Smith-Palmer et al., 1998). In addition, oregano essential oil is active against some food borne pathogens such as *E. coli* (Burt and Reinders, 2003; Hammer et al., 1999; Prudent et al., 1995), *S. typhimurium* (Hammer et al., 1999) and *S. aureus* (Hammer et al., 1999; Prudent et al., 1995). Sage, clove and thyme (Farag et al.,1989; Smith-Palmer et al.,1998; Hammer et al., 1999*;*), lemongrass (Hammer et al., 1999), turmeric (Negi et al., 1999) and tea bush (Bassole et al., 2003) essential oils are among the ones having antibacterial properties, as well. Cosentino (1999) stated that the phenolic components are mainly responsible for the antibacterial properties of essential oils.

Certain oils possess stronger antimicrobial properties than others for meat applications (Burt, 2004). Eugenol and coriander, clove, oregano and thyme oils were found to be effective at levels of 5–20 μL g^{-1} in inhibiting *L. monocytogenes*, *A. hydrophila* and natural spoilage flora in meat products, sometimes causing a marked initial reduction in the number of recoverable cells (Aureli et al., 1992; Stecchini et al., 1993; Hao et al., 1998a,b; Tsigarida et al., 2000; Skandamis and Nychas, 2001) whilst mustard, cilantro, mint and sage oils were less effective or ineffective (Shelef et al., 1984; Tassou et al., 1995; Gill et al., 2002; Lemay et al., 2002). As for fish dishes, applying the same concentrations, oregano oil was found to be

more effective than mint oil (Tassou et al., 1995; Koutsoumanis et al., 1999). Thyme oil also showed antibacterial activity againts *Pseudomonas putida* on cooked shrimps and against natural flora both on surface and in flesh of the whole Asian sea bass (Harpaz et al., 2003; Ouattara et al., 2001). Speaking of dairy products, mint oil at 5–20 μL g^{-1} was found to be effective against *S. enteritidis* in low fat yoghurt and cucumber salad (Tassou et al., 1995). Mint oil inhibited the growth of yoghurt starter culture species at 0.05–5 μL g^{-1} but cinnamon, cardamom and clove oils were much more effective (Bayoumi, 1992). All essential oils (thyme,oregano) and their components (basil methyl chavicol, cinnamaldehyde, thymol) that have been tested on vegetables appeared effective against the natural spoilage flora and food borne pathogens at levels of 0.1–10 μL g^{-1} in washing water (Singh et al., 2002; Wan et al.,1998). Many other studies were made searching for the antibacterial effects of various essential oils and their components on rice and fruits. Sage oil at 0.2–0.5 μL g^{-1} when used against *B. cereus* in rice was ineffective, whereas carvacrol at 0.15–0.75 μL g^{-1} was very effective in extending the lag phase and reducing the final population compared to control (Ultee et al., 2000; Shelef et al., 1984). Carvacrol and cinnamaldehyde were very effective in reducing the viable count of the natural flora on kiwifruit when used at 0.15 μL g^{-1} in dipping solution, but less effective on honeydew melon (Roller and Seedhar, 2002).

Essential oils have been shown to possess antioxidant properties, as well (Capecka et al., 2005; Faleiro et al., 2005; Bendini et al., 2002). The antioxidant activities of essential oils from savory (Gulluce et al., 2003), thyme and basil (Lee and Shibamoto, 2002), cloves and nutmeg (Dorman et al., 2000), and rosemary and cinnamon (Dang et al., 2001) were found similar to those of synthetic antioxidants. The antioxidant properties of 98 individual terpenoids and phenol derivatives present as components of essential oils were compared by studying their ability to inhibit the formation of linoleic acid peroxides (Ruberto and Baratta, 2000). The

antioxidant activities of eugenol, thymol, carvacrol, and methoxyphenol were similar to that of α-tocopherol. Cyclic monoterpene hydrocarbons with two double chains (γ- and α-terpinenes, α-terpinolene, and sabinene) were shown to have very high activity (80-98%) (Ruberto and Baratta, 2000), too. The presence of these compounds in essential oils determines their antioxidant properties (Misharina and Polshkov, 2005).

In addition to their antibacterial and antioxidant properties, owing to their components, essential oils have been demonstrated to exhibit antifungal (Voda et al., 2003; Viuda-Martos et al., 2007; Daferera et al., 2000), antiviral (Bishop, 1995), antimycotic (Mari et al., 2003; Akgül and Kıvanç, 1988) , antiparasitic (Pandey et al., 2000; Pessoa et al., 2002), insecticidal (Konstantopoulou et al., 1992; Karpouhtsis et al., 1998) properties.

There is an increasing demand for safer and more natural food. New methods are being developed for making food safe and 'green'. One such possibility is the use of essential oils as antimicrobial and antioxidant additives. Burt (2004) stated that a number of essential oil components have been registered by the European Commission for use as flavourings in foodstuffs, which are considered to present no risk to the health of the consumer and include amongst others carvacrol, carvone, cinnamaldehyde, citral, p-cymene, eugenol, limonene, menthol and thymol. Estragole and methyl eugenol were deleted from the list in 2001 due to their being genotoxic (Commission Decision of 23 January, 2002; Burt, 2004). The United States Food and Drug Administration (FDA) has classified the substances as generally recognised as safe (GRAS) or as approved food additives. In other countries and if added to food for a purpose other than flavouring, these compounds may be treated as new food additives. Expensive safety and metabolic studies would be necessary for the approval of an essential constituent as a food additive (Burt, 2004). Smid and Gorris (1999) suggested the use of a whole spice or herb or a whole essential oil as an ingredient than to use individual

essential oil components in those countries for the sake of economical considerations.

The use of essential oils in consumer goods is expected to increase in the future due to the rise of ‘green consumerism’, which stimulates the use and development of products derived from plants (Tuley de Silva, 1996). Bassett et al. (1990) stated that this applies to the food and cosmetic sectors as well as medicinal products. International standardisation of the composition of commercially available essential oils would be necessary for reliable applications (Carson and Riley, 2001).

1.2. Oregano (*Origanum vulgare* L.)

Origanum is used throughout the world as a very popular spice, under the name ‘oregano’. Despite the fact that the name 'oregano' is given to many species of various genera, most oregano spice products originate from species of the genus Origanum (Veres et al., 2003) belonging to the family, Lamiaceae (Souza et al., 2007). The genus Origanum is an annual, perennial and shrubby herb that is native to the Mediterranean, Euro-Siberian and Irano-Siberian regions (Sahin et al., 2004). Both morphologically and chemically the genus Origanum is very diverse and many transitional forms occur worldwide (Veres et al., 2003). Ietswaart (1980), based on morphological criteria, recognised 3 groups, 10 sections, 38 species, 6 subspecies and 17 hybrids within the genus. Skoula et al. (1999) stated that the members of the genus are mainly distributed along the Mediterranean region while 75% of them are restricted to the East Mediterranean. 16 species are considered as endemic for the flora of Turkey (Sahin et al., 2004).

Origanum species grow abundantly on stony slopes and in rocky mountain areas at the altitudes of 0–4000 m. Origanum plants belonging to different

species and ecotypes (biotypes) are widely used in agriculture and the pharmaceutical and cosmetic industries as a culinary herb, flavouring substances of food products, alcoholic beverages and perfumery for their spicy fragrance (Sahin et al., 2004). The essential oil of origanum has been shown to possess antimicrobial, antioxidant, and antimutagenic activities (Baratta et al., 1998; Kanazawa et al., 1995; Dapkevicius et al., 1998).

Origanum vulgare L. is the most variable species of the genus and the only one commonly known as `oregano' in most European countries (D'Antuono et al., 2000). Bertelli et al. (2003) stated that it is the most important species in Europe and is widely distributed in Europe and Asia up to a height of 2000 m. In the literature, major components of the Origanu*m vulgare* L. essential oil are specified as carvacrol, thymol, γ-terpinene, p-cymene with approximate compositions of trace-80%, trace-64%, 2-52% and trace-52%, respectively (Lawrence, 1984; Prudent et al., 1995; Charai et al., 1996; Sivropoulou et al., 1996; Kokkini et al., 1997; Russo et al., 1998; Daferera et al., 2000; Demetzos and Perdetzoglou, 2001; Marino et al., 2001). Moreover, these compounds are among the ones responsible for the antibacterial activity of the oil (Burt, 2004). Thymol and carvacrol had been proved to be active against *E. coli, S. typhimurium, S. aureus, L. monocytogenes* and *B. cereus* (Lambert et al., 2001; Pol and Smid, 1999; Cosentino et al.,1999; Kim et al., 1995). Due to their phenolic nature, thymol and carvacrol exhibits antioxidant properties (Faleiro et al., 2005), as well. The structures of the main constituents of *Origanum vulgare* L. essential oil are shown in Fig 3.

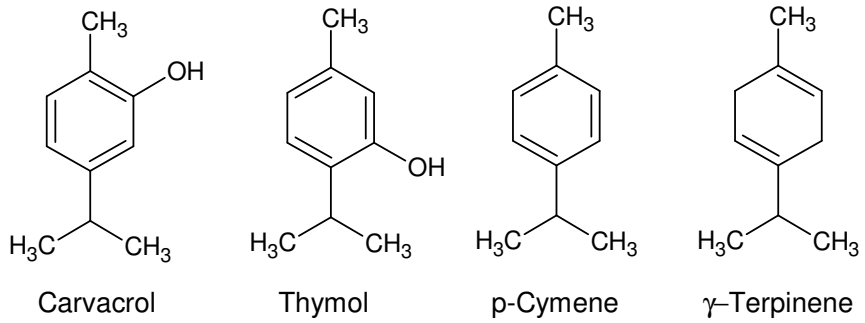


Fig. 3. Structures of the major components of *Origanum vulgare* L. essential oil

1.3. Laurel (*Laurus nobilis* L.)

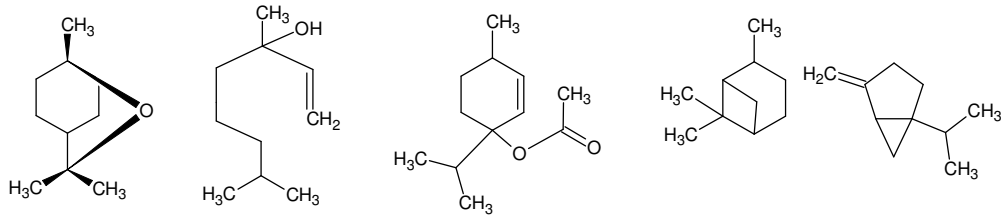
Laurus nobilis L., commonly known as laurel, bay or sweet bay, is an evergreen tree or shrub belonging to the family *Lauraceae* and it is native to the Mediterranean region and Turkey (Kosar et al., 2005a). The species has been esteemed since ancient times. It was dedicated to Apollo, the ancient Greek god of light, and was a symbol of peace and victory used to make wreaths for emperors, generals, and poets (Conforti et al, 2006).

Currently, the plant is cultivated in many Mediterranean countries (Kosar et al., 2005a) and the leaves are harvested principally in Turkey from wild growing plants (Akgül and Kıvanç, 1989). In 2000, Turkey exported 3600 tons of *L. nobilis* leaves with U.S. $7 500 000 income (Kiliç et al., 2004).

Laurel leaf, traditionally, has been used as herbal medicine to promote perspiration, to treat rheumatism, earaches, indigestion, sprains (Fang et al., 2005) and dermatitis (Kiliç et al., 2004). However, with respect to its dosage, care has to be taken because of its allergic effect (Pino et al., 1993). Recent research showed that it can be used in treating diabetes and preventing migraine (Duke, 1997).

Laurel leaf (*Laurus nobilis* L.) has been used as a spice since antiquity, primarily because of its essential oil content (Akgül and Kıvanç, 1989). Dried laurel leaves and their essential oil possess a strong, spicy aroma, and they are therefore widely used as flavor enhancers for foods such as fish (Bouzouita et al, 2001), meats, soups, sauces, and confectionery (Diaz-Maroto et al., 2002). The essential oil of laurel leaves has a fresh-green, strong, sweet-spicy, aromatic-eucalyptaceous odor and a fresh, delicate spicy, camphoraceous- eucalyptaceous taste (Braun et al., 2001). It is known that the essential oil is used widely in the perfume and soap industries (Kosar et al., 2005a) as well as in drugs (Kilic et al., 2004). The soaps are known to be good for acne and have an antidandruff activity (Kiliç et al., 2004).

The essential oil of laurel leaf has been the subject of several studies, and Lawrence (1978, 1980, 1981, 1983, 1987) has reviewed the compounds identified by different authors. Another comparative study of chemical composition of laurel leaf from different geographical origins (Albania, France, Italy and Spain) was reported by Pino et al. (1993), and it was affirmed that the composition of laurel leaf oil depended upon the geographical origin of the leaf (Bouzouita et al., 2001). Generally, 1,8-cineole was found to be the major component of essential oil of laurel leaves, with percentages ranging between 30% and 56%, followed by linalool, α-terpinyl acetate, and several monoterpene hydrocarbons such as β-pinene and sabinene (Fig.4) (Diaz-Maroto et al., 2002). It is also known that benzene compounds such as eugenol, methyl eugenol, and elemicin, present in percentages ranging between 1% and 12%, are responsible for the spicy aroma of laurel leaves and are extremely important factors determining the sensory quality of the leaves (Borges et al., 1992; Biondi et al., 1993; Pino et al., 1993).



1,8-cineole linalool alpha-terpinyl acetate beta-pinene sabinene

Fig. 4. Structures of the major components of *Laurus nobilis* L. essential oil

Several studies have evaluated the potential role of laurel essential oil as an antimicrobial agent (Bouzouita et al., 2003; Simic et al., 2004), and also the antioxidant properties of some leaves extracts (Simic et al., 2003; Skerget et al., 2005) up to now. For instance, among the constituents of the essential oil of *Laurus nobilis* L., α-terpineol was found to be active against *E. coli, S. typhimurium, S. aureus, L. monocytogenes* and *B. cereus* (Cosentino et al.,1999) and eugenol was active against *E. coli, S. Typhimurium and L. Monocytogenes* (Kim et al., 1995). These properties are other factors for laurel to be used in the food industry as a food preservative (Kilic et al., 2004). Laurel leaves are also known to have pharmacological activities such as antifungal, antibacterial, antidiabetes, and antiinflammatory (Fang et al., 2005). Sesquiterpene lactones identified in laurel leaves were found to have different pharmacological properties including inhibitory effects on NO (nitric oxide) production (antiinflammatory) (Matsuda et al., 2000), and inhibitory effects on alcohol absorption (Yoshikawa et al., 2000).

1.4. Rosemary (*Rosmarinus officinalis* L.)

Rosmarinus officinalis L. is a small evergreen shrub which grows wild in most Mediterranean countries, reaching a height of 1.5 m (Atti-Santos et al., 2005). It belongs to the Lamiaceae family, which comprises up to 200 genera and about 3,500 species; it is an aromatic plant with an intense pleasant smell, dark green lavender-like leaves, and a long flowering season extending from April to August (Lo Presti et al., 2005). The name Rosmarinus comes from Latin *ros-roris*, which means dew; it was also called *'antos'* by the ancient Greeks, which is 'the flower' for excellence or 'libanotis' for its smell of incense (Guenther, 1948). The botany of rosemary is rather complex, as there are several species within the Genus *Rosmarinus*, with a range of varieties and forms (Atti-Santos et al., 2005). *Rosmarinus officinalis* L. is one of the most spread species of the genus (Pintore et al., 2002) and the only one that grows naturally in the Mediterranean regions among the other species (Angioni et al., 2004).

Because of its rusticity, *Rosmarinus officinalis* L. grows in every soil type, but prefers a sandy, arid, calcareous, humus-poor soil. Usually the plant is clonally propagated because of the poor germinability of its seeds and the genetic diversity of the seedlings (Flamini et al., 2002) The main producers of rosemary oil are Turkey, Italy, Dalmatia, Spain, Greece, Egypt, France, Portugal and North Africa (Atti-Santos et al., 2005), while the United States, Japan, and some of the European Union countries are the principal importers (Flamini et al., 2002).

Rosmarinus officinalis L. is used fresh, dried or as the essential oil (Bauer et al., 1990). It is used in cosmetics, in traditional medicine for its choreretics, hepatoprotective and antitumorigenic activity and for flavoring food (Ramírez et al., 2006). The leaves are used in the preparation of alcoholic beverages (vermouth), herbal soft drinks, and cooked foods and sauces (Flamini et al., 2002). Additionally, the leaves are used as a tonic

for blood circulation and the nervous system, for chronic weakness, asthenia, and peripheral vascular disorders in folk medicine (Lo Presti et al., 2005). The plant parts used for essential oil production are generally the flowering aerial tops, comprising leaves, twigs, and flowers, collected from spring to late autumn (Flamini et al., 2002), however the highest quality essential oil is obtained from the leaves (Lo Presti et al., 2005). The essential oil is used as a seasoning for foodstuffs such as meat dishes, salami, and sauces. Since it is characterized by unique aromatic characteristics and balsamic properties, the oil is used in perfumery and as a component of disinfectants and insecticides as well (Lo Presti et al., 2005).

Elamrani et al. (2000) stated that many factors can influence the essential oil yield of rosemary, specifying those factors as heredity, part and age of the plant, climatological environment, and isolation method. A large amount of research has been carried out on this plant in terms of chemical composition and the variability of the qualitative and quantitative composition of the essential oil was also attributed to these factors (Lo Presti et al., 2005). The chemical composition of the oil was reported for the first time by Chalchat et al. (1992), who identified 48 constituents. Besides, several studies investigated the composition of rosemary essential oil. The major components of the oil were reported as 1,8-cineole (eucalyptol), α-pinene, camphor, borneol, myrcene, and p-cymene (Katerinopoulos et al., 2005). The structures of the main constituents are shown in Figure 5. Pintore et al. (2002) claimed that two major types of rosemary oil can be distinguished with respect to these main constituents: oils with over 40% of 1,8-cineole (oils from Morocco, Tunisia, Turkey, Greece, Yugoslavia, Italy, France) (Lawrence, 1997; Rezzoug et al., 1998) and oils with approximately equal ratios (20–30%) of 1,8-cineole, α-pinene and camphor (oils from France, Spain, Italy, Greece, Bulgaria) (Lawrence, 1995).

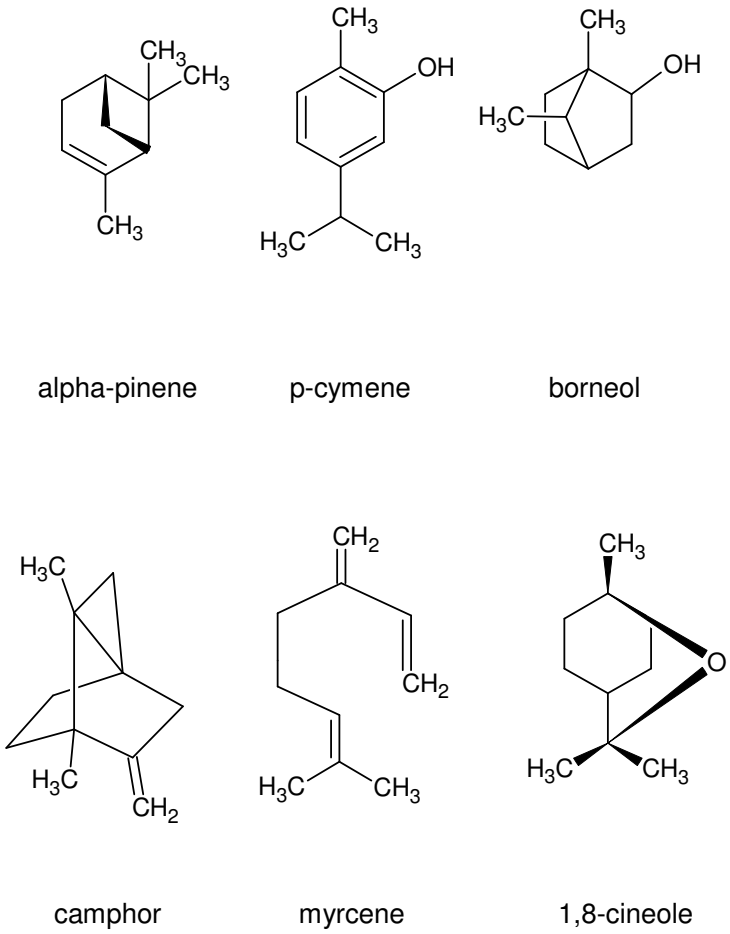


Fig. 5. Structures of the major compounds of *Rosmarinus officinalis* L. essential oil

Rosmarinus officinalis L., has also been shown to exhibit antibacterial activity (Valero and Salmerón, 2003) and to contain several antioxidants (Bicchi et al., 2000). Rosemary essential oil exhibited good microbicidal activity against mycetes and Gram-positive and Gram-negative bacteria and the main active components were reported as 1,8-cineole, camphor, and pinenes (Hethelyi et al., 1989; Panizzi et al., 1993; Caccioni and Guizzardi, 1994; Perrucci et al., 1994; Biavati et al., 1997). In addition, the oil exhibited insecticide properties and in vitro antifungal activity against *Ascosphaera apis* (Larràn et al., 2001). As mentioned before, rosemary

extracts show antioxidative properties, due to the presence of phenolic diterpenes such as rosmarinic acid (Flamini et al., 2002). It is the only spice commercially available for use as an antioxidant in Europe and the United States (Yanishlieva et al., 2006). Rosemary was considered as both lipid antioxidant and metal chelator (Nozaki, 1989). Rosemary extracts were found also to scavenge superoxide radicals (Basaga et al., 1997). Thorsen and Hildebrandt (2003) stated that the quality as antioxidant and the price of commercial rosemary extract was highly correlated to the content of primarily carnosic acid and secondly to the total content of phenolic diterpenes (rosmanol, epirosmanol, etc.) including carnosol. A range of commercial products containing extracts of rosemary are available; some of the products are water dispersible, others are oil soluble, and in order to exploit the synergistic effect, some of them are combined with tocopherols (Yanishlieva et al., 2006).

1.5. Conventional Methods for the Extraction of Essential Oils

Essential oils are complex mixtures of volatile substances generally present at low concentrations. Before such substances can be analyzed, they have to be extracted from the plant matrix (Deng et al., 2006). Various different methods have been used for that purpose up to now. These conventional methods include hydrodistillation (HD), steam distillation (SD) and simultaneous distillation-extraction (SDE). Although soxhlet extraction (SoE) and solvent extraction (SE) have been used to extract neutraceuticals from plant materials, the final product (extract) was not considered as essential oils because of the presence of non-volatile constituents.

Steam distillation is known to be commercially the most common method for the extraction of essential oils. It is extensively used for wide variety of oil bearing plant materials such as seed, root and wood having high boiling

constituents (Singh, 1993). In steam distillation, high-pressure steam is passed in at the base of a still into the space beneath a perforated grid, which supports the charge of the plant material. The steam passes through the plant, heating and saturating it with water. The resulting vapour, a mixture of steam and essential oil vapors, passes out at the top and is conveyed to a condenser (Sovová and Aleksovski, 2006). Temperature of volatilization is lowered by injection of steam into a charge. The components are isolated in accordance with their boiling points. Steam distillation is used to seperate mixtures at a temperature lower than the normal boiling points of their constituents. Therefore, it is advantageous in seperating heat sensitive materials. It is always desirable to use low pressure steam for thermally sensitive oil. In order to reduce the channeling, even distribution of plant materials in the still is necessary, otherwise low oil yields may result (Singh, 1993).

Hydrodistillation with a modified Clevenger apparatus is another widely used conventional method to obtain the essential oil from plant materials. It is suitable for plants which can easily agglutinate and form lumps with live steam (Singh, 1993). In hydrodistillation, contrary to the steam distillation, the plant material is completely immersed in boiling water (Sovová and Aleksovski, 2006). The water is boiled by application of heat and diffusion of essential oils and hot water through the plant membranes occurs (Guenther, 1948). The mixture of water vapor and essential oil vapors is condensed in the condenser and finally collected in a receiver. Lo Presti et al. (2005) stated that hydrodistillation achieves component isolation according to their degree of hydrosolubility rather than to their boiling points. Additionally, this method is believed to protect the oils extracted to a certain degree, since the surrounding water acts as a barrier to prevent them from overheating (Sovová and Aleksovski, 2006).

Among the several techniques that have been developed to isolate volatile compounds, simultaneous distillation–extraction, introduced in 1964 by

Likens and Nickerson, is one of most widely employed (Teixeira et al., 2007). The method has been successfully applied in the extraction of essential oils (Godefroot et al., 1981; Stashenko et al., 2004a; Eikani et al., 2005), aroma compounds (Blanch et al., 1996) and other volatile products (Careri et al., 1999; Barták et al., 2000; Ramos et al., 1998) from numerous matrices. This technique combines steam distillation together with continuous extraction with a solvent or a mixture of solvents (Chaintreau, 2001). This one-step extraction technique is less time consuming and allows a greater reduction of solvent volumes due to the continuous recycling. Moreover, given its particular characteristics, this technique allows to carry out the analysis without a sample clean-up step. The extracts obtained by simultaneous distillation-extraction are free from non-volatile materials (Teixeira et al., 2007).

1.6. Novel Technologies and Extraction of Essential Oils Using Microwaves

Improvement in production technology is an essential element to improve the overall yield and quality of the product. Essential oils used in drug and pharmaceutical industries are acceptable only if they pass pharmaceutical tests. On the other hand, properties like odour and taste become necessary in the case of perfumery and flavory industries and hence they have to pass organoleptic testings. Quality of essential oils is of paramount importance in the food industry, as well. Evidently, the yield and quality of essential oils and their isolates are significantly governed by the way they are extracted and processed (Singh, 1993).

It is known that conventional methods used for the extraction of essential oils and extracts from plant materials have some disadvantages mainly concerned with the quality of the final product. Losses of some volatile

compounds, low extraction efficiency, degradation of unsaturated or ester compounds through thermal or hydrolytic effects and toxic solvent residue in the extract may be encountered using these extraction methods (Ferhat et al., 2007). Moreover, these extraction procedures are time-consuming. These shortcomings have led the researchers to develop new technologies, which use less solvent, time and energy. Novel technologies developed for obtaining neutraceuticals from plant materials include ultrasound-assited extraction (UAE) (Vinatoru, 2001), supercritical fluid extraction (SFE) (Lang & Wai, 2001), accelerated solvent extraction (ASE) (Kaufmann & Christen, 2002; Smith, 2002), and microwave-assisted extraction (MAE) (Kaufmann & Christen, 2002). Recently developed techniques such as microwave-assisted hydrodistillation (MAHD) (Stashenko et al., 2004b), and solvent-free microwave extraction (SFME) (Lucchesi et al., 2004a) are among the novel technologies developed for the extraction of essential oils. Each method have its own advantages and disadvantages.

Supercritical fluid extraction (Fig.6) is analogous to extraction and distillation because both the solubility and volatility effects are utilized. Density and solvent power of supercritical fluids are like those of liquids, whereas, transport properties (viscosity, diffusivity) and compressibility are like those of gases. When the supercritical fluid and sample mixture are brought into contact, soluble components are extracted from the sample mixture. Once the extraction is completed, raffinate and extract phases are separated. Solvent is easily recovered from phases for reuse by making small variations in temperature or pressure or both. Carbon dioxide is generally used as a solvent in supercritical fluid extraction. Its critical conditions are 31°C, 72.8 bar and 0.47 g/cm^3 which permits extraction at around 40°C, depending upon the nature of plant materials (Singh, 1993). Supercritical fluid extration has been used to extract plant materials, especially lipids (Bernardo-Gil et al., 2002), essential oils (Coelho et al., 2003; Sonsuzer et al., 2004) and flavors (Giannuzzo et al., 2003). Wang

and Weller (2006) stated that supercritical fluid extraction can achieve higher yield and quality of essential oils, flavors and natural aromas than conventional steam distillation.

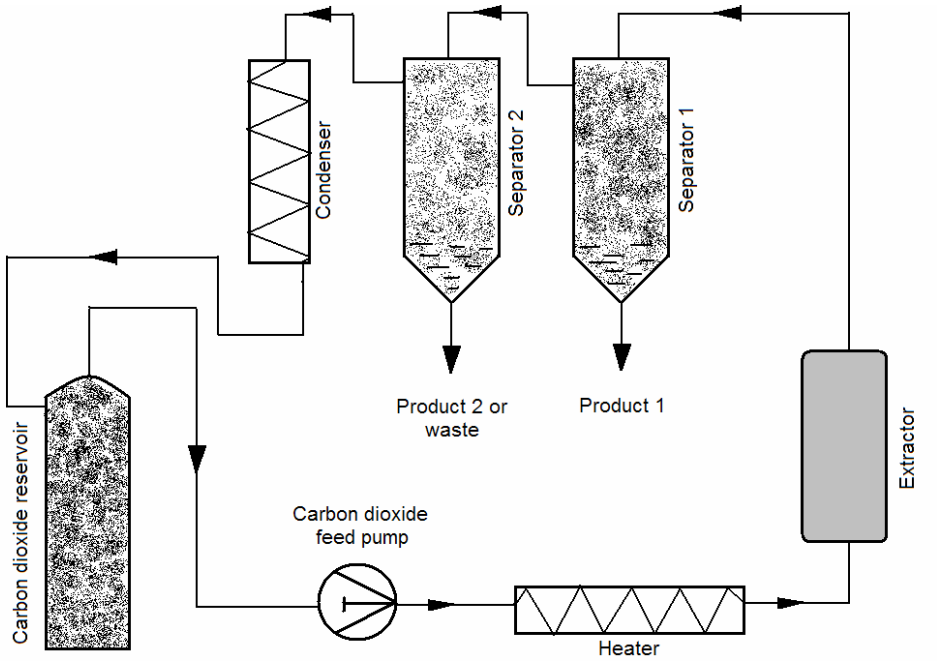


Fig. 6. Schematic diagram of a SFE system

Recently, there has been widespread interest in the application of microwave heating to extraction processes. Microwaves are electromagnetic radiations with a frequency from 0.3 to 300 GHz. Domestic microwave ovens operate at 2.45 GHz, while industrial microwaves operate occasionally at 0.915 GHz in the Europe & USA and at 0.896 GHz in U.K. Microwave energy is also characterized by short wavelengths. Owing to this combination of short wavelength and high frequency, microwave heating is more effective than conventional heating methods. Polar molecules absorb microwave energy more easily causing

resonance and thus generating direct frictional heat. Microwave heating is also more effective because heating proceeds from inside to outside, unlike conventional heating systems where heat flows from outside to inside (Singh, 1993). Much attention has been given to the application of microwave dielectric heating in analytical chemistry owing to its accompanying advantages such as more effective heating, faster energy transfer, selective heating, reduced analysis time, equipment size and energy consumption, faster response to process heating control, faster start-up, simplified manipulation, elimination of process steps and higher purity of the final product (Ferhat et al., 2006).

Microwave-assisted extraction is one of the new methods which makes use of microwave heating for the extraction of neutraceuticals. The technique offers a rapid delivery of energy to a total volume of solid plant matrix and/or solvent with subsequent heating of the solid matrix and/or solvent, efficiently and homogeneously. Because water within the plant material absorbs microwave energy, cell disruption is promoted by internal superheating, which facilitates desorption of chemicals from the matrix, improving the recovery of neutraceuticals (Kaufmann et al., 2001). Up to now, essential oils, aromas, pesticides, phenols, dioxins and other organic compounds have been extracted efficiently from a variety of matrices such as soils, sediments, animal tissues, food and plant materials. All the applications have shown that microwave-assisted solvent extraction is a good alternative to conventional techniques for such materials (Ferhat et al., 2006). There are two types of commercially available microwave-assisted extraction systems: closed extraction vessels under controlled pressure and temperature, and focused microwave ovens at atmospheric pressure (Kaufmann and Christen, 2002). The closed microwave-assisted extraction system is generally used for the extraction under high temperatures. In contrast, the focused microwave-assisted extraction system is operated at a maximum temperature determined by the boiling point of the solvent at atmospheric pressure. If a dry biomaterial is re-

hydrated before extraction, the matrix itself can interact with microwaves and hence facilitate the heating process. The microwave heating leads to the expansion and rupture of cell walls and is followed by the liberation of chemicals into the solvent (Spar Eskilsson & Bjorklund, 2000). In this case, the surrounding solvent can have a low dielectric constant and thus remains cold during extraction. Therefore, this method can be used to extract thermo-sensitive compounds such as essential oils (Wang and Weller, 2006). The main benefits of microwave-assisted extraction technique are reduction of processing time and solvent used. However, there are some drawbacks of the method such as additional filtration or centrifugation necessary to remove the solid residue and poor efficiency of microwaves when the target compounds or the solvents are non-polar (Wang and Weller, 2006).

Stashenko et al. (2004b) developed microwave-assisted hydrodistillation for the extraction of essential oils in plant materials. In this method, the dry plant material and substantial amount of water placed in a Clevenger apparatus was heated inside a microwave oven (which has a hole on the top) for a short period of time to take out the essential oil. Heat is produced by microwave energy so the sample reaches its boiling point very rapidly, leading to very short extraction or distillation times. It is also possible to achieve distillation with the water inside the material. Microwaves interact selectively with the free water molecules present in the gland and vascular systems; this leads to localized heating, and the temperature increases rapidly near or above the boiling point of water. Thus, such systems undergo a dramatic expansion, with subsequent rupture of their walls, allowing the essential oil to flow towards free water (Benkaci-Ali et al., 2006). In the literature, there are reported studies which used microwave-assisted hydrodistillation for the extraction of essential oils from *Anethum graveolens* L., *Coriandum sativum* L. (Kosar et al., 2005a), *Salvia triloba* L., *Laurus nobilis* L. (Kosar et al., 2005b), *Lippia alba* (Mill.) N.E. Brown (Stashenko et al., 2004a), *Lavandula angustifolia*

(Iriti et al., 2006), *Heracleum crenatifolium* Boiss., *H. platytaenium* Boiss., *H. sphondylium* L. subsp. *ternatum* (Velen.) Brummit (Ozek et al., 2005).

More recently, Lucchesi et al. (2004a) developed a new technique combining microwave heating and dry distillation at atmospheric pressure for isolation and concentration of the essential oils in fresh plant materials. They called this method as “solvent-free microwave extraction”. The method involves placing the fresh or rehydrated (by soaking in water for some time and then draining off the excess water) dry plant material in a microwave reactor, which is connected to a Clevenger apparatus outside the microwave oven, without adding any solvent or water. The internal heating of the water within the plant material distends it and makes the glands and oleiferous receptacles burst. This process frees essential oil which is drawn by the water inside the plant material by azeotropic distillation. The vapour then passes through a condenser outside the microwave cavity where it is condensed. The distillate is collected continuously in the receiving flask. The essential oil is collected directly and dried without any solvent extraction step. Solvent free microwave extraction has been used to obtain essential oils from different raw materials. Lucchesi et al. (2004a, 2004b, 2007) analysed the essential oils of three aromatic herbs (basil, garden mint, and thyme), three spices (ajowan, cumin, and star anise) and cardamom seed. The inventors of the technique claimed that solvent-free microwave extraction is neither a modified microwave-assisted extraction nor a modified hydrodistillation which uses a large quantity of water.

More techniques other than the stated ones above have been developed for the extraction of essential oils since the development of solvent-free microwave extraction. These include “improved solvent-free microwave extraction” (ISFME) (Wang et al., 2006b), which uses microwave absorption solid medium such as carbonyl iron powders rather than water, “microwave-assisted steam distillation” (MASD) (Chemat et al., 2006) in

which steam is produced by heating water with microwave radiation, and "microwave-assisted simultaneous distillation-solvent extraction" (MW-SDE) (Ferhat et al., 2007). All of them involve the combination of microwave heating with their corresponding extraction techniques, thus possessing the aforementioned advantages of microwave extraction.

1.7. Quality Parameters of Essential Oils

1.7.1. Yield

Essential oil yield can be defined as the ratio of amount of essential oil extracted to the amount of plant material used. Due to the fact that whole essential oils with high yields are favored, yield is among the most important quality parameters. Today, for this reason, researchers and industries are working on obtaining high quality essential oils by improving the processing technologies.

1.7.2. Composition

Composition of essential oils is of paramount importance in determining their quality. However, the quality criteria change with respect to the final use of the product in the target industry (food, pharmaceutical, cosmetic, etc.). To speak in general, essential oils rich in oxygenated constituents are favored rather than rich in terpene constituents since oxygenated compounds are primarily responsible for the flavor and aroma of the essential oils. Moreover, terpenes are very sensitive compounds to heat and light, so they might decompose easily. In addition to their contribution

to the aroma and flavor of the essential oil, some oxygenated compounds are known to possess high antioxidant and antibacterial activities, as well.

1.7.3. Specific Gravity

Specific gravity is another important criterion of the quality and purity of an essential oil. It can be defined as the ratio of the weight of a given volume of essential oil at 15-25℃ to the weight of an equal volume of distilled water at the same temperature. Among the physicochemical properties, specific gravity has been reported most frequently in the literature. Values for essential oils vary between the limits of 0.696 and 1.188 at 15℃. Generally, pycnometers are used for specific gravity measurements since they offer the most convenient results (Guenther, 1948).

1.7.4. Refractive Index

Refractive index can be defined as the ratio of the velocity of light in a vacuum to its velocity in a transparent specimen. The index of refraction is related to the physical structure of the medium through which light is passing. For this reason, the index of refraction is a characteristic of substances that can be employed in identifying unknowns. The higher the refractive index, the greater the amount of dispersion, which increase the brilliance of a material. Refractive index measurements should be made at constant temperature.

1.7.5. Solubility in Alcohol

Since most essential oils are only slightly soluble in water and are miscible with absolute alcohol, it is possible to determine the number of volumes of dilute alcohol required for the complete solubility of unit volume of oil. The determination of solubility of essential oils in dilute alcohol is a convenient way of evaluation of the quality of the oil. It is known that, in general, oils rich in oxygenated constituents are more readily soluble in dilute alcohol than oils rich in terpenes. Alcohols having strengths of 50% - 60% - 70% - 80% - 90% - 95% and occasionally 65% and 75% are used in determining the solubilities of essential oils. Care should be taken for the temperature of the environment while making the measurements since solubility is affected by temperature (Guenther, 1948).

1.7.6. Optical Rotation

Most essential oils when placed in a beam of polarized light possess the property of rotating the plane of polarization to the right (dextrorotatory) or to the left (laevorotatory). The extent of the optical activity of an oil is determined by a polarimeter and is measured in degrees of rotation. Both the degree of rotation and its direction are important as criteria of purity. Optical rotation readings are usually taken at room temperature since the variation of optical rotation with temperature for most of the essential oils is very small (Guenther, 1948).

1.8. Objectives of the Study

It is known that conventional methods used for the extraction of essential oils from plant materials posses some disadvantages like losses of some volatile compounds, low extraction efficiency, degradation of unsaturated or ester compounds through thermal or hydrolytic effects and presence of toxic solvent residue in the extract. Microwave-assisted hydrodistillation (MAHD) and solvent-free microwave extraction (SFME) are two recently developed techniques, which are thought to overcome these disadvantages.

Oregano *(Origanum vulgare* L.), laurel (*Laurus nobilis* L.) and rosemary (*Rosmarinus officinalis* L.) are generally used in food, cosmetic and pharmaceutical industries. These spices are widely grown and consumed in Turkey. Additionally, essential oils of these plant materials are known to have high antimicrobial and antioxidant effects. Therefore, usage of a safe and economical technique for the extraction of valuable essential oils from these plant materials is extremely important.

In the literature, there are reports indicating the use of SFME for the extraction of essential oils of ajowan, cumin, star anise, basil, garden mint, thyme and cardamom (Lucchesi et al., 2004a, 2004b, 2007) and MAHD for the extraction essential oils from *Lippia alba* (Mill.) N.E. Brown (Stashenko et al., 2004b), three endemic Turkish *Heraclum* species (Ozek et al., 2005), rosemary (Lo Presti et al., 2005), *Salvia triloba* L., *Laurus nobilis* L., dill seed, coriander seed (Kosar et al., 2005a, 2005b) and black cumin seeds (Benkaci-Ali et al., 2007). However, there are no reports on the solvent-free microwave extraction of essential oils from oregano or laurel. Although microwave-assisted hydrodistillation of rosemary has been studied, variation of essential oil constituents during extraction and the effects of different microwave power levels have not been investigated.

Moreover, there are quite few reports on the quantitative analysis of essential oil constituents of oregano, laurel and rosemary.

The objectives of the present study are to examine the applicability of SFME and MAHD methods in the extraction of essential oils from oregano, laurel and rosemary to investigate the effects of microwave power and extraction time on the yield and composition of the final product. In addition, it was aimed to compare these methods with the conventional hydrodistillation regarding to the yield and composition of the extracts. Quality parameters such as specific gravity, refractive index and solubility of essential oils in alcohol were also examined.

CHAPTER 2

MATERIALS AND METHODS

2.1. Materials

The dried *Origanum vulgare* L., *Laurus nobilis* L., and *Rosmarinus officinalis* L. were obtained from Kutas (Kutas Tarim Urunleri Dis Tic. San. A.S., Manisa, Turkey).

Authentic standard materials were used for the qualitative and quantitative analysis of the essential oils. The mixture of α-pinene, 1-octen-3-ol, β-myrcene, α-terpinene, p-cymene, γ-terpinene, 4-terpineol, thymol, carvacrol and β-caryophyllene was purchased from Supelco (Bellefonte, PA, USA) as a custom mix. The other standard materials were β-eudesmol, isopulegol, verbenone (Fluka, Japan), α-humulene, 4-terpineol, 3-carene, bornyl acetate, eugenol, fenchol, terpinolene, (Fluka, Switzerland), α-pinene, β-myrcene, γ-terpinene, methyleugenol, cuminaldehyde, p-cymene (Fluka, US), valencene (Fluka, Israel), myrtenal, copaene, o-cimene, limonene (Fluka, Germany), β-caryophyllene (Fluka, Spain), thymol, borneol, α-terpineol, 1,8-cineole, camphor, β-pinene, methyl jasmonate, linalool (Sigma-Aldrich, Steinheim, Germany) and camphene (Supelco, Bellefonte, PA, USA). Nonane (internal standard), hexane, sodium sulfate anhydrous and ethanol were purchased from Fluka (Buchs, Switzerland), Merck (Darmstadt, Germany), Riedel-de Haën (Seelze, Germany), and Merck (Darmstadt, Germany), respectively.

2.2. Methods

For the extraction of essential oils of *Origanum vulgare* L. and *Laurus nobilis* L., solvent-free microwave extraction (SFME) technique was performed. However, it was observed that SFME was unsuccessful in dry rosemary leaves since the plant material did not absorb enough water in the water soaking step prior to extraction due to its arid nature and burning was observed even for short extraction times. Therefore, MAHD was performed rather than SFME for the extraction of the essential oil from *Rosmarinus officinalis* L.

2.2.1. Solvent-free Microwave Extraction (SFME)

The microwave oven used for SFME was White-Westinghouse (Pittsburg, USA) operating at 2450 MHz with a maximum delivered power of 622 W (measured using IMPI-2L test (Buffler, 1993)). In SFME, to study the effect of microwave power, four power levels, 100% (622W), 80% (498 W), 60% (373 W) and 40% (249 W) were studied for *Origanum vulgare* L. and two power levels (100% and 40%) were studied for *Laurus nobilis* L.

The dimensions of the interior cavity are 29 x 37 x 40 cm. Microwave oven was modified by drilling a hole at the top. Flat bottom flask having a capacity of 1000 mL was placed in the oven and connected to Clevenger apparatus through the hole. After the Clevenger apparatus was placed into the oven, the hole around the neck of the flask was covered with polytetrafloraethylene (teflon) to prevent leakage of microwave. For SFME, 50 g of dried *Origanum vulgare* L. was soaked in 700 mL distilled water at room temperature for 1 h in order to hydrate the external layers of the plant material and the excess water was drained off. In the case of *Laurus nobilis* L., 150 g of dried sample was soaked in 700 mL distilled water for 1

h at room temperature. The moistened plant material was placed in flat-bottom flask connected to the Clevenger apparatus. During the process, the vapor passes through the condenser outside the microwave cavity where it is condensed. The process was continued until no more essential oil was obtained. Schematic diagram of the process is illustrated in Figure 7. Experiments were performed starting from the beginning each time to collect the essential oil samples during extraction since taking continuous data was not possible. For each condition, experiments were replicated twice. The essential oil was collected in amber colored vials, dehydrated with anhydrous sodium sulfate, capped under nitrogen and kept at 4°C until being analyzed.

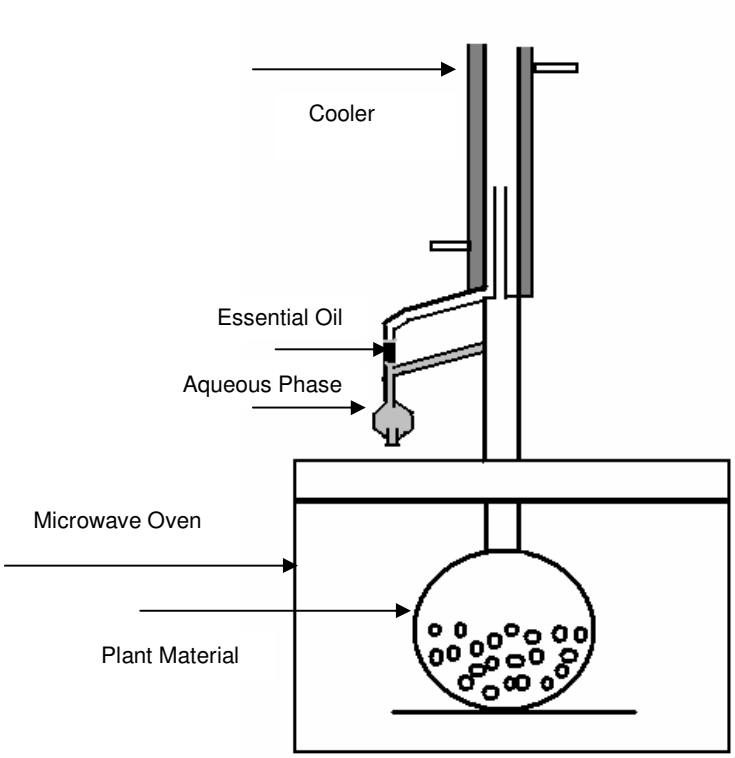


Fig. 7. Schematic diagram of the SFME & MAHD processes

2.2.2. Microwave-assisted Hydrodistillation (MAHD)

The same experimental set-up used in SFME was utilized for the MAHD process (Fig.7). To investigate the effect of microwave power, two power levels (100% and 40%) were studied for *Rosmarinus officinalis* L.

The procedure is the same as in SFME except the saoking step of plant material in water for 1 h. Instead, 350 mL of distilled water was added to 50 g of *Rosmarinus officinalis* L. The process was continued until no more essential oil was obtained. Experiments were performed starting from the beginning each time to collect the essential oil samples during extraction since taking continuous data was not possible. For each condition, experiments were conducted twice. The essential oil was collected in amber colored vials, dehydrated with anhydrous sodium sulfate, capped under nitrogen and kept at 4°C until being analyzed.

2.2.3. Hydrodistillation

In conventional hydrodistillation, dried plant materials were hydrodistilled using a Clevenger apparatus. Solid:water ratios were 1:10 for *Origanum vulgare* L. and *Laurus nobilis* L. and 1:7 for *Rosmarinus officinalis* L. A heater (Termal Laboratory Equipments, Istanbul,Turkey) with a maximum power of 200 W was used for this purpose. For each condition, experiments were conducted twice. The essential oil was collected in amber colored vials, dehydrated with anhydrous sodium sulfate, capped under nitrogen and kept at 4°C until being analyzed.

2.2.4. Analysis

2.2.4.1. Particle Size Distribution

Particle size distribution was obtained by performing a sieve analysis. A set of test sieves (Endecotts Ltd., England) was placed on an automatic shaker (Endecotts Octagon 200 Test Sieve Shaker, England). The sieve having the largest diameter of openings (8 mm) was placed at the top and the one having the smallest diameter of openings (425 micron) was placed at the bottom. The sizes of the openings of the other sieves were as follows: 4 mm, 2 mm, 1 mm, 850 micron. A pan was located under the last sieve for the particles passing the finest sieve.

50 g of *Origanum vulgare* L., 100 g of *Laurus nobilis* L. and 100 g of *Rosmarinus officinalis* L. leaves were seperately placed on the top sieve and the set was shaken for 30 min. Once the process was finished, the particles retained on each sieve were removed and weighed. Mass fractions of the retained particles were calculated.

Particle size analysis was performed using the differential analysis method. Since the particle size ranges differ from sieve to sieve, a plot of particle size range versus $W_i/(D_{p(i+1)} - D_{pi})$ was drawn, where W_i is the mass fraction and $(D_{p(i+1)} - D_{pi})$ is the particle size range in sieve 'i' (McCabe et al., 1993; Sahin and Sumnu, 2006).

Sauter mean diameter ($\overline{D}_s$) of the plant materials was calculated using the following formula:

$$\overline{D}_s = \frac{1}{\sum (W_i \overline{D}_{pi})} \qquad (2.1)$$

where $\overline{D}_{pi}$ is the average particle diameter described as the following formula:

$$\overline{D}_{pi} = \frac{D_{pi} + D_{p(i+1)}}{2} \tag{2.2}$$

2.2.4.2. IMPI-2L Test

The maximum power of the microwave oven was determined using the IMPI (International Microwave Power Institute) 2L test (Buffler, 1993). The oven was operated at 100% power for 5 min with a load of 2000 ± 5 g water placed in two 1-L Pyrex beakers. The oven cavity was wiped with a wet cloth. The subsequent procedure is as follows: Two beakers of 1-L capacity, each containing 1000 g of water, were placed in the center of the oven, side by side and touching each other in the width dimension of the cavity. Initial temperature of the water in the beakers was 20℃ ± 2℃. The oven was operated for 2 min 2 s at 100% power level. Final temperatures were measured immediately after the oven was turned off. 3 replications were made and the oven cavity was wiped with a wet cloth each time. The power was calculated using the following formula:

$$P(W) = \frac{70(\Delta T_1 + \Delta T_2)}{2} \tag{2.3}$$

where ΔT_1 and ΔT_2 are the temperature rises of the water in the two beakers calculated by subtracting the initial water temperature from final temperature (Buffler, 1993).

2.2.4.3. GC/GC-MS Analysis

The essential oils obtained were analyzed by gas chromatography (Agilent Technologies 6890N Network GC System, USA) and gas chromatography coupled to mass spectrometry (Agilent Technologies 6890N Network GC System coupled to Agilent Technologies 5973 Network Mass Selective Detector, USA). In order to perform quantitative analysis with FID at the same time with the component characterization of MSD, a two hole ferrule was used in which two columns were placed. By this way, injection of the sample from one injection block was distributed equally into two columns. The capillary columns used for both of the analysis were HP-5MS (30m × 0.25mm × 0.25µm) with a 5% phenyl methyl siloxane stationary phase. The samples were injected to the columns with an Agilent Tecnologies 7683B Series Injector (Thailand). The components of the essential oils were identified by comparison of their retention times with those of available authentic standards and with library matching of their mass spectra (NIST98, Wiley7n, Flavor2). The data were analyzed by a software program, MSD ChemStation.

GC-MS conditions for *Origanum vulgare* L. were as follows: carrier gas, He; flow rate, 1 mL/min; splitless; injection volume 1 µL; injection temperature 250°C; oven temperature program, from 50°C to 150°C with 3°C/min, holding at 150°C for 10 min, to 250°C with 10°C/min; MSD transfer line temperature, 250°C; MSD quadrupole temperature, 150°C; ionization temperature, 230°C; ionization mode, electronic impact at 70 eV. Solvent delay was for 4 min. The GC analysis was performed with the following conditions: flow rate, 0.6 mL/min; FID temperature, 250°C; make-up gas type, He with a make-up flow rate of 45 mL/ min.

GC-MS conditions for *Laurus nobilis* L. were as follows: carrier gas, He; flow rate, 1.2 mL/min; splitless; injection volume 1 µL; injection

temperature 250°C; oven temperature program, holding at 60°C for 5 min, and rising to 210°C with 2°C/min; MSD transfer line temperature, 230°C; MSD quadrupole temperature, 150°C; ionization temperature, 230°C; ionization mode, electronic impact at 70 eV. Solvent delay was for 4.5 min. The GC analysis was performed with the following conditions: flow rate, 0.8 mL/min; FID temperature, 260°C; make-up gas type, He with a make-up flow rate of 45 mL/ min.

GC-MS conditions for *Rosmarinus officinalis* L. were as follows: carrier gas, He; flow rate, 0.8 mL/min; splitless; injection volume 1 µL; injection temperature 250°C; oven temperature program, holding at 50°C for 2 min, rising to 225°C with 3°C/min; MSD transfer line temperature, 250°C; MSD quadrupole temperature, 150°C; ionization temperature, 230°C; ionization mode, electronic impact at 70 eV. Solvent delay was for 4.5 min. The GC analysis was performed with the following conditions: flow rate, 0.4 mL/min; FID temperature, 275°C; make-up gas type, He with a make-up flow rate of 45 mL/ min.

For the quantitative analysis of *Origanum vulgare* L. essential oil, calibration solutions composed of the custom mix (α-pinene, 1-octen-3-ol, β-myrcene, α-terpinene, p-cymene, γ-terpinene, 4-terpineol, thymol, carvacrol, β-caryophyllene, Supelco, USA) and internal standard (nonane) were prepared in 7 concentrations and injected into the columns with the GC method explained above. The concentration of the internal standard in each solution was constant (229.668 ppm). A calibration curve ($r^2 \geq 0,998$) was obtained for each component in the solution, which was used for the quantitation of the corresponding components in the oregano essential oil. For the quantitation of the other constituents of oregano essential oil, the approach of Schoenmakers et al. (2000) was used. They claimed that the response of FID to a large number of compounds within a component class is very similar and highly constant, therefore, the response factor of a component within a class can be used for the quantitation of the other

components in the same class. Following this approach, monoterpene hydrocarbons, phenols, alcohols and sesquiterpenes in the essential oil of *Origanum vulgare* L. were quantified using the relative response factors of α-terpinene (~1.24), thymol (~1.26), 1-octen-3-ol (~1.53) and β-caryophyllene (~1.26), respectively. 3-octanone (ketone) was quantified by assuming its relative response factor as 1. The relative response factor is described as follows:

$$f_i = \frac{A_s W_i}{A_i W_s} \tag{2.4}$$

where f_i is the relative response factor of a target component; A_i and A_s are the integrated peak area of a target component and internal standard, respectively; W_i and W_s are the mass of a target component and internal standard, respectively.

For the quantitative analysis of *Laurus nobilis* L. essential oil, calibration solutions composed of eugenol, γ-terpinene, cuminal, 1,8-cineole, β-pinene, p-cymene, β-caryophyllene, bornyl acetate, methyl eugenol, α-pinene, linalool, α-terpineol, myrtenal, 4-terpineol, camphor , β-eudesmol (external standards) and nonane (internal standard) were prepared in 7 concentrations and injected into the columns with the corresponding GC method explained above. The concentration of the internal standard in each solution was constant (238.855 ppm). A calibration curve ($r^2 \geq 0{,}998$) was obtained for each component in the solution, which was used for the quantitation of the corresponding components in the laurel essential oil. Following the same approach stated above, monoterpene hydrocarbons, phenols, alcohols, ketones, aldehydes, esters, ethers and sesquiterpenes in the essential oil of *Laurus nobilis* L. were quantified using the relative response factors of β-pinene (~1.23), eugenol (~1.48), 4-terpineol (~1.43), camphor (~1.34), myrtenal (~1,18), bornyl acetate (~1.6), 1,8-cineole (~1.46) and β-caryophyllene (~1.26), respectively. For the quantitation of

lactones the relative response factor of β-caryophyllene (sesquiterpene) was used.

For the quantitative analysis of *Rosmarinus officinalis* L. essential oil, calibration solutions composed of γ-terpinene, methyl jasmonate, verbenone, 1,8-cineole, β-pinene, p-cymene, β-caryophyllene, bornyl acetate, methyl eugenol, carvacrol, 4-terpineol, camphor , fenchol (external standards) and nonane (internal standard) were prepared in 7 concentrations and injected into the columns with the corresponding GC method explained above. The concentration of the internal standard in each solution was constant (238.855 ppm). A calibration curve ($r^2 \geq 0,998$) was obtained for each component in the solution, which was used for the quantitation of the corresponding components in the rosemary essential oil. Following the same approach stated above, monoterpene hydrocarbons, phenols, alcohols, ketones, esters, ethers and sesquiterpenes in the essential oil of *Rosmarinus officinalis* L. were quantified using the relative response factors of β-pinene (~0.943), carvacrol (~1.073), 4-terpineol (~1.092), camphor (~1.059), bornyl acetate (~1.245), 1,8-cineole (~1.068) and β-caryophyllene (~0.935), respectively. For the quantitation of aldehydes, the relative response factor of camphor was used since Zhu et al. (2005) claimed that the relative response factors of aldehydes were close to those of ketones.

5 µL of *Origanum vulgare* L. essential oil was diluted with 1545 µL hexane and 10 µL of 5 % (v/v) internal standard (nonane) solution was added to the sample in order to minimize the injection errors. For the analysis of *Laurus nobilis* L. essential oil, 5 µL of oil was diluted with 1445 µL hexane and 50 µL of 1 % (v/v) nonane solution was added to the sample. Finally, for the the analysis of *Rosmarinus officinalis* L. essential oil, 5 µL of oil was diluted with 1445 µL hexane and 50 µL of 1 % (v/v) nonane solution was added to the sample. The samples were injected with their corresponding GC methods.

2.2.4.4. Specific Gravity Measurements

Specific gravities of the essential oils of *Origanum vulgare* L., *Laurus nobilis* L. and *Rosmarinus officinalis* L. were calculated by dividing the weight of 10 µL essential oil to that of 10 µL distilled water. Weight measurements were made in triplicate using a highly sensitive balance (Denver Instrument, Germany) at 22 ± 2°C.

2.2.4.5. Refractive Index Measurements

Refractive index measurements were made in triplicate using the Bellingham Stanley Ltd. RFM 330 refractometer (England). Measurement temperatures were 25 ± 2°C.

2.2.4.6. Measurements of Solubility in Alcohol

Solubility of the essential oil in 85% (v/v) ethanol was determined by using the method of Institute of Turkish Standards (TS 780). 1 mL of oil was diluted with 0.1 mL of 85% ethanol and throughly shaken each time. The dilution procedure is continued until a clear mixture was obtained. The volume of alcohol (V) used to obtain a completely clear solution was recorded. Once the clear solution was obtained, dilution process was continued this time with 0.5 mL of ethanol until 20 times of the volume of previously added alcohol was attained. The solution was throughly shaken each time 0.5 mL ethanol was added. No turbidity or opalescence was observed during the process. The measurements were made at 22-23°C. Three replications were made. The results were expressed as:

1 volume of essential oil soluble in V volumes or more of 85% ethanol.

2.2.5. Statistical Analysis

The results (essential oil yields, chemical classes of compounds and physical properties) were statistically evaluated by analysis of variance (ANOVA). The assumptions (normality and constant variance of the residuals) of ANOVA were checked. Normality assumption was checked by Anderson-Darling test. Once the normality or constant variance assumption was not satisfied for residuals, Box-Cox transformation was performed. Whenever significance was obtained, Tukey test ($p \leq 0.05$) was performed using MINITAB for Windows (Version 13).

CHAPTER 3

RESULTS AND DISCUSSION

In this section, the particle size distribution results of oregano, laurel and rosemary leaves were given. Additionally, the results obtained in the extraction (by SFME, MAHD and hydrodistillation) of essential oils from these plant materials in terms of yield, composition and physical properties such as specific gravity, refractive index and solubility in alcohol were given and discussed. The effects of microwave power and time on yield, composition and the physical properties of the essential oils were also discussed.

3.1. Oregano (*Origanum vulgare* L.)

3.1.1. Particle Size Distribution of Oregano Leaves

Particle size distribution of oregano leaves is given in Figure 8. Sauter mean diameter ($\overline{D}_s$) of the leaves was calculated as 1727.5 µm.

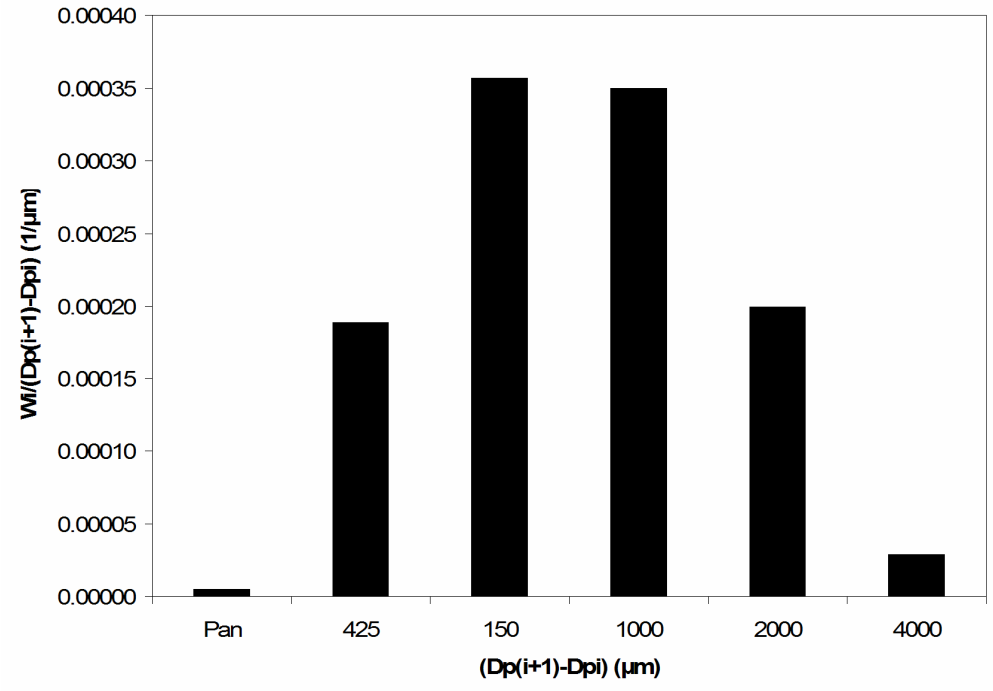


Fig. 8. Particle size distribution of *Origanum vulgare* L. leaves

3.1.2. Effect of extraction on quality parameters of oregano essential oil

3.1.2.1. Yield

Effects of microwave power level and duration of the SFME process on the essential oil yield of oregano were investigated. The amount of water absorbed by dry oregano leaves was about 290 % of its initial weight when soaked in water for 1 h. The maximum yields obtained were found as 0.054, 0.053, 0.052 and 0.049 mL oil/g oregano, for 100% (622 W), 80% (498 W), 60% (373 W) and 40% (249 W) power levels, respectively. The yield was 0.048 mL oil/g oregano in hydrodistillation method. The results were found to be significantly different ($p \leq 0.05$) except for the yields obtained in hydrodistillation and SFME working at 40% power level (Table

A.1-2). Therefore, it can be concluded that SFME method offered higher yield essential oil from *Origanum vulgare* L. as compared to conventional hydrodistillation. The decrease in the yield with lower microwave power and hydrodistillation process can be explained by the loss of some of the volatile compounds due to longer processing time.

Figure 9 shows the variation of essential oil yield with time in SFME and hydrodistillation processes. As it is clearly seen from the graph, the initial extraction rate increased with increase in microwave power. This is doubtlessly due to the rapid generation of heat inside the moistened oregano with the absorption of microwave energy and the subsequent formation of a higher pressure gradient inside the plant material when subjected to higher microwave power levels. The essential oils must have been thrown out of the glands quicker due to this increased pressure gradient. Another observation was that the time to reach the maximum yield was significantly higher in hydrodistillation (3 h) than in SFME and decreased as the microwave power increased (35 min for 622 W, 50 min for 249 W). The reason for the 80% reduction in the time of the extraction process in SFME method than in hydrodistillation is again the high pressure gradient formed inside the plant material. The decrease in processing time with an increase in power can be explained by the same reason; as microwave power increases the pressure gradient increases, which causes a rise in the extraction rate (Ferhat et al., 2006; Lucchesi et al., 2007).

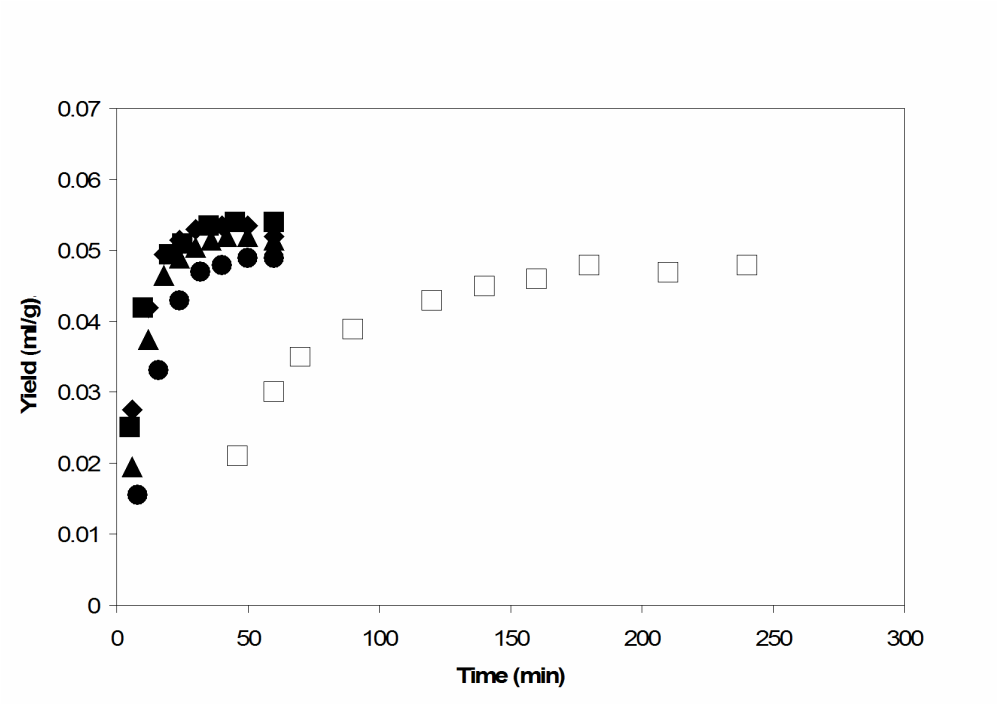


Fig. 9. Variation of essential oil yield of *Origanum vulgare* L. during hydrodistillation and solvent-free microwave extraction (SFME) at different power levels (■, SFME-100% power level[a]; ♦, SFME-80% power level[a]; ▲, SFME-60% power level[ac]; ●, SFME-40% power level[bc]; □, hydrodistillation[b])

3.1.2.2. Composition

The total ion chromatogram of the origanum essential oil is given in Figure 10. The composition of the essential oil of *Origanum vulgare* L. obtained by SFME and conventional hydrodistillation methods are given in Table 1. As it is observed from the table, the composition of the essential oils obtained by both of the methods are almost the same. The main components of the essential oil of *Origanum vulgare* L. were determined as thymol (650-750 mg/mL oil) followed by p-cymene (60-85 mg/mL oil), carvacrol (40-60 mg/mL oil), γ-terpinene (35-50 mg/mL oil), β-myrcene (~15 mg/mL oil) and α-terpinene (10-15 mg/mL oil). It was also found that the essential oil was composed mainly of oxygenated compounds (80-85

%) while monoterpene hydrocarbons and sesquiterpenes constituted 13-16 % and 1-1.6 % of it, respectively. This situation explains the pungent odour of *Origanum vulgare* L. since oxygenated compounds are the ones responsible for the characteristic aroma of essential oils, monoterpenes contribute to their fragrance to a very small extent (Lucchesi et al., 2004a).

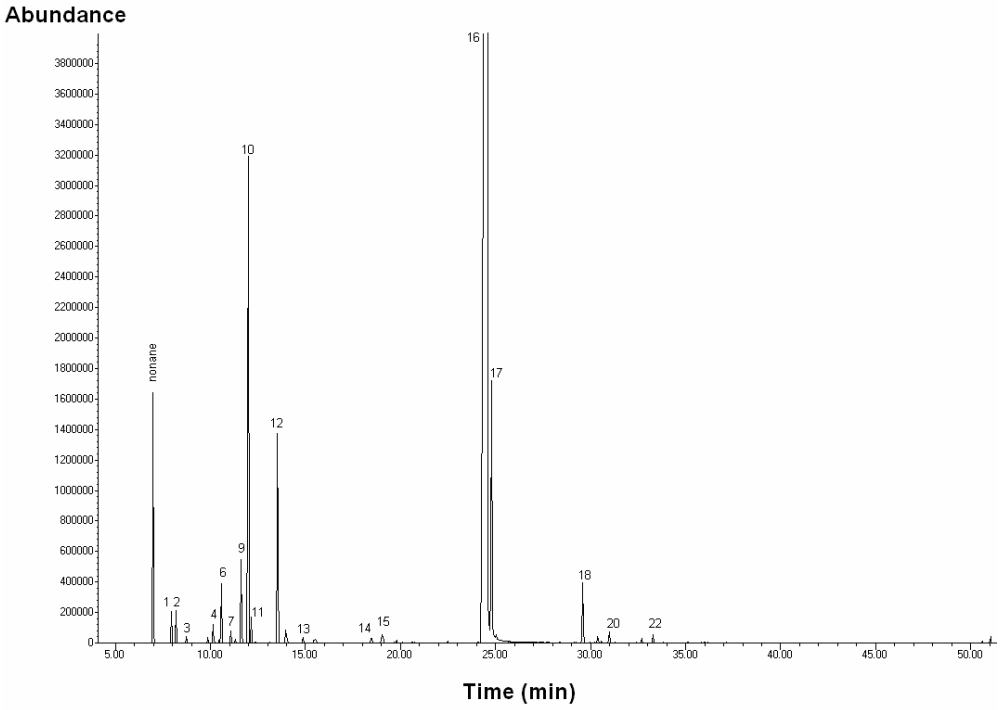


Fig.10. Total ion chromatogram (obtained by GC-MS analysis) of the *Origanum vulgare* L. essential oil extracted by SFME at 100% power level (1: α-thujene, 2: α-pinene, 3: camphene, 4: 1-octen-3-ol, 6: β-myrcene, 7: α-phellandrene, 9: α-terpinene, 10: p-cymene, 11: β-phellandrene, 12: γ-terpinene, 13: terpinolene, 14: borneol, 15: 4-terpineol, 16: thymol, 17: carvacrol, 18: β-caryophyllene, 20: α-humulene, 22: β-bisabolene)

Table 1. Compounds present in the essential oil of *Origanum vulgare* L. obtained by different methods and their concentrations

				Hydrodistillation			SFME-100% power level			SFME-80% power level			SFME-60% power level			SFME-40% power level		
				46 (min)	*90 (min)*	*180 (min)*	*5 (min)*	*20 (min)*	*45 (min)*	*6 (min)*	*18 (min)*	*60 (min)*	*6 (min)*	*24 (min)*	*60 (min)*	*8 (min)*	*24 (min)*	*60 (min)*
No	**Compounds**	**RT 1 (min)**	**RT 2 (min)**	**(mg/ml)**	**(mg/ml)**	**(mg/ml)**	**(mg/ml)**	**(mg/ml)**	**(mg/ml)**	**(mg/ml)**	**(mg/ml)**	**(mg/ml)**	**(mg/ml)**	**(mg/ml)**	**(mg/ml)**	**(mg/ml)**	**(mg/ml)**	**(mg/ml)**
1	α-thujene	7.949	12.275	8.572	2.460	3.921	6.406	2.058	5.533	8.388	8.090	4.160	10.751	9.555	4.645	9.945	-	2.800
2	α-pinene	8.184	12.579	12.212	6.093	6.515	7.299	3.519	6.844	8.363	8.262	4.477	11.372	10.488	5.608	10.095	1.332	3.059
3	camphene	8.744	13.221	2.542	1.318	1.225	1.724	-	1.271	1.914	1.729	-	2.498	2.116	1.543	2.205	-	-
4	1-Octen-3-ol	10.123	14.527	27.108	13.778	10.747	27.481	11.489	11.915	29.077	15.631	8.997	34.910	20.416	13.090	32.248	10.067	13.450
5	3-Octanone	10.421	14.903	7.064	3.375	-	6.924	-	1.776	7.272	2.519	-	8.681	4.946	2.200	8.383	-	2.291
6	β-myrcene	10.57	15.082	26.373	14.559	13.581	19.336	11.249	16.210	23.592	20.771	13.104	28.628	27.038	17.751	28.915	6.661	12.171
7	α-phellandrene	11.068	15.711	3.684	1.939	1.846	2.916	1.661	2.293	3.390	2.911	1.877	4.047	3.712	2.493	4.099	-	1.704
8	3-Carene	11.308	16	1.521	-	-	1.248	-	1.089	1.310	1.494	-	1.565	1.487	1.300	1.523	-	-
9	α-terpinene	11.617	16.277	25.122	14.212	12.010	19.338	10.249	14.550	21.901	18.361	11.677	25.623	23.225	16.405	26.662	6.463	10.711
10	p-cymene	12.012	16.655	147.005	78.115	67.737	125.273	64.052	76.658	144.500	96.310	59.754	166.007	126.263	84.797	157.997	44.970	66.858
11	β-phellandrene	12.149	16.844	9.105	5.302	4.831	7.395	4.377	5.583	8.793	6.946	4.499	10.203	9.029	6.189	10.116	2.875	4.541
12	γ-terpinene	13.539	18.242	65.756	37.832	33.041	48.477	29.999	41.922	59.941	54.180	36.264	70.482	68.155	49.260	77.590	20.973	36.039
13	Terpinolene	14.867	19.617	2.426	1.714	1.368	1.781	-	1.602	1.909	1.897	-	2.193	1.934	1.956	2.225	-	1.566
14	borneol	18.466	23.228	5.795	3.586	2.690	5.837	2.968	3.192	6.426	4.167	2.553	5.872	5.346	3.724	5.116	2.895	3.791
15	4-terpineol	19.027	23.753	10.940	6.369	4.834	7.048	3.416	3.671	7.476	4.666	2.937	7.153	6.145	4.721	6.437	3.480	4.766
16	thymol	24.549	28.92	581.286	684.230	702.017	732.250	879.728	715.837	543.897	676.199	753.692	526.767	450.489	651.440	427.253	832.878	672.858
17	carvacrol	24.795	29.201	46.160	46.931	44.608	51.775	47.234	54.368	53.173	60.511	42.780	40.407	77.114	63.038	31.992	44.409	59.751
18	β-caryophyllene	29.59	34.204	7.281	8.003	9.493	4.643	10.277	13.566	6.178	11.400	10.215	6.129	15.288	16.015	6.788	9.273	13.093
19	aromadendrene	30.385	34.973	-	1.342	1.634	-	3.012	3.013	-	2.405	2.485	-	2.313	3.175	-	2.398	2.624
20	α-humulene	30.98	35.559	1.510	1.713	1.883	1.226	2.039	2.680	1.351	2.336	1.996	1.419	3.139	3.206	1.395	1.848	2.669
21	ledene	32.691	37.214	-	-	-	-	-	-	-	-	-	-	-	1.481	-	-	-
22	β-bisabolene	33.286	37.706	-	-	1.242	-	-	1.944	-	1.845	1.451	-	1.798	2.273	-	-	1.853
	% of total			99.060	99.585	99.381	97.977	98.369	98.624	97.555	98.574	98.944	97.723	97.715	98.327	97.759	98.676	98.699
	monoterpene hydrocarbons			304.318	163.544	146.076	208.794	127.164	132.317	310.782	151.172	135.812	390.653	272.652	132.100	350.627	83.274	102.502
	oxygenated compounds			678.353	758.270	764.897	979.120	944.835	778.248	639.328	860.334	810.959	642.489	552.147	820.212	466.783	893.729	815.857
	sesquiterpenes			8.790	11.058	14.251	3.825	15.328	15.835	8.161	9.499	16.148	8.917	21.569	17.166	8.234	13.519	14.599

RT 1: Retention time in min on HP-5MS column obtained by MSD; RT 2: Retention time in min on HP-5MS column obtained by FID

A general trend was obtained in the variation of the amounts of monoterpene hydrocarbons and sesquiterpenes with the time of extraction in both SFME and hydrodistillation methods. It was observed that monoterpene hydrocarbons decreased while sesquiterpenes increased with increase in time (Figure 11-15). Monoterpene hydrocarbons being the most volatile substances were preferably extracted at the beginning of the process whereas higher molecular weight compounds were extracted later. The increase in sesquiterpenes during the extraction period is probably due to their high molecular weights, low volatilities and lower solubilities in water than the other constituents. They need time to reach their maximum levels in the essential oil. The decrease in monoterpene hydrocarbon concentration during the later stages of extraction may be due to the enhanced leaching of substances which were more difficult to be extracted (oxygenated compounds and sesquiterpenes). In addition, monoterpene hydrocarbons are known to be very unstable against heat and light, so the decrese in their amount may also be explained by their probable degradation due to the thermal or hydrolytic effects.

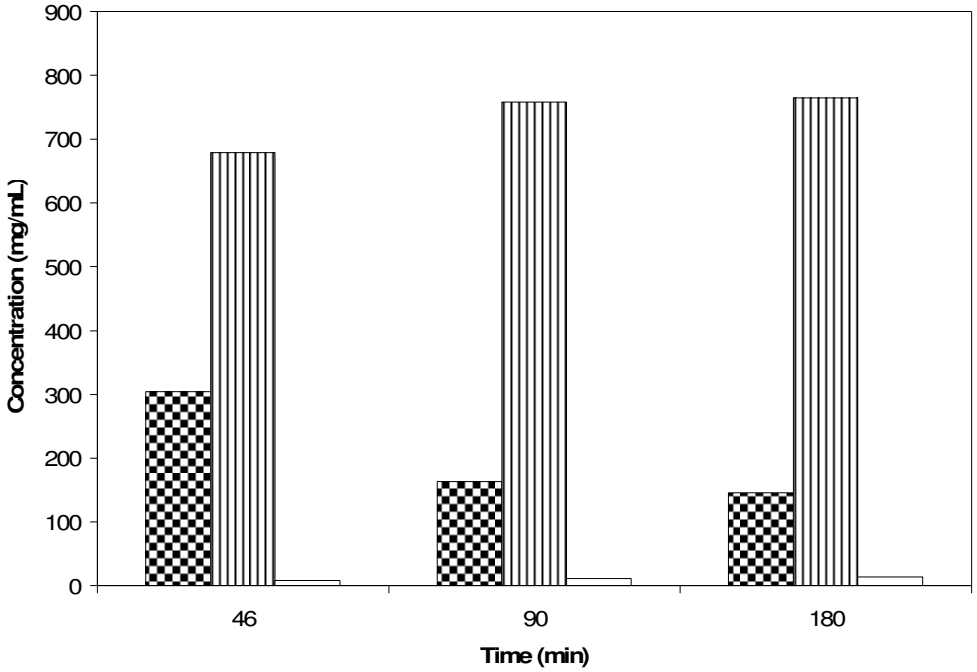


Fig.11. Variation of component classes with time in the essential oil of *Origanum vulgare* L. obtained by hydrodistillation (▩, monoterpene hydrocarbons; ||||, oxygenated compounds; □, sesquiterpenes)

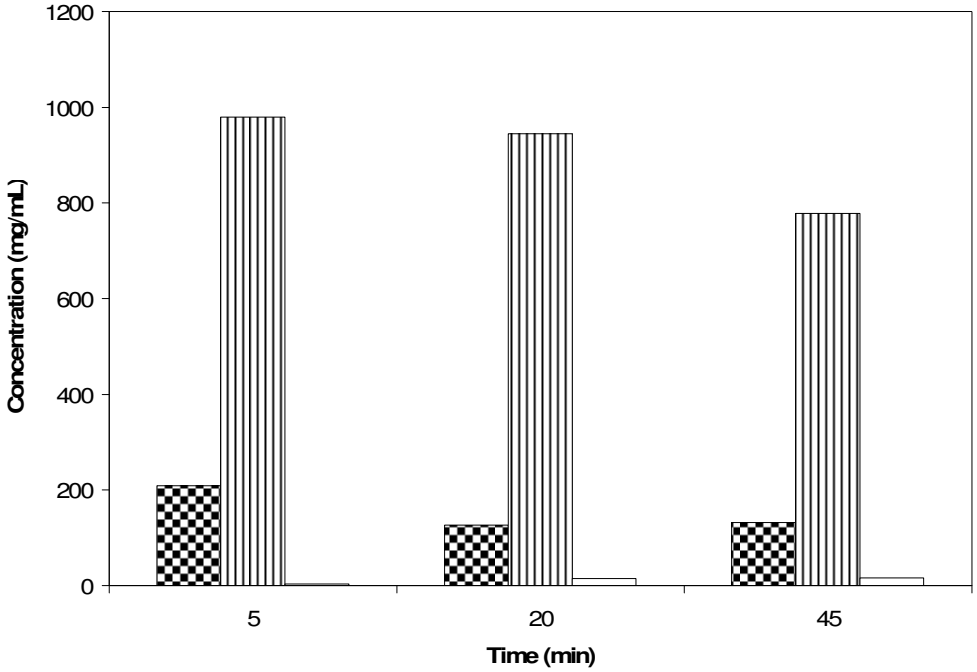


Fig. 12. Variation of component classes with time in the essential oil of *Origanum vulgare* L. obtained by SFME at 100% power level (▩, monoterpene hydrocarbons; ||||, oxygenated compounds; □, sesquiterpenes)

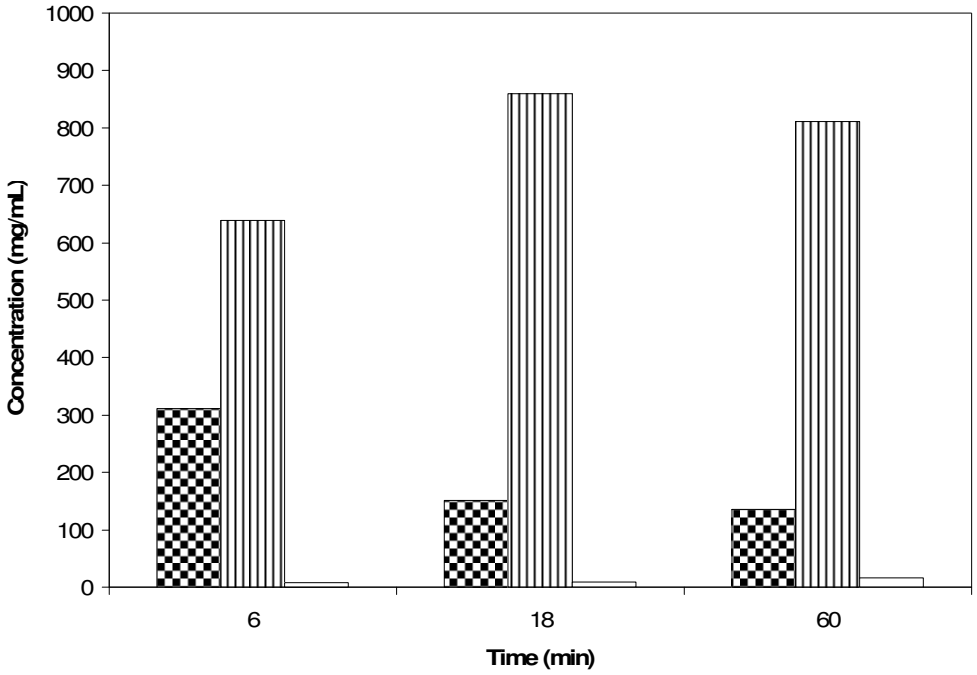


Fig. 13. Variation of component classes with time in the essential oil of *Origanum vulgare* L. obtained by SFME at 80% power level (▩, monoterpene hydrocarbons; ||||, oxygenated compounds; □, sesquiterpenes)

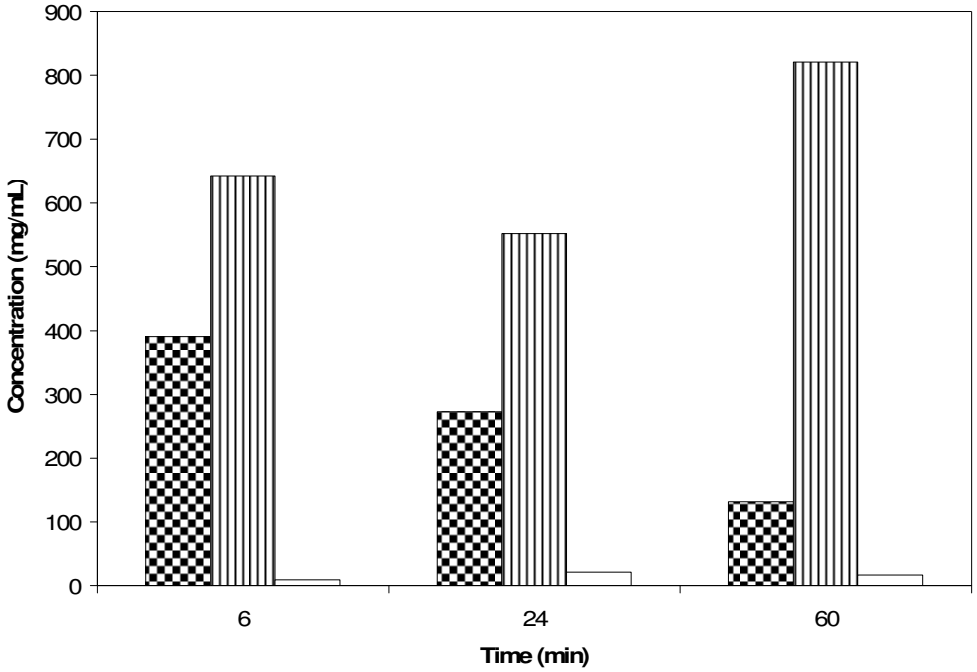


Fig. 14. Variation of component classes with time in the essential oil of *Origanum vulgare* L. obtained by SFME at 60% power level (▩, monoterpene hydrocarbons; ||||, oxygenated compounds; □, sesquiterpenes)

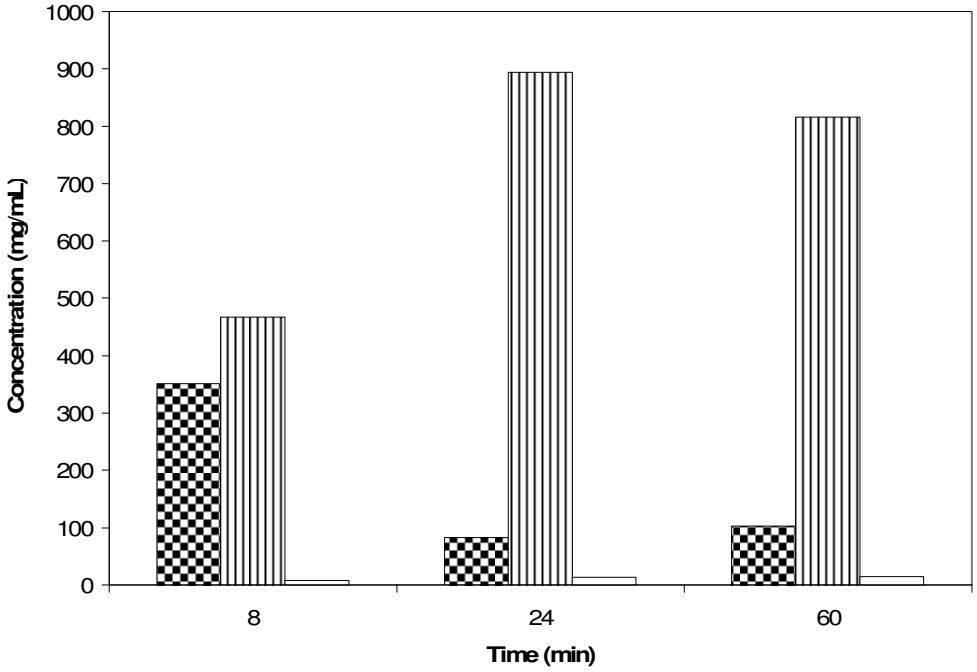


Fig. 15. Variation of component classes with time in the essential oil of *Origanum vulgare* L. obtained by SFME at 40% power level (▩, monoterpene hydrocarbons; ||||, oxygenated compounds; □, sesquiterpenes)

It is hard to say that a perfect trend was obtained in the variation of the amounts of oxygenated compounds with time. In general, their concentrations increased to some extent. However, the extraction of oxygenated compounds were very fast in SFME at 100% power level because of pressure build-up inside the plant material. Therefore, their concentrations seemed to decrease slightly with time in SFME working at 100% power level as the concentrations of sesquiterpenes increased. It was found that, approximately, 95% of the oxygenated compounds in the essential oil of *Origanum vulgare* L. were made up of phenols (thymol and carvacrol), while the rest consisted of alcohols and ketones. Generally, an increase with time in the amount of phenols and a decrease in the amount of alcohols and ketones were observed (Table 1). The increase in the amount of oxygenated compounds with time is due to the increase in the

amounts of phenols (thymol and carvacrol) since they are the main components. It was also found out that, about 70% of the monoterpene hydrocarbons in the oregano essential oil were made up of p-cymene and γ-terpinene. These two monoterpene hydrocarbons are known to be the precursors of thymol and carvacrol in the species of *Origanum* and *Thymus* (Burt, 2004). Therefore, the increase in the amounts of thymol and carvacrol with time might be due to the decrease in the amounts of p-cymene and γ-terpinene resulting from a couple of reactions (aromatization of γ-terpinene to p-cymene followed by hydroxylation of p-cymene (Nhu-Trang et al., 2006)) activated under the thermal or hydrolytic effects. That would also explain the decrease in the amount of monoterpene hydrocarbons with time since they consist mostly of p-cymene and γ-terpinene, as stated before.

Figure 16 shows the variation of monoterpene hydrocarbons, oxygenated compounds and sesquiterpenes with the method of extraction. Statistically, no significant difference in their corresponding concentrations was observed between the methods and power levels studied ($p \leq 0.05$) (Table A.3-5). This shows that SFME did not change the quality of the essential oil of *Origanum vulgare* L., but decreased the process time substantially. Therefore, it can be concluded that SFME is a good alternative for the extraction of essential oil from *Origanum vulgare* L.

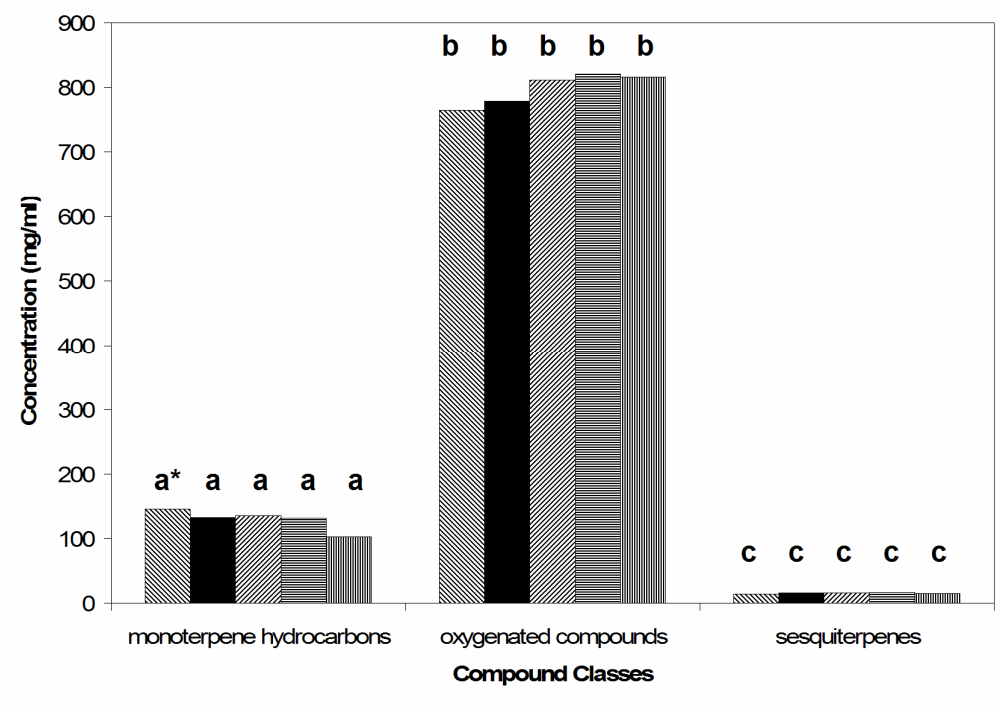


Fig.16. Variation of compound classes in the essential oil of *Origanum vulgare* L. with respect to different extraction methods (▧, hydrodistillation-180 min; ■, SFME-100% power level-45 min; ▨, SFME-80% power level-60 min; ▤, SFME-60% power level-60 min; ▥, SFME-40% power level-60 min) (* means bars with different letters within each compound class are significantly different ($p \leq 0.05$))

3.1.2.3. Other parameters

3.1.2.3.1. Specific gravity

Variation of specific gravity values of oreganum essential oil with time obtained by SFME at different power levels and conventional hydrodistillation is shown in Figure 17. Although most of the values practically maintained constant during the extraction process, a slight increase with time was observed in both of the methods, as it can be seen from the figure. This is probably caused by the fact that as extraction proceeded in both of the methods, monoterpene hydrocarbons decreased

with time and sesquiterpenes together with oxygenated compounds increased to some extent. Since oxygenated compounds and sesquiterpenes have higher molecular weights than the monoterpene hydrocarbons, specific gravity of the oil increased as they were gradually being extracted. These results are in good agreement with those of Gamarra et al. (2006) who worked on lemon essential oil.

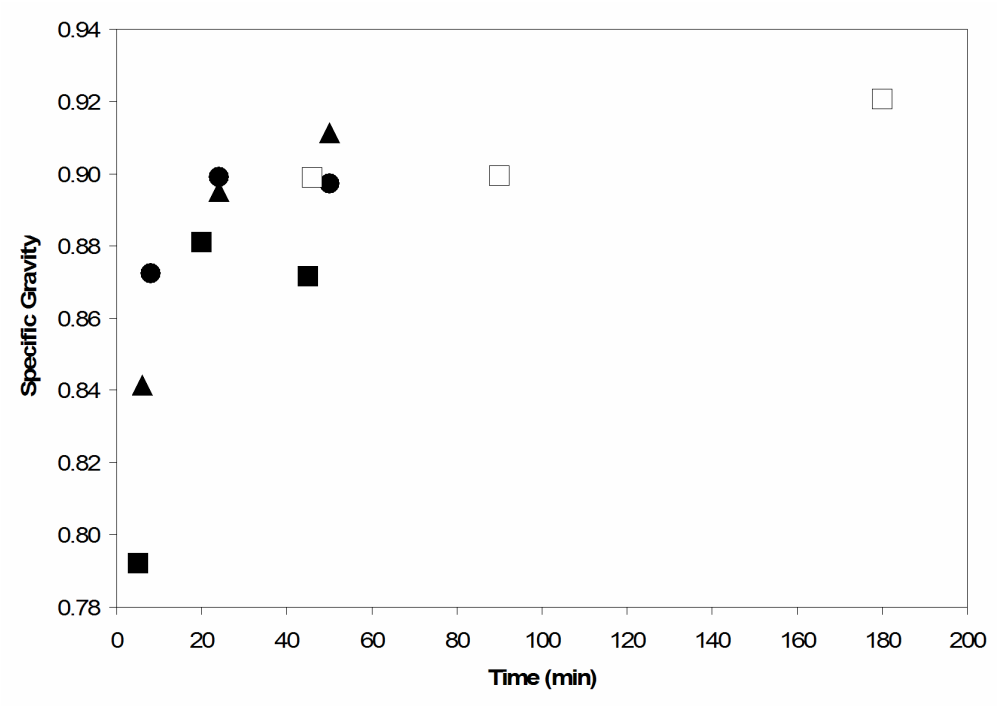


Fig. 17. Variation of specific gravity of the *Origanum vulgare* L. essential oil with time obtained by different methods (■, SFME-100% power[a]; ▲, SFME-60% power[a]; ●, SFME-40% power[a]; □, hydrodistillation[a])

Figure 18 shows the specific gravity values of oregano essential oil obtained by hydrodistillation and SFME at different power levels. The mean values for the specific gravities of the oregano oil extracted by hydrodistillation, SFME at 100%, 60% and 40% power levels were found as 0.921, 0.871, 0.911 and 0.897, respectively. Statistically, no significant difference was found between these values ($p \leq 0.05$) (Table A.6).

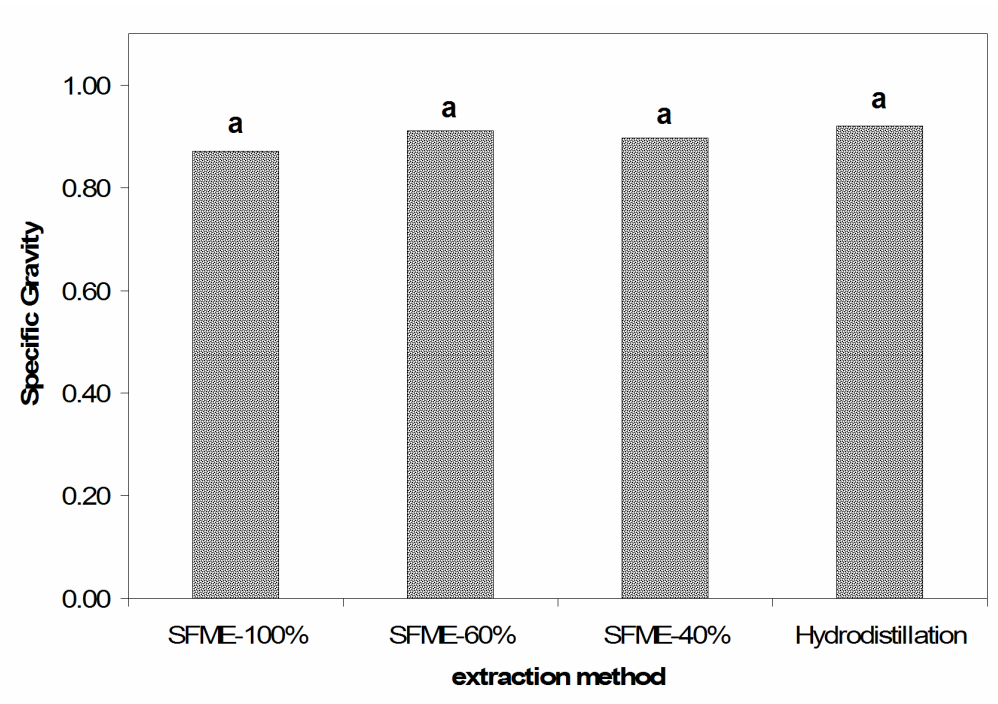


Fig. 18. Specific gravity values of *Origanum vulgare* L. essential oil extracted by different methods

3.1.2.3.2. Refractive index

Variation of refractive index values of oregano essential oil with time obtained by SFME at different power levels and conventional hydrodistillation is shown in Figure 19. Like the specific gravity values, a slight increase with time was observed in both of the methods although most of the values maintained practically constant. Lower values at the first stages of extraction is probably due to the higher amounts of monoterpene hydrocarbons and lower amounts of oxygenated compounds and sesquiterpenes compared to the last stages (Gamarra et al, 2006). This is also in good agreement with the statement of Castilho et al. (2005). They noted that refractive indices of natural fats and oils are related to their average degree of unsaturation in an almost linear way, and tend to increase as the number of double bonds increase. It is known that as

chain length increases, the number of double bonds increases in terpenes. Since sesquiterpenes (15 carbon atoms) are bigger molecules than monoterpenes and their oxygenated forms (10 carbon atoms), it can be concluded that they are more unsaturated than monoterpenes. Therefore, refractive index values are expected to increase with the amount of sesquiterpenes.

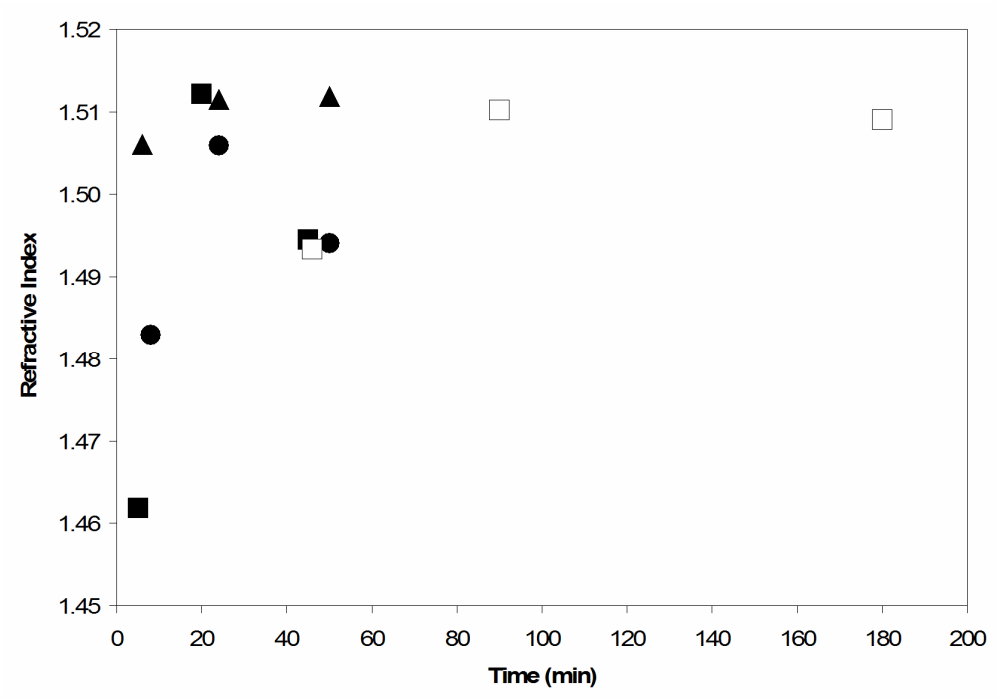


Fig. 19. Variation of refractive index of the *Origanum vulgare* L. essential oil with time obtained by different methods (■, SFME-100% power[a]; ▲, SFME-60% power[a]; ●, SFME-40% power[a]; □, hydrodistillation[a])

Figure 20 shows the refractive index values of oregano essential oil obtained by hydrodistillation and SFME at different power levels. The mean values for the refractive index of the oregano oil extracted by hydrodistillation, SFME at 100%, 60% and 40% power levels were found as 1.509, 1.495, 1.512 and 1.494, respectively. Statistically, no significant difference was found between these values ($p \leq 0.05$) (Table A.7).

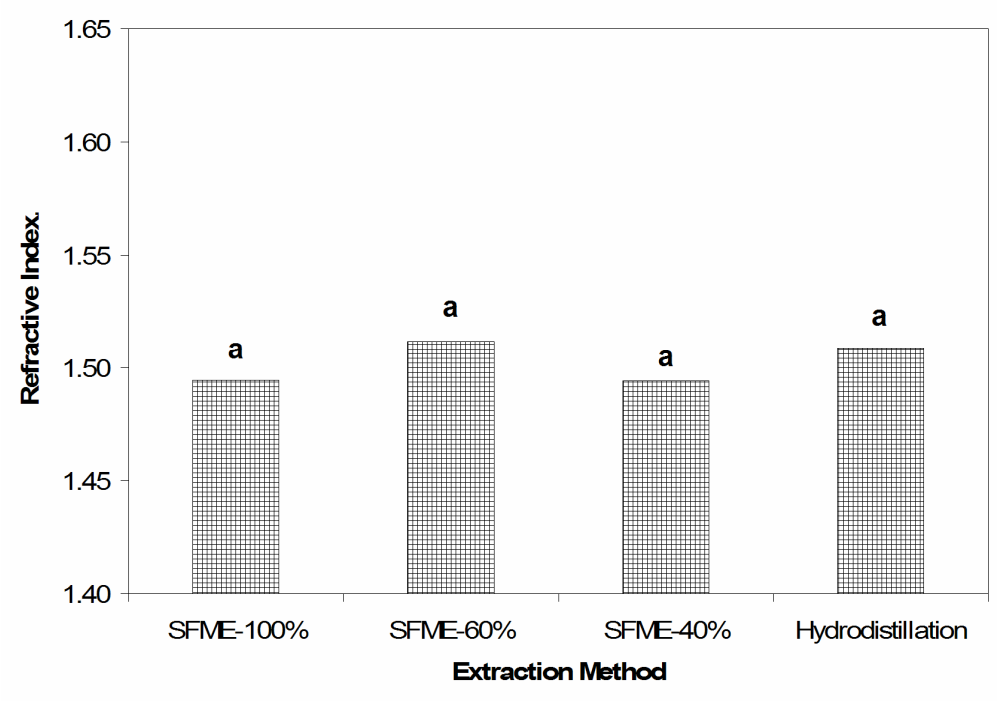


Fig.20. Refractive index values of *Origanum vulgare* L. essential oil extracted by different methods

3.1.2.3.3. Solubility in ethanol

Figure 21 shows the values of solubility of oreganum essential oil in ethanol extracted by different methods. 1 volume of oregano essential oil extracted by hydrodistillation, SFME at 100%, 60% and 40% power levels were found to be soluble in 1.3 to 1.4 volumes, 1.5 to 2 volumes, 1.2 to 1.6 volumes and 1.3 to 1.7 volumes of ethanol, respectively. The difference among the solubility of oregano essential oil obtained using hydrodistillation or SFME at different power levels were found to be insignificant ($p \leq 0.05$) (Table A.8).

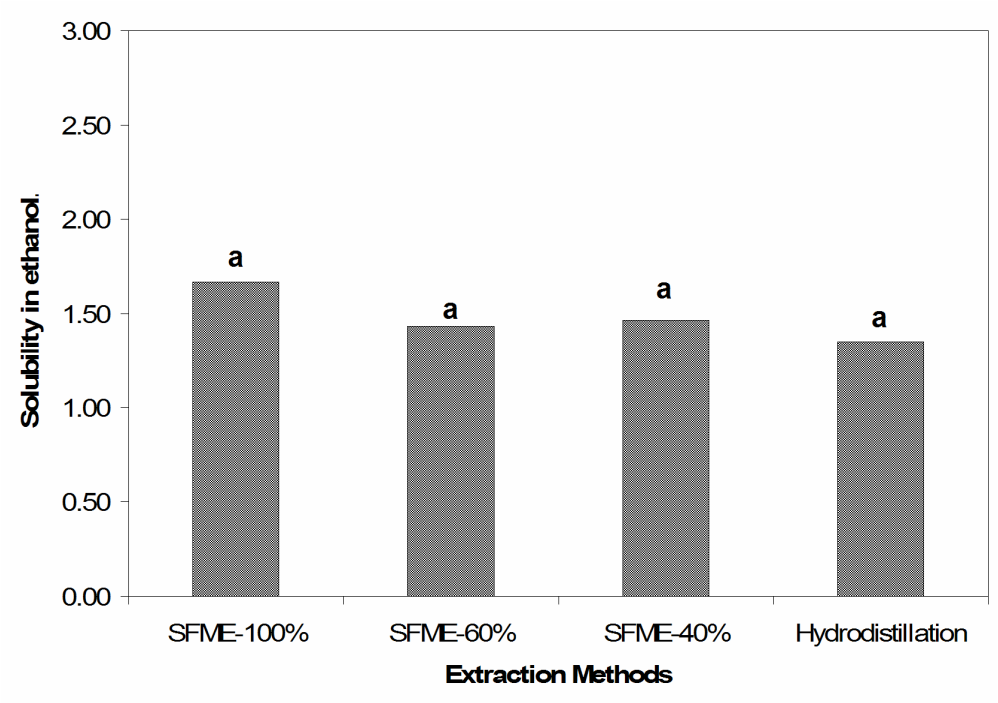


Fig.21. Solubility in ethanol values of *Origanum vulgare* L. essential oil extracted by different methods

3.2. Laurel (*Lauris nobilis* L.)

3.2.1. Particle Size Distribution of laurel leaves

Particle size distribution of laurel leaves is given in Figure 22. Sauter mean diameter ($\overline{D}_s$) of the leaves was calculated as 2103.4 µm.

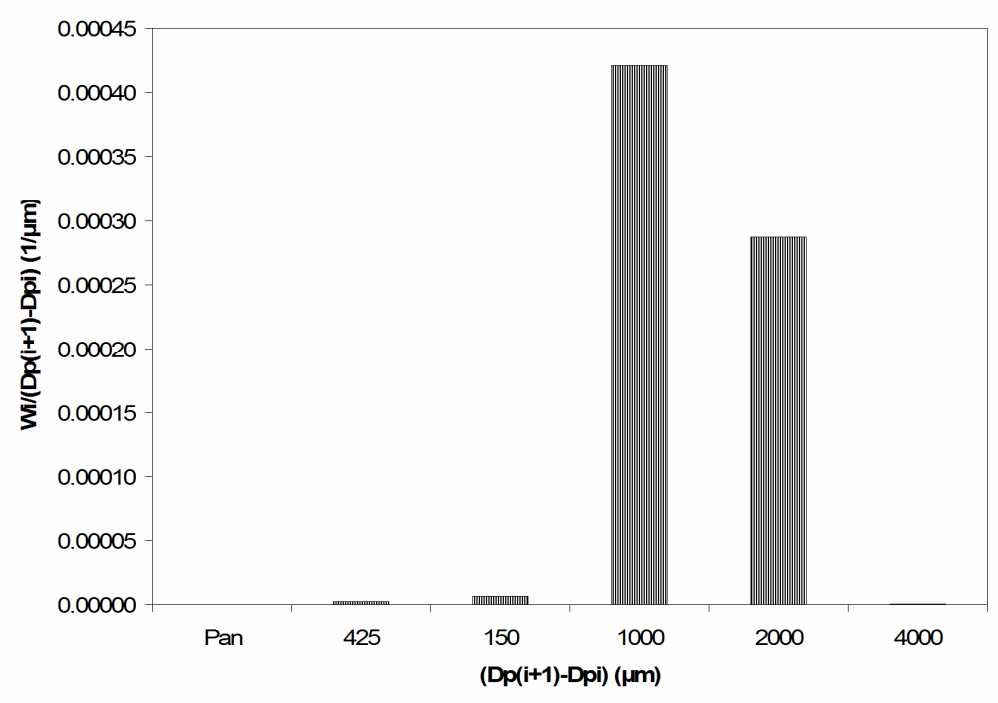


Fig. 22. Particle size distribution of *Laurus nobilis* L. leaves

3.2.2. Effect of extraction on quality parameters of laurel essential oil

3.2.2.1. Yield

Finding out that there was no significant difference in the yields of essential oil of oregano extracted by different power levels, it was decided to work with only two power levels (100% and 40%) for laurel. The amount of water absorbed by dry laurel leaves was about 135 % of its initial weight when soaked in water for 1 h. The variation of essential oil yield (mL oil/g laurel) with time during hydrodistillation and SFME processes is given in Figure 23. The maximum yields obtained in SFME for 100% and 40% power levels were 0.0235 and 0.022 mL oil/g laurel, respectively. In hydrodistillation method, the maximum yield was found to be 0.022 mL

oil/g laurel. Statistically, no significant difference was obtained between these values ($p \leq 0.05$) (Table A.9).

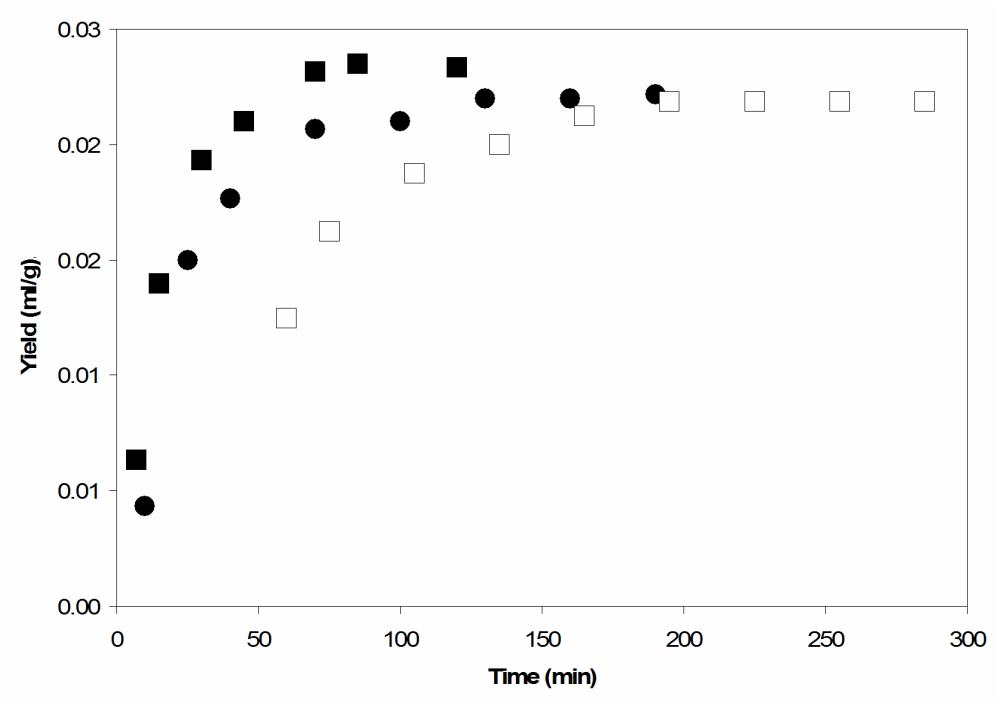


Fig. 23. Variation of essential oil yield of *Laurus nobilis* L. during hydrodistillation and solvent-free microwave extraction (SFME) at different power levels (■, SFME-100% power[a]; ●, SFME-40% power[a]; □, hydrodistillation[a])

As in the case of oregano, the initial extraction rate increased with the increase in microwave power. This is due to the higher pressure gradient formed inside the plant material when it was subjected to higher microwave power levels, as discussed before. The essential oils must have been thrown out of the glands quicker due to this high pressure gradient.

It was found that the time needed for the complete extraction of essential oil of laurel at 100% power was 85 min, while it was 130 min in 40%

power. This observation verifies the expectation for longer extraction times with lower microwave powers, which was discussed before. In the case of hydrodistillation, the process time was 195 min. Therefore, the extraction time seems to be reduced by 55-60% in the case of SFME.

3.2.2.2. Composition

Total ion chromatogram of the oil obtained by GC-MS analysis is given in Figure 24.The composition of the essential oil of *Laurus nobilis* L. obtained by SFME and conventional hydrodistillation methods are given in Table 2. Due to the lack of some of the authentic compound standards, only about 90% of the essential oil could be characterized. The composition of the essential oils obtained by both of the methods were found to be almost the same. The main components of the essential oil of *Laurus nobilis* L. were determined as 1,8-cineole (630-730 mg/mL oil) followed by α-terpinyl acetate (90-115 mg/mL oil), sabinene (45-55 mg/mL oil), α-pinene (30-40 mg/mL oil), 4-terpineol (35-40 mg/mL oil) and β-pinene (~30 mg/mL oil). It was also found that the essential oil was composed mainly of oxygenated compounds (≥75 %) while monoterpene hydrocarbons and sesquiterpenes constituted about ≥15 % and ≥0.15 % of it, respectively. Doubtlessly, *Laurus nobilis* L. essential oil owes its pungent odor to these high amount of oxygenated compounds as in the case of oregano.

A general trend was obtained in the variation of the amounts of monoterpene hydrocarbons and oxygenated compounds with the time of extraction in both SFME and hydrodistillation methods. It was observed that monoterpene hydrocarbons increased, while oxygenated compounds decreased with time slightly. No sesquiterpenes were detected in the essential oil extracted by hydrodistillation while they were detected only in the final stages of extraction in SFME (Figure 25-27). The oxygenated compounds detected in laurel essential oil were found to be mainly

composed of ethers (70-75%), especially of 1,8-cineole (~ 70%). The rest of the oxygenated constituents of the oil were determined as alcohols (linalool, pinocarveol/trans-pinocarveol, borneol, 4-terpineol, α-terpineol, cumic alcohol, β-eudesmol), aldehydes (myrtenal, cuminal), ketones (camphor, sabina ketone, pinocarvone carvone), esters (bornyl acetate, α-terpinyl acetate), lactones (dehydrocostuslactone, eremanthin) and phenols (eugenol, trans/cis-methyl isoeugenol) (Table 2). Among these, although it was very small, only ethers and ketones showed a reduction in their amounts as the process continued. Since ethers constituted the main components of the oxygenated compounds present in the laurel essential oil, the decrease with time in the amount of oxygenated components must be primarily due to the decrease in the amount of ethers, especially 1,8-cineole. They might have gone under some degradation reactions caused by high temperature and/or hydrolytic effects.

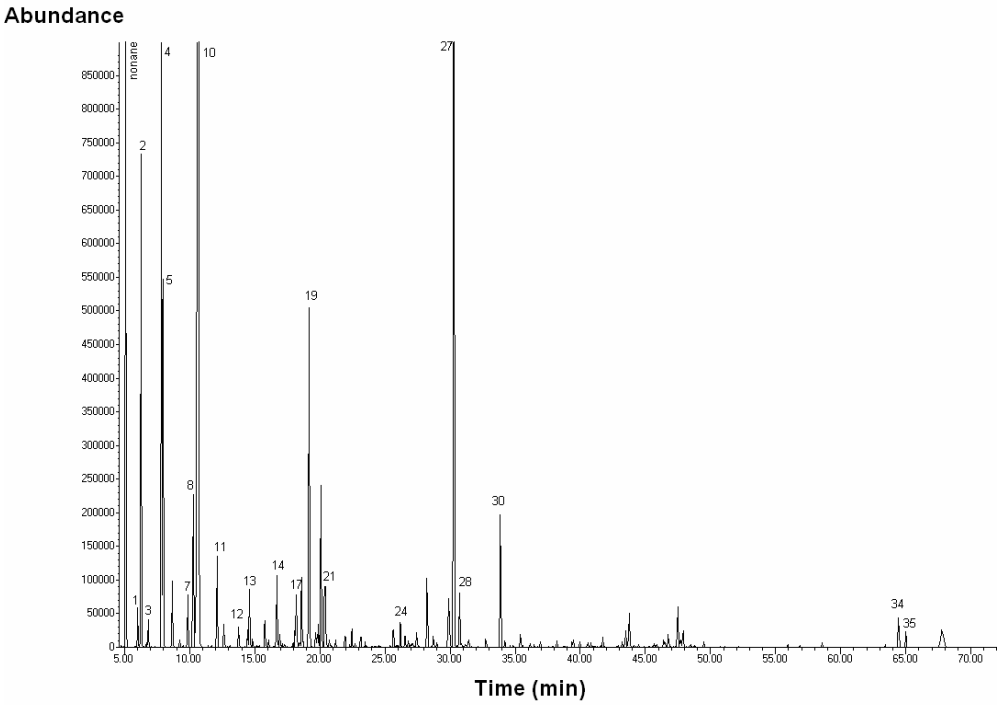


Fig. 24. Total ion chromatogram (obtained by GC-MS analysis) of the *Laurus nobilis* L. essential oil extracted by SFME at 100% power level (1: α-thujene, 2: α-pinene, 3: camphene, 4: sabinene, 5: β-pinene, 7: α-terpinene, 8: p-cymene, 10: 1,8-cineole, 11: γ-terpinene, 12: terpinolene, 13: linalool, 14: pinocarveol/trans-pinocarveol, 17: pinocarvone, 19: 4-terpineol, 21: myrtenal, 24: bornyl acetate, 27: α-terpinyl acetate, 28: eugenol, 30: methyl eugenol, 34: dehydrocostuslactone(?), 35: erementhin(?)

Table 2. Compounds present in the essential oil of *Laurus nobilis* L. obtained by different methods and their concentrations

				Hydrodistillation			SFME-100% power level			SFME-40% power level		
				60 (min)	*75 (min)*	*195 (min)*	*7 (min)*	*15 (min)*	*85 (min)*	*10 (min)*	*25 (min)*	*130 (min)*
No	**Compounds**	**RT 1 (min)**	**RT 2 (min)**	**(mg/ml)**	**(mg/ml)**	**(mg/ml)**	**(mg/ml)**	**(mg/ml)**	**(mg/ml)**	**(mg/ml)**	**(mg/ml)**	**(mg/ml)**
1	alpha-thujene	6.043	9.852	2.447	2.189	3.448	1.697	1.840	3.289	2.004	2.555	2.665
2	alpha-pinene	6.287	10.179	19.517	24.243	38.981	9.729	19.977	38.889	9.716	20.036	32.622
3	camphene	6.837	10.909	1.943	2.547	2.874	1.285	1.924	2.918	1.571	1.917	2.429
4	sabinene	7.875	12.186	39.496	43.163	47.032	25.591	41.582	53.446	26.364	41.023	46.693
5	beta-pinene	7.986	12.348	20.397	24.263	32.354	11.106	20.339	33.284	12.165	20.882	30.212
6	alpha-phellandrene	9.273	13.868	-	-	1.582	-	-	-	-	1.310	0.900
7	alpha-terpinene	9.882	14.580	3.050	3.258	4.915	1.604	2.184	4.459	2.078	2.152	4.246
8	p-cymene	10.300	15.051	10.425	11.301	12.786	6.732	9.482	13.413	7.535	10.328	12.702
9	limonene		15.325	-	-	-	-	-	-	-	-	8.165
10	1,8-cineole	10.708	15.507	933.170	843.320	630.237	853.899	878.790	731.748	1011.908	874.094	653.048
11	gamma-terpinene	12.133	17.120	5.769	6.495	8.885	3.207	3.981	8.181	2.684	4.460	8.742
12	terpinolene	13.795	18.953	1.571	1.628	2.915	-	1.084	2.301	-	1.187	2.603
13	linalool	14.627	19.878	2.219	2.691	2.399	2.469	2.994	2.172	2.273	2.969	2.478
14	pinocarveol/trans-pinocarveol	16.729	22.116	10.259	11.441	9.828	11.118	13.460	11.296	9.088	13.042	12.072
15	camphor	16.946	22.355	1.180	1.182	0.886	1.356	1.288	-	-	1.170	1.025
16	sabina ketone	18.090	23.405	3.439	4.228	3.674	5.770	6.023	4.401	4.470	5.462	5.720
17	pinocarvone	18.233	23.680	8.537	8.487	6.300	9.004	9.521	8.027	7.932	9.534	8.027
18	borneol	18.476	23.984	7.256	9.160	8.809	9.928	11.579	11.461	6.802	7.957	12.245
19	4-terpineol	19.207	24.649	33.333	38.225	36.776	28.464	36.812	40.473	24.930	36.401	42.561
20	alpha-terpineol		25.756	1.850	2.148	2.222	2.266	2.831	2.899	1.349	2.485	3.060
21	myrtenal	20.430	25.877	10.103	10.916	10.091	9.633	12.342	12.500	8.346	11.552	12.494
22	cuminal	23.168	28.966	-	3.273	3.057	-	3.788	3.431	-	3.504	3.503
23	carvone	23.517	31.030	2.488	2.234	2.748	1.403	1.953	3.013	1.946	2.623	3.328
24	bornyl acetate	26.197	31.656	3.560	4.238	4.536	3.000	4.305	4.830	3.201	4.050	4.365
25	cumic alcohol	26.557	31.935	-	-	1.622	-	1.483	2.642	-	1.176	2.473
26	pseudolimonene	28.225	33.636	4.894	5.370	7.136	3.869	5.728	8.024	3.158	5.754	8.639
27	alpha-terpinyl acetate	30.301	35.676	45.688	64.401	90.276	40.758	68.171	107.300	35.080	66.680	115.321
28	eugenol	30.719	36.107	5.840	8.012	12.153	7.491	11.084	16.364	5.405	8.517	17.321

Table 2. (continued)

29	elemene(?)	32.752	38.223	-	-	-	-	-	1.314	-	-	1.930
30	methyl eugenol	33.864	39.078	5.316	9.228	12.075	6.795	10.080	14.757	5.054	8.087	14.933
31	beta-caryophyllene	34.214	39.793	-	-	-	-	-	2.283	-	-	3.101
32	trans/cis-methyl isoeugenol	39.382	44.690	-	-	-	-	-	1.631	-	-	0.996
33	beta-eudesmol	47.696	53.238	-	3.258	4.043	-	-	4.838	-	-	3.893
34	dehydrocostuslactone(?)	64.429	69.523	-	-	0.972	-	-	5.153	-	-	1.826
35	eremanthin(?)	64.990	70.082	-	-	-	-	-	3.052	-	-	-
	% of total			95.028	93.550	91.264	92.907	92.212	88.685	93.350	91.860	88.749
	monoterpene hydrocarbons			261.380	302.905	373.689	212.771	289.665	413.579	206.162	286.746	408.687
	oxygenated compounds			1079.550	1038.924	859.794	1000.150	1086.583	1025.012	1132.836	1067.387	947.364
	sesquiterpenes			0.000	0.000	0.000	0.000	0.000	3.597	0.000	0.000	5.030

RT 1: Retention time in min on HP-5MS column obtained by MSD; RT 2: Retention time in min on HP-5MS column obtained by FID

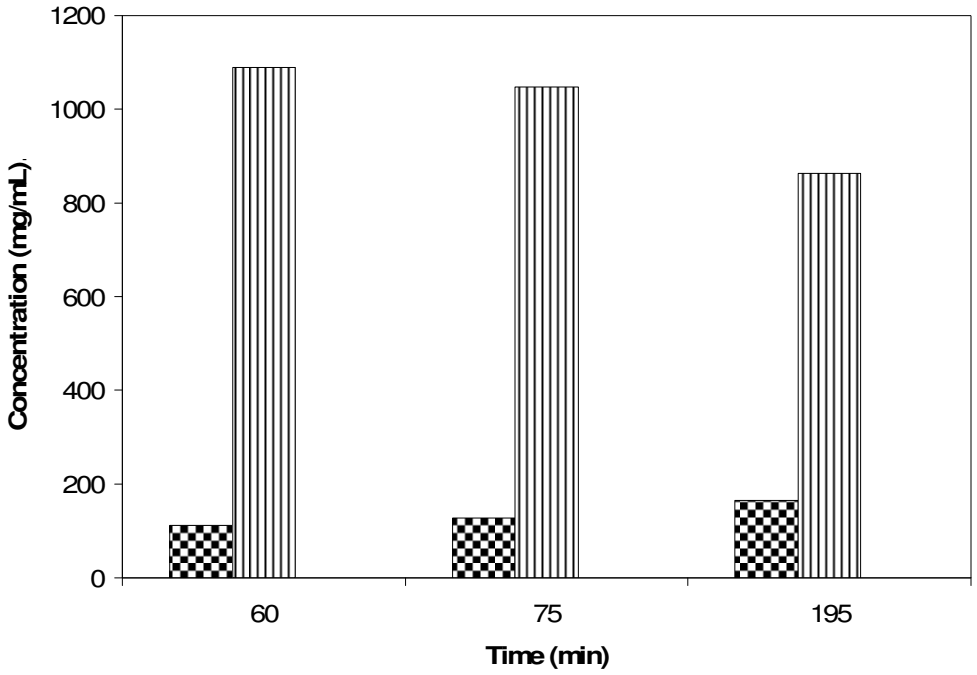


Fig. 25. Variation of component classes with time in the essential oil of *Laurus nobilis* L. obtained hydrodistillation (▩, monoterpene hydrocarbons; ||||, oxygenated compounds; □, sesquiterpenes)

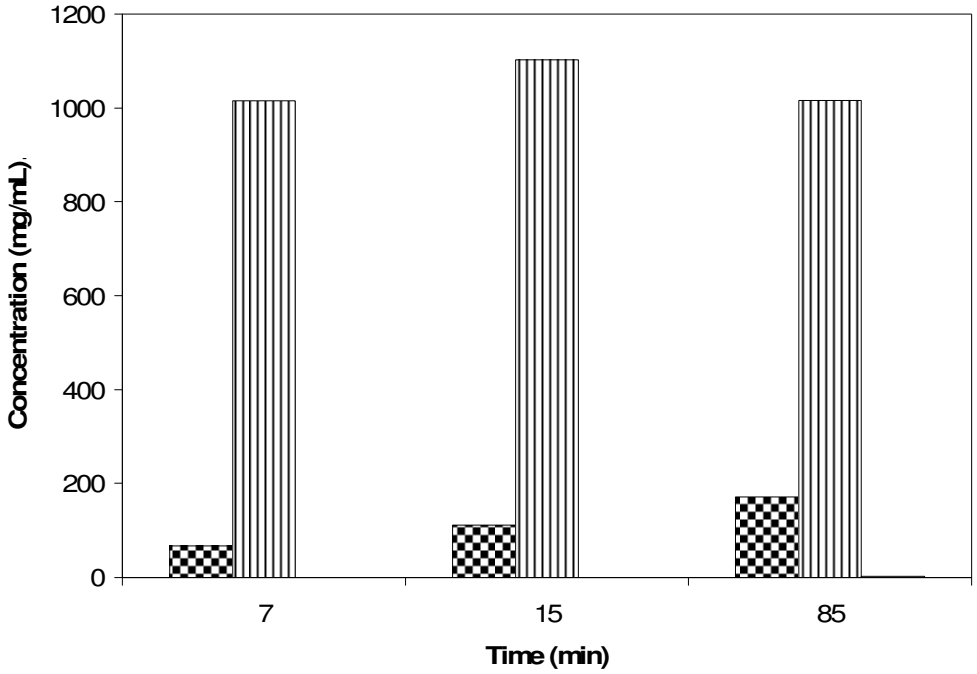


Fig. 26. Variation of component classes with time in the essential oil of *Laurus nobilis* L. obtained SFME-100% power level (▩, monoterpene hydrocarbons; ||||, oxygenated compounds; □, sesquiterpenes)

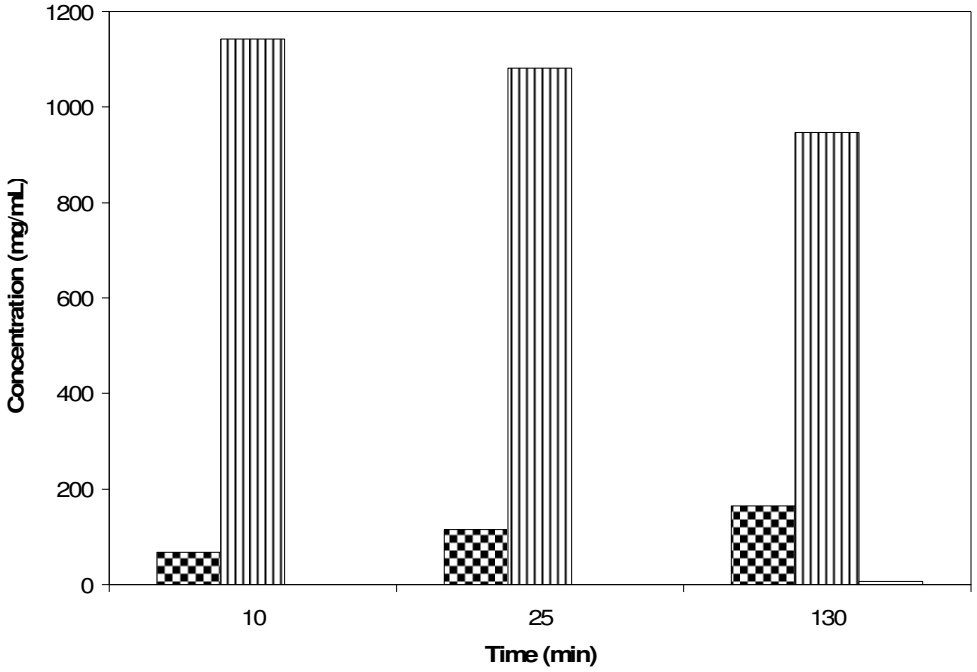


Fig. 27. Variation of component classes with time in the essential oil of *Laurus nobilis* L. obtained SFME-40% power level (▩, monoterpene hydrocarbons; ||||, oxygenated compounds; □, sesquiterpenes)

Sesquiterpenes, have lower volatilities, higher molecular weights and thus lower solubilities in water than the other constituents. They need time to reach their maximum levels in the essential oil. Therefore, they were detected only in the final stages of SFME.

Figure 28 and figure 29 show the variation of monoterpene hydrocarbons, oxygenated compounds and sesquiterpenes detected in the laurel essential oil with respect to different methods of extraction. No significant difference in the amount of monoterpene hydrocarbons and oxygenated compounds was observed between the methods and power levels studied ($p \leq 0.05$) (Table A.10-11). However, no sesquiterpenes were extracted in the hydrodistillation method while they were obtained only in the later stages of SFME (Table 2). This is probably because sesquiterpenes are high molecular weight compounds with low polarities. As it is known, in

hydrodistillation, isolation of compounds is achieved according to their degree of polarity (which determines their degree of solubility in water) rather than their boiling points. Therefore, the extraction of sesquiterpenes could not be achieved in hydrodistillation most probably because of their high molecular weight nature since it is hard to extract high molecular weight compounds. However, they could be extracted in SFME probably because of the high pressure build-up inside the glands, which induced the secretion of compounds with the rapid rupture of glands. Sesquiterpenes are generally known to have anti-inflammatory and anti-allergenic effects, thus their presence in foods is not so advantageous as compared to the presence of other constituents which possess antimicrobial and antioxidant effects. Therefore, the SFME process can be stopped in the later stages whenever a product free of sesquiterpenes is preferred. This would be advantageous also in shortening the process time.

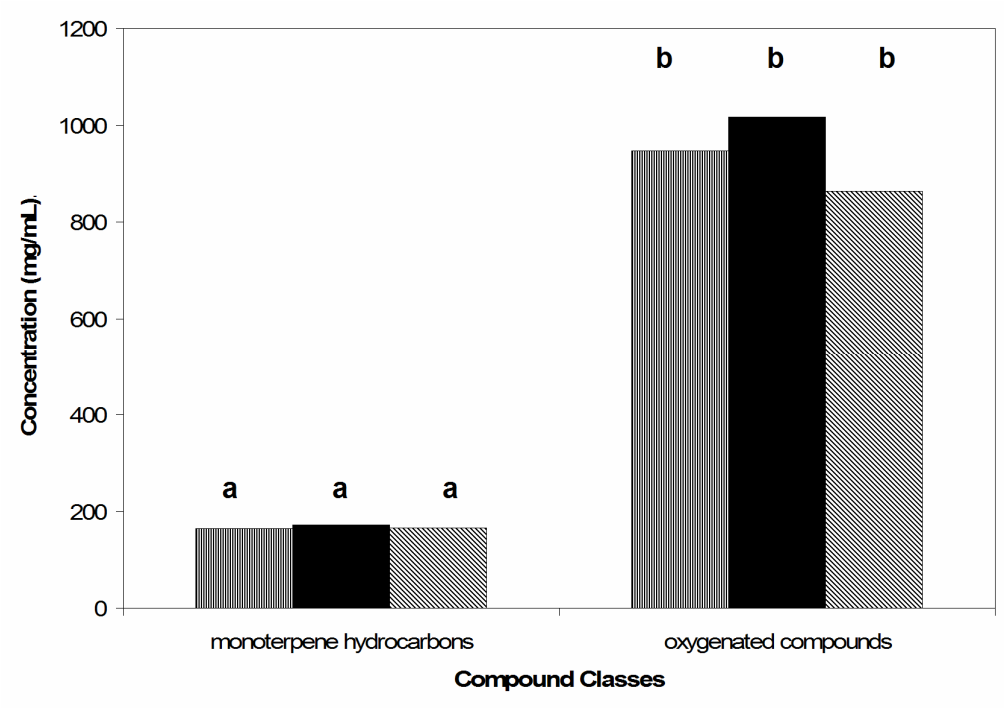


Fig. 28. Variation of monoterpene hydrocarbons and oxygenated compounds in the essential oil of *Laurus nobilis* L. with respect to different extraction methods (▨, hydrodistillation; ■, SFME-100% power level; ▥, SFME-40% power level)

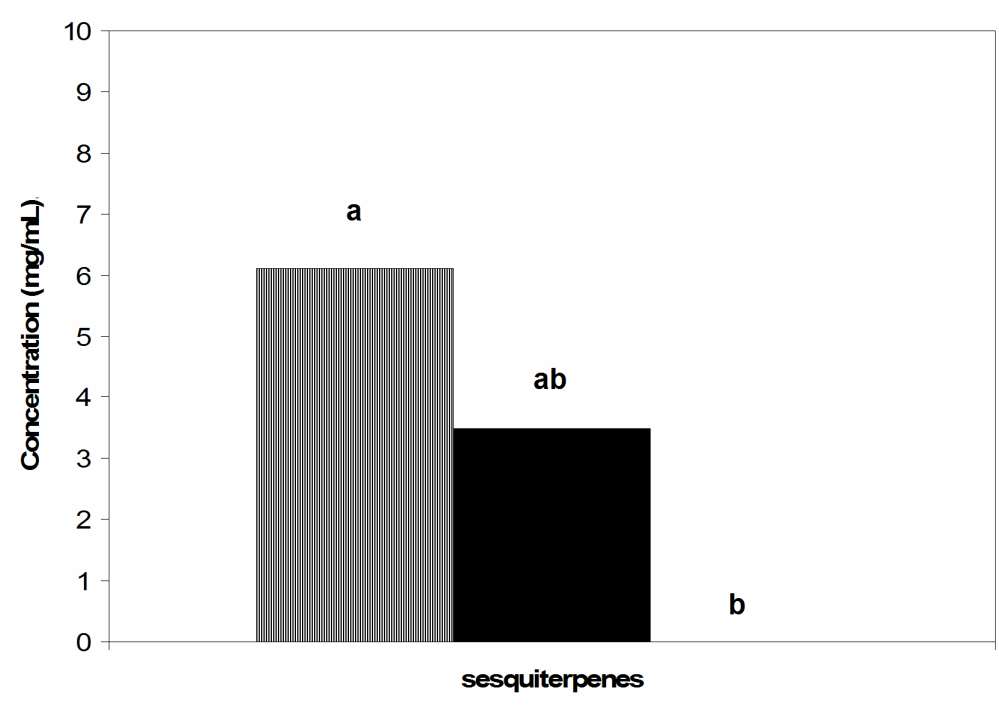


Fig. 29. Variation of sesquiterpenes in the essential oil of *Laurus nobilis* L. with respect to different extraction methods (▨, hydrodistillation; ■, SFME-100% power level; ▥, SFME-40% power level) (Table A.12-13)

It can be concluded that SFME is a good alternative for the extraction of essential oils from *Laurus nobilis* L. since it provides essential oils of almost the same quality with conventional hydrodistillation while reducing the time of the process drastically.

3.2.2.3. Other parameters

3.2.2.3.1. Specific gravity

Variation of specific gravity values of laurel essential oil with time obtained by SFME at different power levels and conventional hydrodistillation is shown in Figure 30. Although the values practically maintained constant

during the extraction process, a slight increase with time in the first stages of extraction was observed in both of the methods. This is probably due to the fact that compounds were gradually extracted as the process continued. Since no sesquiterpenes were extracted in hydrodistillation method, the specific gravity of the oil obtained by hydrodistillation was slightly lower.

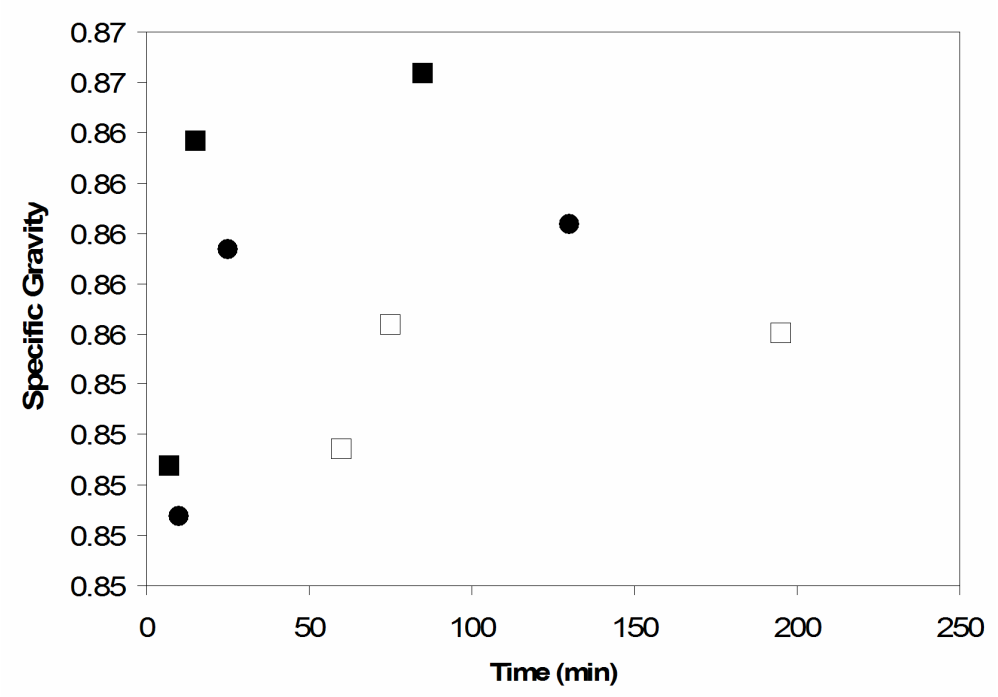


Fig. 30. Variation of specific gravity of the *Laurus nobilis* L. essential oil with time obtained by different methods (■, SFME-100% power[a]; ●, SFME-40% power[a]; □, hydrodistillation[a])

Figure 31 shows the specific gravity values of laurel essential oil obtained by hydrodistillation and SFME at different power levels. The mean values for the specific gravities of the laurel oil extracted by hydrodistillation, SFME at 100% power level and at 40% power level were found as 0.856, 0.867 and 0.861, respectively. Statistically, no significant difference was determined between these values ($p \leq 0.05$) (Table A.14). Specific gravity

values of the laurel essential oil was similar to the values given in literature (Castilho et al., 2005).

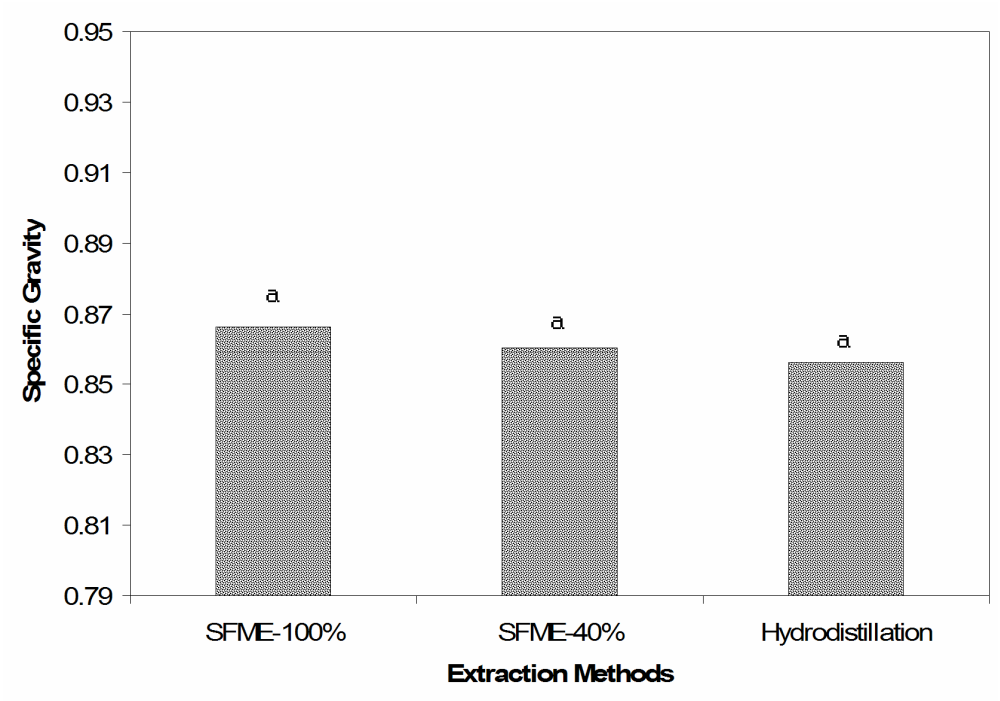


Fig.31. Specific gravity values of *Laurus nobilis* L. essential oil extracted by different methods

3.2.2.3.2. Refractive index

Variation of refractive index values of laurel essential oil with time obtained by SFME at different power levels and conventional hydrodistillation is shown in Figure 32. Like the specific gravity values, a slight increase with time in the first stages of extraction was observed in both of the methods although most of the values maintained practically constant. This is probably due to the fact that compounds were gradually extracted as the process continued. As time increased, the extraction of unsaturated compounds increased, correspondingly increasing the refractive index

values. The refractive index of the essential oil obtained by hydrodistillation was lower since no sesquiterpenes were extracted in hydrodistillation method.

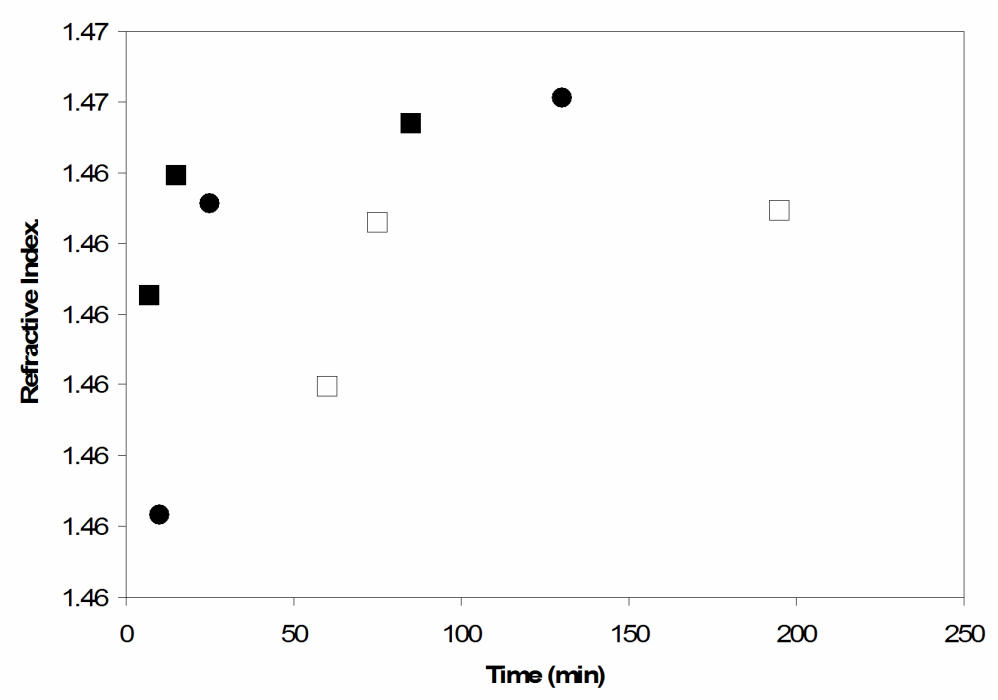


Fig. 32. Variation of refractive index of the *Laurus nobilis* L. essential oil with time obtained by different methods (■, SFME-100% power[a]; ●, SFME-40% power[a]; □, hydrodistillation[a])

Figure 33 shows the refractive index values of laurel essential oil obtained by hydrodistillation and SFME at different power levels. The mean values for the refractive index of the laurel oil extracted by hydrodistillation, SFME at 100% power level and at 40% power level were found as 1.464, 1.465 and 1.465, respectively. Statistically, no significant difference was found between these values ($p \leq 0.05$) (Table A.15).

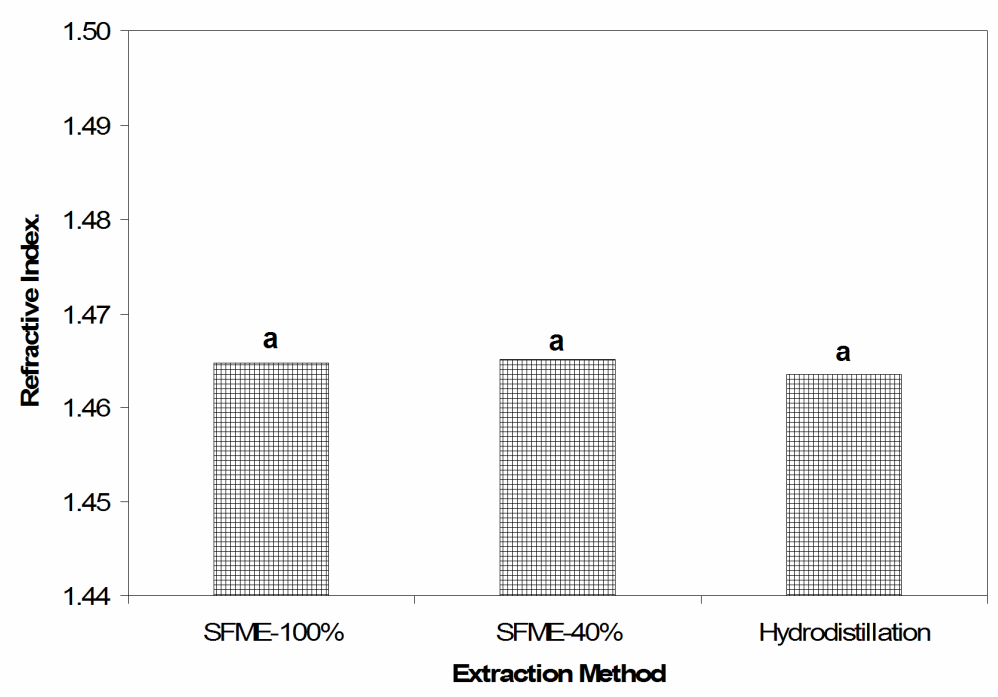


Fig.33. Refractive index values of *Laurus nobilis* L. essential oil extracted by different methods

3.3. Rosemary (*Rosmarinus officinalis* L.)

3.3.1. Particle Size Distribution of Rosemary Leaves

Particle size distribution of rosemary leaves is given in Figure 34. Sauter mean diameter ($\overline{D}_s$) of the leaves was calculated as 1392.4 µm.

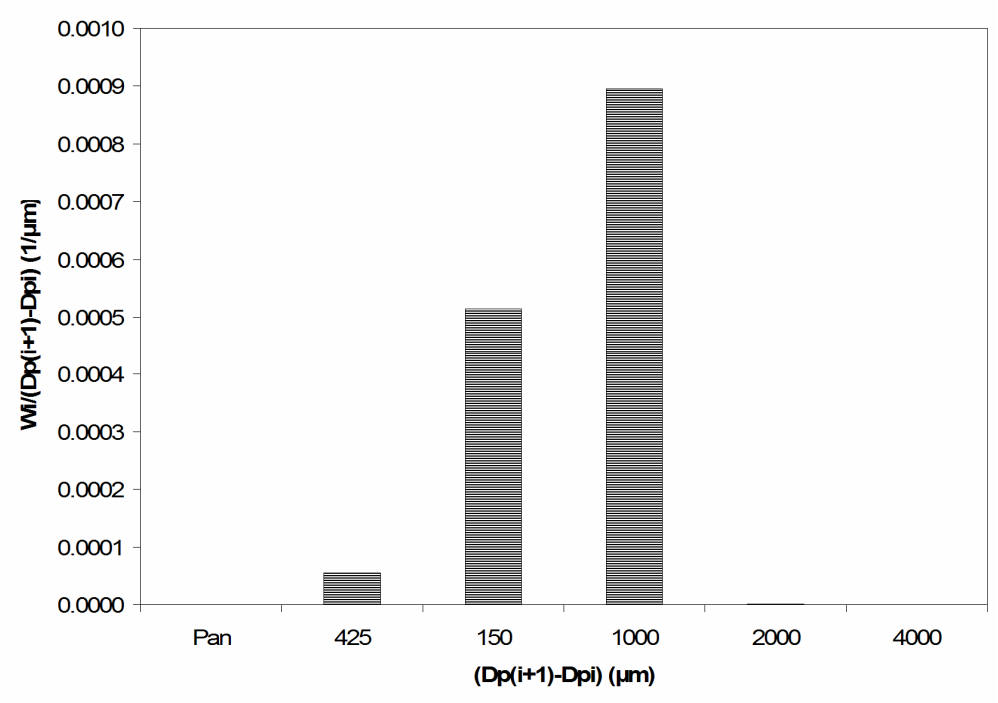


Fig. 34. Particle size distribution of *Rosmarinus officinalis* L. leaves

3.3.2. Effect of extraction on quality parameters of rosemary essential oil

3.3.2.1. Yield

It was found that solvent-free microwave extraction (SFME) method was unsuccessful in dry rosemary since the plant material did not absorb much water in the preliminary water soaking step due to its nature and burning was observed even for short extraction times. Therefore, it was decided to perform microwave-assisted hydrodistillation (MAHD) rather than SFME in rosemary. Figure 35 shows the variation of oil yield with processing time during the conventional hydrodistillation and MAHD methods.

The maximum essential oil yields obtained in MAHD performed at 100% and 40% power levels were found as 0.026 mL oil/g rosemary and 0.021 mL oil/g rosemary, respectively. The maximum yield obtained in hydrodistillation was 0.026 mL oil/g rosemary. There was no significant difference between the yields obtained with MAHD at 100% power level and hydrodistillation, while the yield obtained at 40% power level was found to be significantly different ($p \leq 0.05$) (Table A.16-17). The reason for lower essential oil yield in MAHD at 40% power level might be explained as follows: the magnetron shuts itself in definite periods when operated at lower power levels than 100%. During the longer waiting periods at 40% power level, condenser which is made of glass may also be heated by conduction. Therefore, some of the more volatile constituents of the oil might evaporate and be lost during these waiting periods.

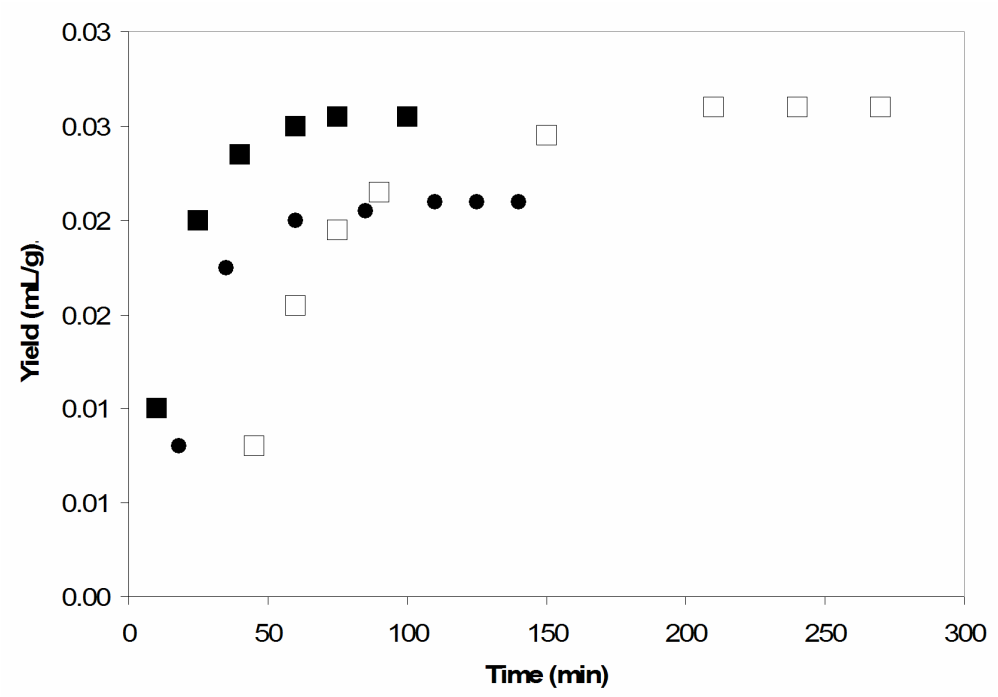


Fig. 35. Variation of essential oil yield of *Rosmarinus officinalis* L. during hydrodistillation and microwave-assisted hydrodistillation (MAHD) at different power levels (■, MAHD-100% power[a]; ●, MAHD-40% power[b]; □, hydrodistillation[a])

Higher initial extraction rate at higher power level was observed as in the case of oregano and laurel. The reason was the formation of a higher pressure gradient with higher power level as discussed before. The time needed for the complete extraction of rosemary essential oil was found as 75 min for MAHD at 100% power level and 110 min for 40% power level. In hydrodistillation, the duration of the process was found to be 210 min. Therefore, the extraction time was reduced by about 65% by using microwave.

3.3.2.2. Composition

Total ion chromatogram of the oil obtained by GC-MS analysis is given in Figure 36. The composition of the essential oil of *Rosmarinus officinalis* L. obtained by MAHD and conventional hydrodistillation methods are given in Table 3. Approximately, 98% of the oil could be characterized. The composition of the essential oils obtained by both of the methods were found, qualitatively, almost the same, whereas some quantitative differences were observed. The main components of the essential oil of *Rosmarinus officinalis* L. were determined as 1,8-cineole (430-500 mg/mL oil) followed by camphor (150-210 mg/mL oil), α-pinene (65-85 mg/mL oil), α-terpineol (35-55 mg/mL oil), borneol (35-51 mg/mL oil), β-pinene (~33 mg/mL oil), and camphene (20-25 mg/mL oil). It was also found that the essential oil was composed mainly of oxygenated compounds (77-82%) while monoterpene hydrocarbons and sesquiterpenes constituted 17-22 % and 0.5-0.7% of it, respectively. It is clear that *Rosmarinus officinalis* L. essential oil owes its pungent odor to these high amount of oxygenated compounds as in the case of oregano and laurel.

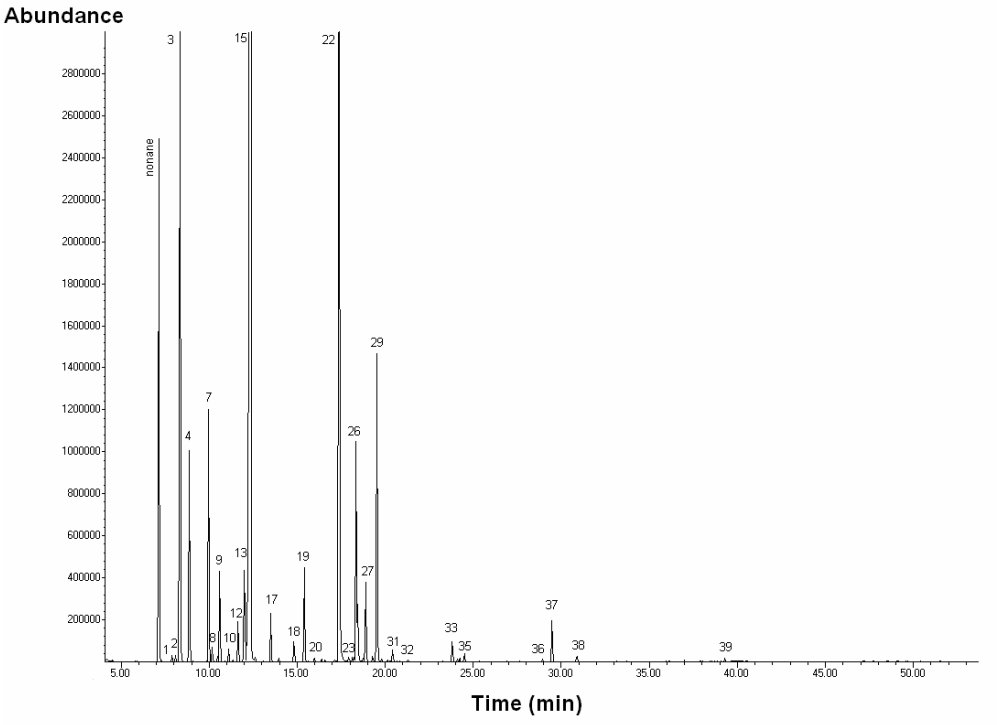


Fig. 36. Total ion chromatogram (obtained by GC-MS analysis) of the *Rosmarinus officinalis* L. essential oil extracted by MAHD at 100% power level (1: tricyclene, 2: α-thujene, 3: α-pinene, 4: camphene, 7: β-pinene, 8: 1-octen-3-ol, 9: β-myrcene, 10: α-phellandrene, 12: α-terpinene, 13: p-cymene, 15: 1,8-cineole, 17: γ-terpinene, 18: terpinolene, 19: linalool, 20: fenchol, 22: camphor, 23: isopulegol, 26: borneol, 27: 4-terpineol, 29: α-terpineol, 31: verbenone, 32: citronellol(?), 33: bornyl acetate, 35: carvacrol, 36: methyl eugenol, 37: β-caryophyllene, 38: α-humulene, 39: methyl jasmonate)

Table 3. Compounds present in the essential oil of *Rosmarinus officinalis* L. obtained by different methods and their concentrations

				Hydrodistillation			MAHD-100% power level			MAHD-40% power level		
				45 (min)	*75 (min)*	*210 (min)*	*10 (min)*	*25 (min)*	*75 (min)*	*18 (min)*	*35 (min)*	*125 (min)*
No	**Compounds**	**RT 1 (min)**	**RT 2 (min)**	**(mg/ml)**	**(mg/ml)**	**(mg/ml)**	**(mg/ml)**	**(mg/ml)**	**(mg/ml)**	**(mg/ml)**	**(mg/ml)**	**(mg/ml)**
1	tricyclene	7.910	13.401	0.903	0.649	0.837	1.177	1.170	1.523	1.481	0.813	0.659
2	alpha-thujene	8.095	13.549	0.841	0.489	0.718	0.991	0.981	0.868	1.324	0.542	0.922
3	alpha-pinene	8.350	13.889	79.625	53.477	85.710	120.695	78.877	84.323	100.176	55.743	66.427
4	camphene	8.874	14.534	20.404	15.777	25.464	30.442	21.682	24.211	25.990	16.425	21.003
5	verbenene (?)	9.101	14.810	-	-	0.281	-	0.181	-	-	-	-
6	sabinene (?)	9.869	15.629	-	-	-	0.248	0.208	0.224	0.330	-	-
7	beta-pinene	9.970	15.760	27.696	21.647	32.847	41.549	31.049	33.161	37.266	25.532	32.859
8	1-octen-3-ol	10.176	16.096	2.118	1.435	1.423	1.787	1.537	1.332	2.345	1.406	1.637
9	beta-myrcene	10.605	16.291	9.108	6.786	11.579	14.622	10.272	11.234	13.003	9.581	12.416
10	alpha-phellandrene	11.119	16.949	1.300	0.953	1.799	1.817	1.460	1.643	1.898	1.385	2.090
11	3-carene	11.378	17.213	0.000	0.000	0.742	0.661	0.235	0.000	0.260	-	0.319
12	alpha-terpinene	11.648	17.502	3.059	2.484	4.846	4.324	3.593	4.279	3.949	3.073	5.020
13	p-cymene	12.014	17.875	7.556	7.277	11.010	9.536	8.160	10.046	8.372	8.352	11.651
14	limonene	-	18.080	4.138	7.131	15.151	16.454	11.683	14.826	6.270	5.438	15.654
15	1,8-cineole	12.363	18.234	538.797	461.529	436.875	409.754	441.957	450.188	461.570	461.844	498.831
16	cis-Ocimene	12.644	18.909	-	-	-	-	-	-	-	-	-
17	gamma-terpinene	13.502	19.407	3.521	3.284	6.219	4.764	4.529	5.582	4.457	4.449	6.810
18	terpinolene	14.826	20.771	1.763	1.728	5.332	2.447	2.592	3.081	2.209	2.275	3.723
19	linalool	15.413	21.214	13.520	13.385	14.352	12.248	15.251	13.583	14.756	16.750	17.438
20	fenchol	15.975	21.955	1.532	1.206	2.342	1.049	1.394	1.509	1.269	1.613	1.632
21	campholaldehyde (?)	16.589	22.528	0.591	0.226	0.696	0.297	-	0.586	0.436	-	0.726
22	camphor	17.399	23.420	139.592	150.045	153.297	149.583	173.693	167.174	157.466	190.386	208.697
23	isopulegol	17.939	23.903	1.074	0.912	0.887	1.059	1.057	0.843	1.263	1.110	1.427
24	pinocamphone/isopinocamphone	18.146	24.140	1.030	0.784	0.727	0.843	0.724	0.764	0.907	0.917	1.118
25	pinocarvone/trans-pinocarvone	18.236	24.252	0.299	-	-	0.344	-	-	-	-	0.435
26	borneol	18.352	24.330	21.488	26.936	35.582	28.329	38.437	39.672	28.329	39.752	51.643
27	4-terpineol	18.892	24.837	7.568	8.820	10.497	8.153	10.727	11.294	8.444	12.081	14.928
28	p-cymene-8-ol (?)	19.295	25.143	0.223	0.316	0.893	0.857	0.832	1.145	0.712	-	0.782
29	alpha-terpineol	19.539	25.401	18.104	25.886	36.869	26.356	40.711	41.003	25.611	41.425	54.200

Table 3. (continued)

30	myrtenol (?)	19.814	25.937	-	-	0.401	0.199	0.626	0.886	-	0.302	1.195
31	verbenone	20.418	26.315	1.389	1.7865	2.9385	2.133	2.8755	3.273	1.7685	2.37	3.7125
32	citronellol (?)	21.312	26.916	-	-	0.824459259	-	0.578129826	0.846105154	-	0.543471239	0.994083584
33	bornyl acetate	23.812	29.552	2.241	2.253	3.2115	2.991	3.006	3.261	2.9865	3.204	4.6185
34	thymol	24.108	29.794	-	-	-	-	0.68692941	0.665915351	-	0.256734558	1.112471649
35	carvacrol	24.5	30.068	1.005	0.975	1.1115	1.197	1.2705	1.617	1.0725	1.2765	1.863
36	methyl eugenol	28.964	34.339	-	-	1.0395	-	0.978	1.1685	-	0.5775	1.458
37	beta-caryophyllene	29.478	35.195	2.6685	1.923	4.035	4.7955	4.0215	4.8945	3.939	4.3365	6.192
38	alpha-humulene	30.886	36.534	0.169433005	0.153087555	0.480769374	0.651675182	0.414703386	0.850271214	0.413007986	0.513157198	0.94080399
39	methyl jasmonate	38.496	43.609	-	-	1.0785	-	-	2.643	-	-	2.895
	% of total			99.49176509	99.38255075	96.68546349	99.05299893	97.61412913	97.89157564	98.76336118	97.78032623	97.5032591
	monoterpene hydrocarbons			159.9125319	121.6799879	202.534682	249.7267823	176.6717458	194.9988948	206.9846789	133.6080648	179.5537594
	oxygenated compounds			750.5698686	696.494566	705.0443739	647.1772676	736.3394915	743.4509655	708.9340372	775.8137516	871.341967
	sesquiterpenes			2.837933005	2.076087555	4.515769374	5.447175182	4.436203386	5.744771214	4.352007986	4.849657198	7.13280399

RT 1: Retention time in min on HP-5MS column obtained by MSD; RT 2: Retention time in min on HP-5MS column obtained by FID

A general trend was obtained in the variation of the amounts of monoterpene hydrocarbons and sesquiterpenes with the time of extraction in MAHD and hydrodistillation. It was observed that sesquiterpenes increased with increase in time (Table 3). This is probably due to their high molecular weights, low volatilities and lower solubilities in water than the other constituents. They need time to reach their maximum levels in the essential oil.

In the case of oxygenated compounds, an increase in their concentrations with time was observed in MAHD, while the opposite trend was observed in conventional hydrodistillation (Figure 37-39). The oxygenated compounds detected in rosemary essential oil were found to be mainly composed of ethers (~ 60%), ketones (22-24 %), alcohols (15-17 %). Ethers were found to be constituted almost entirely of 1,8-cineole. The other oxygenated constituents of the oil were determined as aldehydes, esters, and phenols (Table 3). Since the oxygenated compounds were mainly composed of ethers, ketones and alcohols, the slight increase in their concentrations in MAHD can be attributed to the increase in the amount of the constituents stated above, especially 1,8-cineole. It is interesting to observe that 1,8-cineole concentration (thus, the oxygenated compound concentration) decreased with time in laurel essential oil while its concentration (and the oxygenated compound concentration) increased with time in rosemary oil which was extracted by MAHD method. In rosemary leaves, 1,8-cineole extraction was probably more difficult than its extraction from laurel leaves because of its plant matrix characteristics. Therefore, a couple of minutes may have not been enough for the entire extraction of 1,8-cineole from rosemary leaves as it was in the case of laurel leaves in which the going on process probably caused decomposition of the compound as noted before. In contrast to the situation in MAHD, as mentioned earlier, the oxygenated compounds in rosemary essential oil seemed to slightly decrease with time in

hydrodistillation. This is again caused by the decrease in the amounts of ethers, specifically 1,8-cineole, since other constituents of the oxygenated compounds increase with time. The opposite trends observed in both of the methods for oxygenated compounds might be due to the differences in extraction mechanisms of these methods and/or the noise factors involved in conventional hydrodistillation method, such as light. The system in MAHD does not contact with light since it is placed inside the microwave oven while the system in hydrodistillation does. Therefore, contact of the system with light might be among the reasons for the decomposition of 1,8-cineole with time in hydrodistillation.

In the overall, monoterpene hydrocarbons decreased with time in MAHD, while they slightly increased with time in hydrodistillation. The concentration of monoterpene hydrocarbons seems to reduce with respect to time in the case of MAHD which may be due to the extraction of substances which were more difficult to be isolated (oxygenated compounds and sesquiterpenes). This dilution effect was not observed in the case of hydrodistillation since the concentration of oxygenated compounds decreased during extraction.

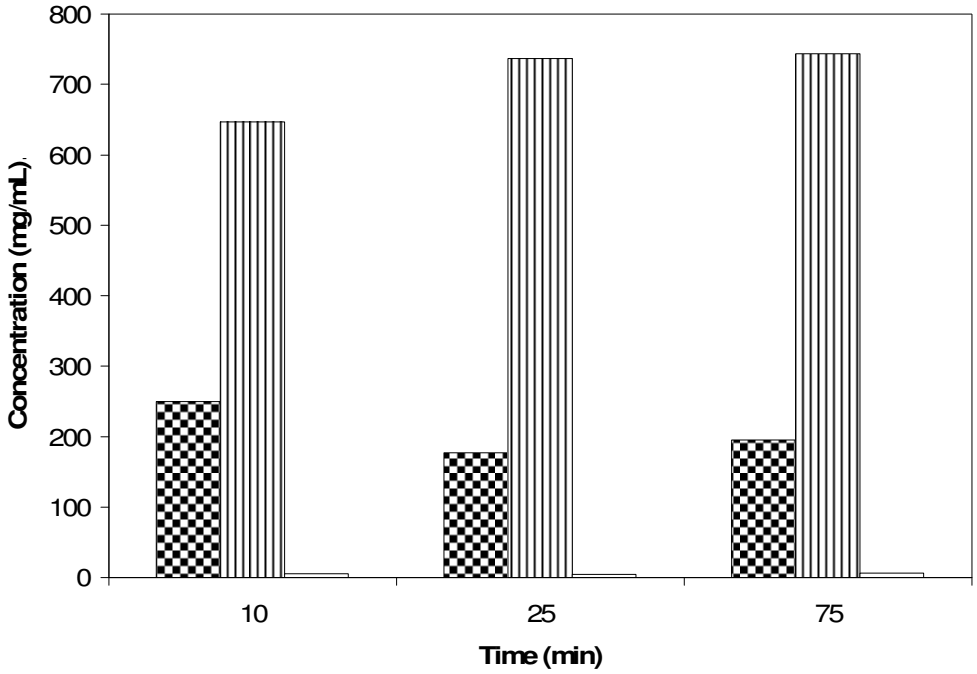


Fig. 37. Variation of component classes with time in the essential oil of *Rosmarinus officinalis* L. obtained by MAHD at 100% power level (▩, monoterpene hydrocarbons; ||||, oxygenated compounds; □, sesquiterpenes)

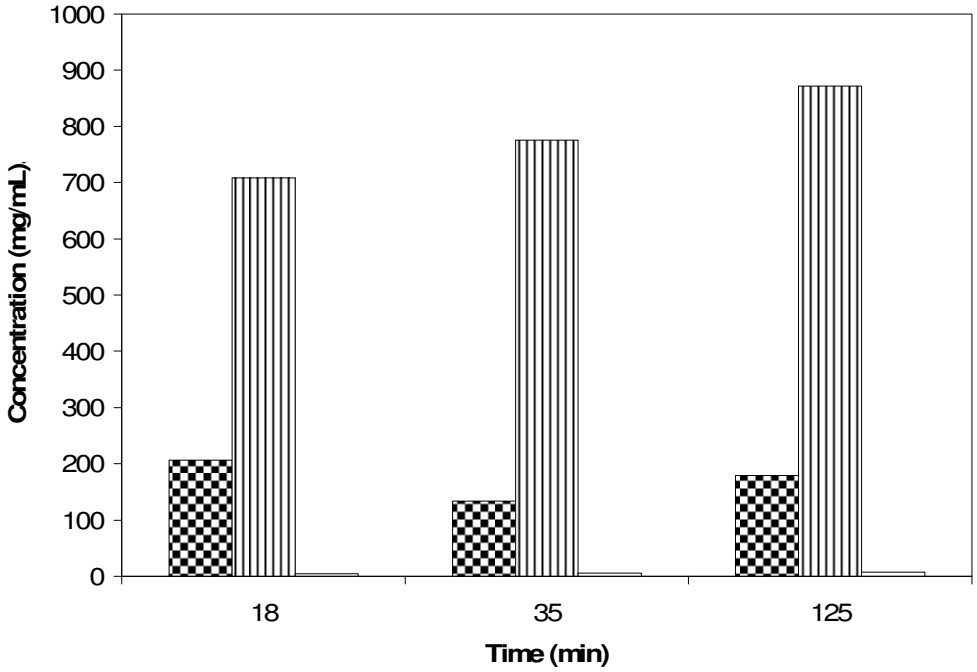


Fig. 38. Variation of component classes with time in the essential oil of *Rosmarinus officinalis* L. obtained by MAHD at 40% power level (▩, monoterpene hydrocarbons; ||||, oxygenated compounds; □, sesquiterpenes)

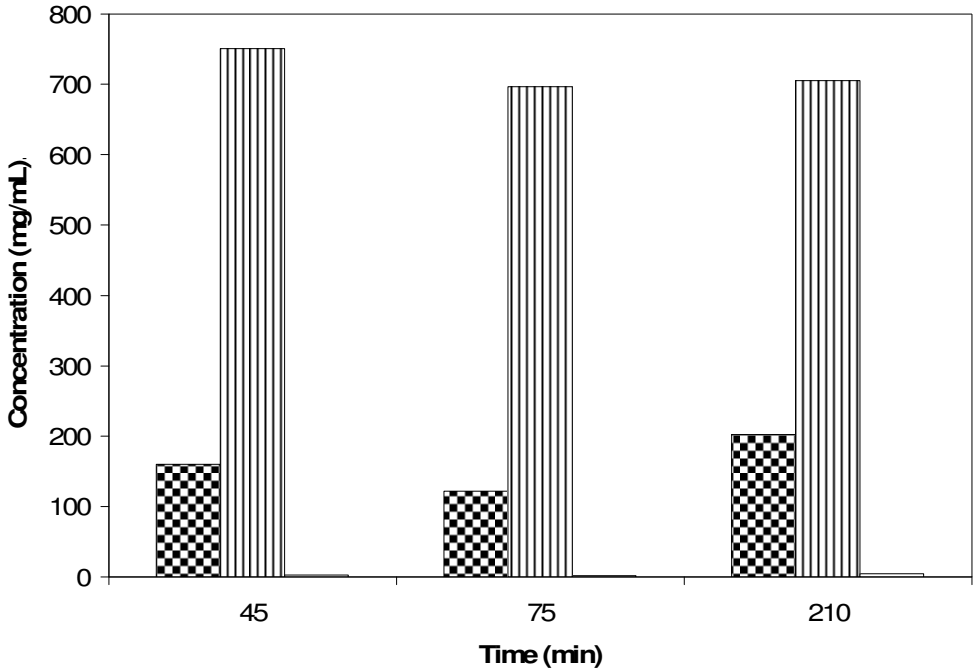


Fig. 39. Variation of component classes with time in the essential oil of *Rosmarinus officinalis* L. obtained by hydrodistillation (▩, monoterpene hydrocarbons; ||||, oxygenated compounds; □, sesquiterpenes)

Figure 40 shows the variation of monoterpene hydrocarbons, oxygenated compounds and sesquiterpenes detected in the rosemary essential oil with respect to the method of extraction. In all compound classes, significant differences were observed between the methods and power levels studied ($p \leq 0.05$) (Table A.18-23). The essential oil extracted by MAHD was found to contain significantly higher amounts of oxygenated compounds than the one obtained by hydrodistillation (Table A.20-21). This is probably due to the small differences in the extraction mechanisms of the methods. In conventional hydrodistillation, extraction is achieved by dissolving the essential oil constituents in water and subsequent volatilization of the mixture, while in MAHD, additionally, direct volatilization of the essential oil constituents from olifereous glands might occur due to the high pressure gradients formed inside the plant material. Therefore, it may not be wrong to say that hydrodistillation was incapable of the entire extraction of

oxygenated compounds in rosemary leaves. The reason for significantly higher amounts of oxygenated compounds obtained in MAHD at 40% power level than at 100% power level might be the longer extraction times involved in 40% power level. Oxygenated compounds in essential oils might be the products of either oxidation or hydrolysis (Wang et al. 2006a) and it is known that as time increases the possibility of occurence of these reactions increases.

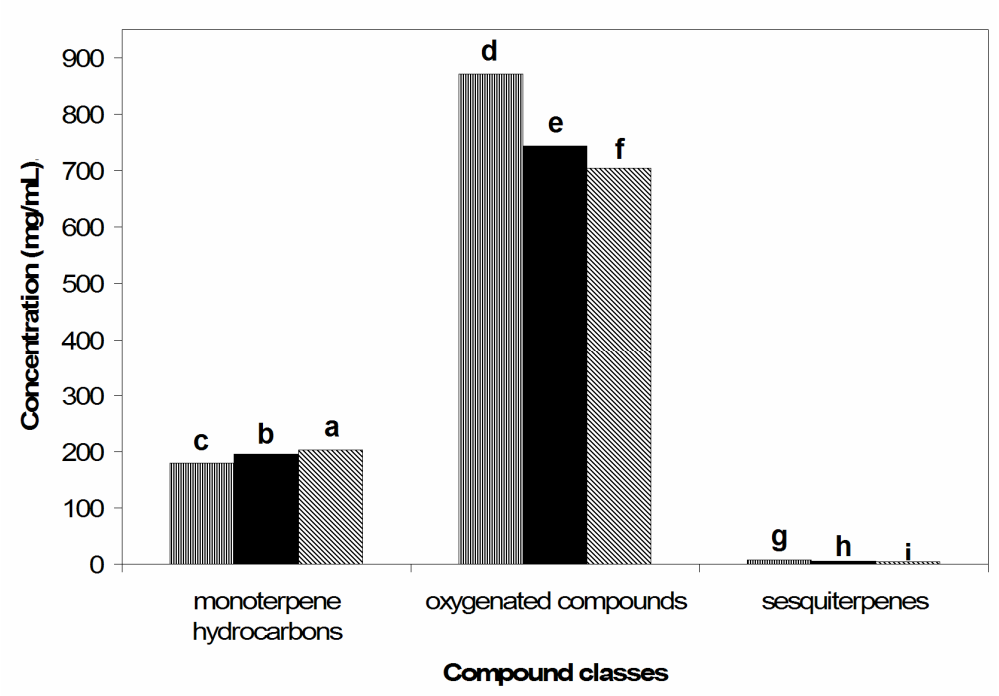


Fig. 40. Variation of compound classes in the essential oil of *Rosmarinus officinalis* L. with respect to different extraction methods (▨, hydrodistillation; ■, MAHD-100% power level; ▥, MAHD-40% power level)

Significantly lower amounts of monoterpene hydrocarbons were detected in the rosemary essential oil obtained by MAHD than in the one obtained by hydrodistillation ($p \leq 0.05$) (Table A.18-19). This might be due to the diminution of thermal or hydrolytic effects because of significantly shorter

extraction times in MAHD than in conventional hydrodistillation. As stated before, monoterpene hydrocarbons are unstable against thermal effects, so it is highly possible that they have gone under some degradation reactions. However, it is known that too long extraction times may also cause decomposition of the other constituents of essential oils such as oxygenated compounds. Therefore, the increase in the concentration of monoterpene hydrocarbons in hydrodistillation may be caused by the decomposition of some of the oxygenated compounds with the effect of long processing times. In contrast to this, significantly lower amounts of monoterpene hydrocarbons were detected in the oil extracted by MAHD at 40% power level than the one extracted at 100% power level although the time of extraction was longer in the former. This might be explained as follows: degradation of monoterpene hydrocarbons may have become dominant over the decomposition of oxygenated compounds in MAHD since the extraction time was fairly shorter than hydrodistillation. Thus, as the time of extraction increased in MAHD, the possibility of degradation of monoterpene hydrocarbons increased which might be the reason of significantly lower amounts of monoterpene hydrocarbons at 40% power level than at 100% power level.

Lastly, significantly higher amounts of sesquiterpenes were detected in the essential oil of rosemary extracted with MAHD than the one extracted with conventional hydrodistillation (Table A.22-23). This is probably because sesquiterpenes are high molecular compounds with low polarities. As mentioned before, in hydrodistillation, isolation of compounds is achieved according to their degree of polarity (which determines their degree of solubility in water) rather than their boiling points. Therefore, the entire extraction of sesquiterpenes might have not been achieved in hydrodistillation most propably bacause they could not totally dissolve in water. Additionally, the oil extracted with MAHD at 40% power level was found to contain significantly higher amounts of sesquiterpenes than the one extracted at 100% power level. This is probably due to the longer

extraction times involved in 40% power level. In addition, in some cases, a compound may be chemically linked to a substrate, so its extraction rate would depend on the decomposition rate of the chemical bond (Simard et al., 1988). Therefore, further extraction of sesquiterpenes might have been achieved with time.

It can be concluded that MAHD is a good alternative for the extraction of essential oils from *Rosmarinus officinalis* L. since it provides essential oils of higher quality (higher oxygenated compounds, lower monterpene hydrocarbons) compared to conventional hydrodistillation while reducing the time of the process drastically.

3.3.2.3. Other parameters

3.3.2.3.1. Specific gravity

Variation of specific gravity values of rosemary essential oil with time obtained by MAHD at different power levels and conventional hydrodistillation is shown in Figure 41. Although the values practically maintained constant during the extraction process, a slight increase with time, especially in MAHD, was observed. This is probably caused by the fact that as MAHD proceeded, monoterpene hydrocarbons decreased with time and sesquiterpenes together with oxygenated compounds increased. Since oxygenated compounds and especially sesquiterpenes have higher molecular weights than the monoterpene hydrocarbons, specific gravity of the oil increased as they were gradually being extracted.

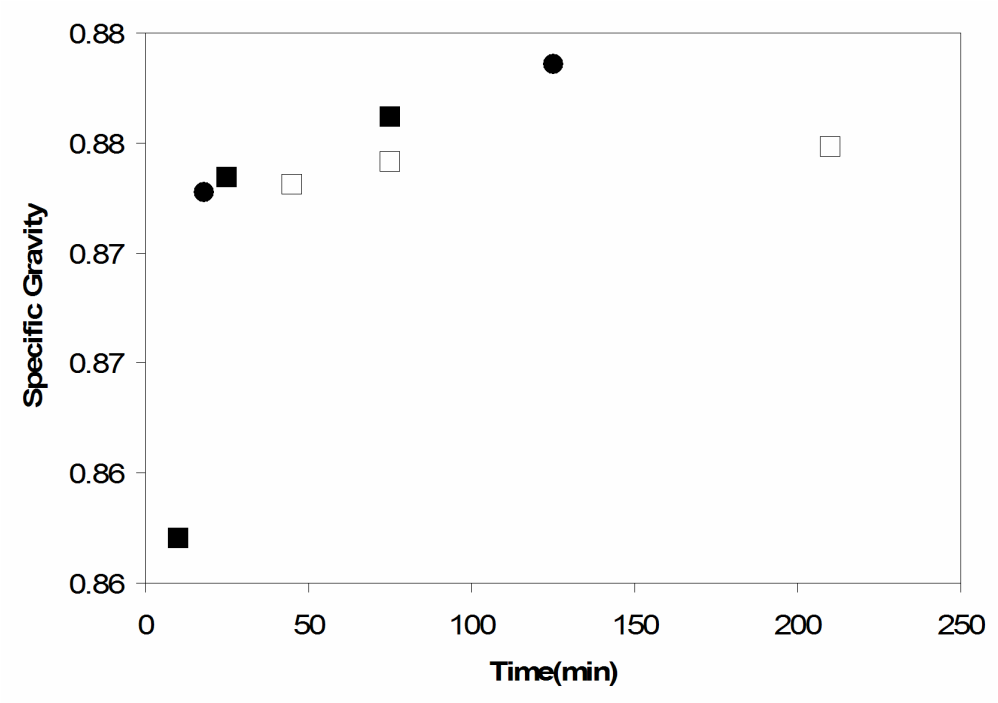


Fig. 41. Variation of specific gravity of the *Rosmarinus officinalis* L. essential oil with time obtained by different methods (■, MAHD-100% power[a]; ●, MAHD-40% power[a]; □, hydrodistillation[a])

Figure 42 shows the specific gravity values of rosemary essential oil obtained by hydrodistillation and MAHD at different power levels. The mean values for the specific gravities of the rosemary oil extracted by hydrodistillation, MAHD at 100% power level and at 40% power level were found as 0.875, 0.876, and 0.879, respectively. Statistically, no significant difference was found between these values ($p \leq 0.05$) (Table A.24). The values were found to be in good agreement with the ones for the essential oil of *Rosmarinus officinalis* L. in literature (Atti-Santos et al., 2005).

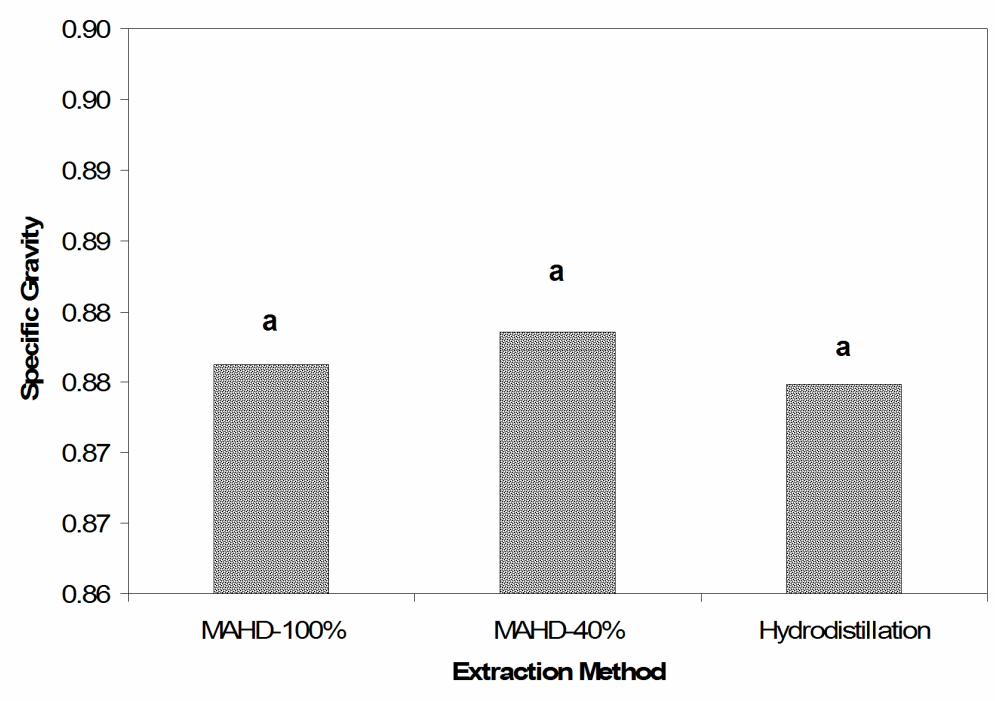


Fig. 42. Specific gravity values of *Rosmarinus officinalis* L. essential oil extracted by different methods

3.3.2.3.2. Refractive index

Variation of refractive index values of rosemary essential oil with time obtained by MAHD at different power levels and conventional hydrodistillation is shown in Figure 43. Like the specific gravity values, a slight increase with time was observed in both of the methods although the values can be assumed to be maintained practically constant. Lower values at the first stages of extraction is probably due to the higher amounts of monoterpene hydrocarbons and lower amounts of oxygenated compounds and sesquiterpenes compared to the last stages (Gamarra et al, 2006).

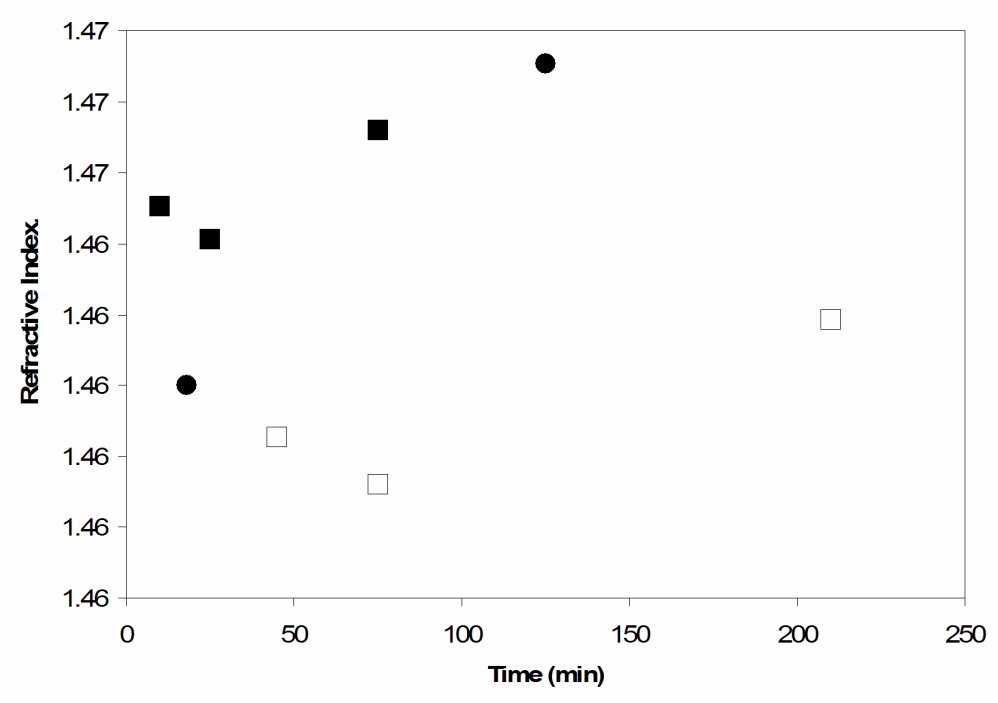


Fig. 43. Variation of refractive index of the *Rosmarinus officinalis* L. essential oil with time obtained by different methods (■, MAHD-100% power; ●, MAHD-40% power; □, hydrodistillation)

Figure 44 shows the refractive index values of rosemary essential oil obtained by hydrodistillation and MAHD at different power levels. The mean values for the refractive index of the rosemary oil extracted by hydrodistillation, MAHD at 100% power level and at 40% power level were found as 1.465, 1.465 and 1.466, respectively. Statistically, no significant difference was found between these values ($p \leq 0.05$) (Table A.25). The values were found to be in good agreement with the ones for the essential oil of *Rosmarinus officinalis* L. in literature (Atti-Santos et al., 2005).

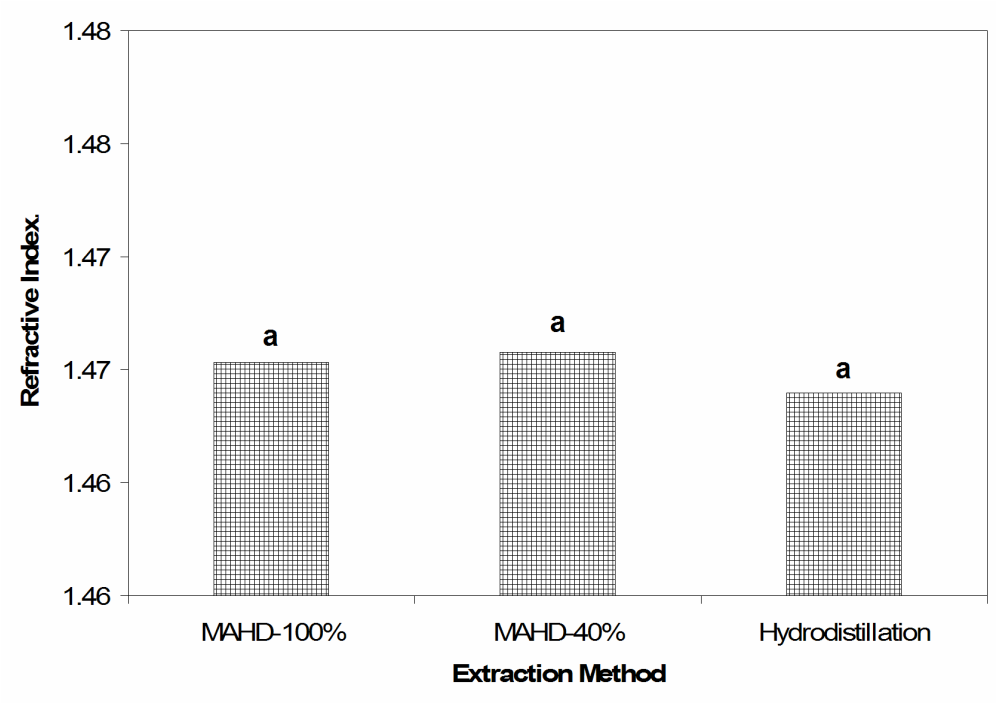


Fig. 44. Refractive index values of *Rosmarinus officinalis* L. essential oil extracted by different methods

CHAPTER 4

CONCLUSION

SFME of dried *Origanum vulgare* L. and *Laurus nobilis* L. and MAHD of dried *Rosmarinus officinalis* L. were compared with the conventional hydrodistillation in terms of process time, yield, composition and other quality parameters of the essential oils.

For *Origanum vulgare* L., SFME offered significantly higher essential oil yields as compared to conventional hydrodistillation while reducing the conventional process time drastically. The main aroma compound in the essential oil of *Origanum vulgare* L. was found to be thymol. The compositions of the essential oils in both of the methods were found to be almost the same which means SFME did not affect quality of the final product. Therefore, it can be concluded that SFME, which is a simple and time-saving method, can be a good alternative for the extraction of essential oils from *Origanum vulgare* L.

In the case of *Laurus nobilis* L., no significant differences were obtained in the essential oil yields obtained by SFME and hydrodistillation. Main constituent of the essential oil of *Laurus nobilis* L. was found to be 1,8-cineole. Compositions of the laurel essential oils obtained by both of the methods were found to be the same except for sesquiterpenes. No sesquiterpenes were extracted in hydrodistillation. Since the presence of sesquiterpenes is not very advantageous as compared to the presence of other constituents which possess antimicrobial and antioxidant effects, the SFME can be stopped in the later stages. This would be advantageous also in shortening the process time. It can be concluded that SFME is a good alternative for the extraction of essential oils from *Laurus nobilis* L. since it provided essential oils of almost the same quality with

conventional hydrodistillation while reducing the time of the process substantially.

Finding out that SFME was unsuccessful on dry rosemary leaves due to their arid nature, MAHD was performed instead for the extraction of essential oil from *Rosmarinus officinalis* L. No significant differences were obtained in the essential oil yields obtained by MAHD at higher power level and conventional hydrodistillation and the process time was found to be reduced substantially. Rosemary essential oil was mainly composed of 1,8-cineole and camphor. Significantly higher amounts of oxygenated compounds and sesquiterpenes and significantly lower amounts of monoterpene hydrocarbons were detected in the essential oils extracted by MAHD than by hydrodistillation, which means that MAHD offered higher quality essential oils than conventional hydrodistillation. Therefore, MAHD is a good alternative for the extraction of essential oils from *Rosmarinus officinalis* L. since it provides essential oils of higher quality compared to conventional hydrodistillation and reduces the process time significantly.

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APPENDIX A

ANOVA AND TUKEY'S PAIRWISE COMPARISON TEST TABLES

Table A.1. ANOVA results for yield values obtained for *Origanum vulgare* L. (Box-Cox transformed)

Analysis of Variance for yield					
Source	*DF*	*SS*	*MS*	*F*	*P*
method	*4*	*4.391E+12*	*1.098E+12*	*14.08*	*0.006*
Error	*5*	*3.899E+11*	*7.798E+10*		
Total	*9*	*4.781E+12*			

Table A.2. Tukey's pairwise comparison test results for yield values obtained in the extraction of *Origanum vulgare* L.

	Hydrodistillation	*SFME-100%p*	*SFME-40%p*	*SFME-60%p*
SFME-100%p	*533215* *2772467*			
SFME-40%p	*-748369* *1490883*	*-2401211* *-161958*		
SFME-60%p	*186557* *2425809*	*-1466285* *772968*	*-184700* *2054552*	
SFME-80%p	*413386* *2652638*	*-1239455* *999797*	*42129* *2281382*	*-892797* *1346456*

Table A.3. ANOVA results for monoterpene hydrocarbon concentrations obtained in the extraction of *Origanum vulgare* L.

Analysis of Variance for monoterpene hydrocarbons					
Source	*DF*	*SS*	*MS*	*F*	*P*
method	*4*	*4387*	*1097*	*0.44*	*0.776*
Error	*5*	*12427*	*2485*		
Total	*9*	*16815*			

Table A.4. ANOVA results for oxygenated compound concentrations obtained in the extraction of *Origanum vulgare* L.

Analysis of Variance for oxygenated compounds					
Source	*DF*	*SS*	*MS*	*F*	*P*
method	*4*	*6578*	*1644*	*0.29*	*0.874*
Error	*5*	*28495*	*5699*		
Total	*9*	*35072*			

Table A.5. ANOVA results for sesquiterpene concentrations obtained in the extraction of *Origanum vulgare* L.

Analysis of Variance for sesquiterpenes					
Source	*DF*	*SS*	*MS*	*F*	*P*
method	*4*	*167.8*	*42.0*	*0.82*	*0.566*
Error	*5*	*257.3*	*51.5*		
Total	*9*	*425.1*			

Table A.6. ANOVA results for specific gravity values obtained in the extraction of *Origanum vulgare* L.

Analysis of Variance for specific gravity					
Source	*DF*	*SS*	*MS*	*F*	*P*
method	*3*	*0.00412*	*0.00137*	*0.76*	*0.546*
Error	*8*	*0.01442*	*0.00180*		
Total	*11*	*0.01854*			

Table A.7. ANOVA results for refractive index values obtained in the extraction of *Origanum vulgare* L.

Analysis of Variance for refractive index					
Source	*DF*	*SS*	*MS*	*F*	*P*
method	*3*	*0.0007970*	*0.0002657*	*3.68*	*0.062*
Error	*8*	*0.0005776*	*0.0000722*		
Total	*11*	*0.0013746*			

Table A.8. ANOVA results for solubility in ethanol values obtained in the extraction of *Origanum vulgare* L.

Analysis of Variance for solubility in ethanol					
Source	*DF*	*SS*	*MS*	*F*	*P*
method	*3*	*0.1441*	*0.0480*	*0.97*	*0.457*
Error	*7*	*0.3450*	*0.0493*		
Total	*10*	*0.4891*			

Table A.9. ANOVA results for yield values obtained for *Laurus nobilis* L.

Analysis of Variance for yield					
Source	*DF*	*SS*	*MS*	*F*	*P*
method	*2*	*0.0000033*	*0.0000016*	*5.86*	*0.092*
Error	*3*	*0.0000008*	*0.0000003*		
Total	*5*	*0.0000041*			

Table A.10. ANOVA results for monoterpene hydrocarbon concentrations obtained in the extraction of *Laurus nobilis* L.

Analysis of Variance for monoterpene hydrocarbons					
Source	*DF*	*SS*	*MS*	*F*	*P*
method	*2*	*124*	*62*	*0.22*	*0.818*
Error	*3*	*861*	*287*		
Total	*5*	*984*			

Table A.11. ANOVA results for oxygenated compound concentrations obtained in the extraction of *Laurus nobilis* L.

Analysis of Variance for oxygenated compounds					
Source	*DF*	*SS*	*MS*	*F*	*P*
method	*2*	*23391*	*11695*	*1.19*	*0.417*
Error	*3*	*29552*	*9851*		
Total	*5*	*52943*			

Table A.12. ANOVA results for sesquiterpene concentrations obtained in the extraction of *Laurus nobilis* L.

Analysis of Variance for sesquiterpenes					
Source	*DF*	*SS*	*MS*	*F*	*P*
method	*2*	*37.840*	*18.920*	*24.67*	*0.014*
Error	*3*	*2.301*	*0.767*		
Total	*5*	*40.141*			

Table A.13. Tukey's pairwise comparison test results for sesquiterpene concentrations obtained in the extraction of *Laurus nobilis* L.

	Hydrodistillation	*SFME-100%p*
SFME-100%p	*-6.1149* *1.2048*	
SFME-40%p	*-9.7720* *-2.4523*	*-7.3169* *0.0027*

Table A.14. ANOVA results for specific gravity values obtained in the extraction of *Laurus nobilis* L.

Analysis of Variance for specific gravity					
Source	*DF*	*SS*	*MS*	*F*	*P*
method	*2*	*0.000170*	*0.000085*	*0.21*	*0.814*
Error	*6*	*0.002390*	*0.000398*		
Total	*8*	*0.002560*			

Table A.15. ANOVA results for refractive index values obtained in the extraction of *Laurus nobilis* L.

Analysis of Variance for refractive index					
Source	*DF*	*SS*	*MS*	*F*	*P*
method	*2*	*0.0000042*	*0.0000021*	*0.15*	*0.864*
Error	*6*	*0.0000845*	*0.0000141*		
Total	*8*	*0.0000887*			

Table A.16. ANOVA results for yield values obtained for *Rosmarinus officinalis* L.

Analysis of Variance for yield					
Source	*DF*	*SS*	*MS*	*F*	*P*
method	*2*	*0.0000303*	*0.0000152*	*18.20*	*0.021*
Error	*3*	*0.0000025*	*0.0000008*		
Total	*5*	*0.0000328*			

Table A.17. Tukey's pairwise comparison test results for yield values obtained in the extraction of *Rosmarinus officinalis* L.

	Hydrodistillation	*MAHD-100%p*
MAHD-100%p	*-0.0033149* *0.0043149*	
MAHD-40%p	*0.0011851* *0.0088149*	*0.0006851* *0.0083149*

Table A.18. ANOVA results for monoterpene hydrocarbon concentrations obtained in the extraction of *Rosmarinus officinalis* L.

Analysis of Variance for monoterpene hydrocarbons					
Source	*DF*	*SS*	*MS*	*F*	*P*
method	*2*	*548.98*	*274.49*	*93.96*	*0.002*
Error	*3*	*8.76*	*2.92*		
Total	*5*	*557.74*			

Table A.19. Tukey's pairwise comparison test results for monoterpene hydrocarbon concentrations obtained in the extraction of *Rosmarinus officinalis* L.

	Hydrodistillation	*MAHD-100%p*
MAHD-100%p	*0.393* *14.678*	
MAHD-40%p	*15.838* *30.123*	*8.303* *22.588*

Table A.20. ANOVA results for oxygenated compound concentrations obtained in the extraction of *Rosmarinus officinalis* L.

Analysis of Variance for oxygenated compounds					
Source	*DF*	*SS*	*MS*	*F*	*P*
method	*2*	*30324.0*	*15162.0*	*252.29*	*0.000*
Error	*3*	*180.3*	*60.1*		
Total	*5*	*30504.3*			

Table A.21. Tukey's pairwise comparison test results for oxygenated compound concentrations obtained in the extraction of *Rosmarinus officinalis* L.

	Hydrodistillation	*MAHD-100%p*
MAHD-100%p	*-70.80* *-6.01*	
MAHD-40%p	*-198.69* *-133.90*	*-160.29* *-95.49*

Table A.22. ANOVA results for sesquiterpene concentrations obtained in the extraction of *Rosmarinus officinalis* L.

Analysis of Variance for sesquiterpenes					
Source	*DF*	*SS*	*MS*	*F*	*P*
method	*2*	*6.8573*	*3.4287*	*49.00*	*0.005*
Error	*3*	*0.2099*	*0.0700*		
Total	*5*	*7.0672*			

Table A.23. Tukey's pairwise comparison test results for sesquiterpene concentrations obtained in the extraction of *Rosmarinus officinalis* L.

	Hydrodistillation	*MAHD-100%p*
MAHD-100%p	*-2.3344* *-0.1236*	
MAHD-40%p	*-3.7225* *-1.5116*	*-2.4935* *-0.2826*

Table A.24. ANOVA results for specific gravity values obtained in the extraction of *Rosmarinus officinalis* L.

Analysis of Variance for specific gravity					
Source	*DF*	*SS*	*MS*	*F*	*P*
method	*2*	*0.0000221*	*0.0000111*	*0.19*	*0.834*
Error	*6*	*0.0003543*	*0.0000590*		
Total	*8*	*0.0003764*			

Table A.25. ANOVA results for refractive index values obtained in the extraction of *Rosmarinus officinalis* L.

Analysis of Variance for refractive index					
Source	*DF*	*SS*	*MS*	*F*	*P*
method	*2*	*0.0000019*	*0.0000010*	*0.09*	*0.915*
Error	*6*	*0.0000648*	*0.0000108*		
Total	*8*	*0.0000667*			